LIDIANE COVELL

METABOLIC RESPONSES TO NITROGEN DEFICIENCY AND RAPAMYCIN TREATMENT IN FRESHWATER MICROALGAE STRAINS

Thesis submitted to the Plant Physiology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

Adviser: Adriano Nunes Nesi

Co-adviser: Marcelo Gomes Marçal Vieira Vaz

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BIOGRAPHY

Lidiane Covell, daughter of Adenir da Silva Lessa and Vanir Ribeiro Magalhães, was born on May 1, 1978, in the city of Viçosa, Minas Gerais. She concluded high school in her birth city in 1997.

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“Es gibt einen Antrieb, der stärker ist als Dampf, als Elektrizität und als Atomenergie: Der Wille”
("There is a driving force more powerful than steam, electricity and atomic energy: the will.")
Albert Einstein

ABSTRACT

COVELL, Lidiane, D.Sc., Universidade Federal de Viçosa, February 2022. **Metabolic responses to nitrogen deficiency and rapamycin treatment in freshwater microalgae strains.** Adviser: Adriano Nunes Nesi. Co-adviser: Marcelo Gomes Marçal Vieira Vaz.

Nutrient deprivation causes significant stress to microalgae, which responds by altering their metabolic pathways. Following N deprivation, the accumulation of starch and triacylglycerols (TAGs) are significantly altered following reprogramming of cellular metabolism. In addition, the adaptation of cell growth and proliferation to environmental changes is essential for the surviving of biological systems. The evolutionary conserved Ser/Thr protein kinase (Target of Rapamycin - TOR) is crucial for the signaling pathway that integrates the sensing of growth signals to the regulation of metabolism and cellular growth. The TOR pathway controls cell growth by promoting anabolic processes, including protein synthesis and ribosome biogenesis, and inhibiting catabolic processes such as autophagy. Therefore, a greater understanding of the regulation of growth and metabolic pathways under N deprivation and TOR inhibition is necessary to understand the physiology and the mechanisms involved in the biosynthesis of storage compounds in Brazilian native microalgae. In this study, native microalgae, *Scenedesmus obliquus* BR003, *Chlorella vulgaris* BR017, *Chlamydomonas* sp. BR020 and *Monoraphidium* sp.BR023, and one consider model, *Chlamydomonas reinhardtii* CC503 were selected to evaluate the growth parameters, biochemical composition, and metabolite profile under different nitrogen concentrations and TOR pathway inhibition by rapamycin. Whereas N depletion leads to typical stress-related responses in all strains, including reduction of cell growth, chlorophyll and protein, differential accumulation of primary compounds, membrane lipid composition and fatty acid were also observed. The metabolic and physiological analysis showed not only differential sensitivity to the absence of N in relation to N-replete and N-saturated treatments but also differences between strains. The response of growth, dynamics, metabolism profile, and lipid profile to TOR-inhibition by rapamycin in the strains showed a decrease in both cell growth and strong changes in carbon and nitrogen partitioning in the direction of rapid conversion into carbon and nitrogen storage through an accumulation of starch, triacylglycerol and amino acids. Interestingly, the strains showed different metabolite levels, confirming the difference in reserve compound accumulation of each strain. Based on the results, this study indicates that, due to their metabolic profile, microalgae have different

responses in cell growth and metabolism, thus, a better understanding of the metabolic pathways in different strains is needed for biotechnological use.

Keywords: Chlorophytes. *Chlamydomonas reinhardtii*. Primary- and lipid- metabolism. Rapamycin. N-deprivation.

RESUMO

COVELL, Lidiane, D.Sc., Universidade Federal de Viçosa, fevereiro de 2022. **Respostas metabólicas à deficiência de nitrogênio e tratamento de rapamicina em linhagens de microalgas de água doce.** Orientador: Adriano Nunes Nesi. Coorientador: Marcelo Gomes Marçal Vieira Vaz.

A privação de nutrientes leva a diferentes estresses às microalgas, que respondem alterando as vias metabólicas. Após a privação de nitrogênio (N), os acúmulos de amido e triacilgliceróis (TAGs) são significativamente alterados pela reprogramação do metabolismo celular. Além disso, a adaptação do crescimento e proliferação celular às mudanças ambientais é essencial para a sobrevivência dos sistemas biológicos. A proteína quinase Ser/Thr evolutivamente conservada "Alvo da Rapamicina" (TOR) é crucial para a via de sinalização que integra a detecção de sinais de crescimento à regulação do metabolismo e crescimento celular. A via TOR controla o crescimento celular promovendo processos anabólicos, incluindo síntese de proteínas e biogênese de ribossomos, e inibindo processos catabólicos, como autofagia. Portanto, um maior entendimento da regulação do crescimento e das vias metabólicas sob privação de N e inibição de TOR é necessário para entender a fisiologia e os mecanismos envolvidos na biossíntese de compostos de armazenamento em microalgas nativas brasileiras. Neste estudo, microalgas nativas, *Scenedesmus obliquus* BR003, *Chlorella vulgaris* BR017, *Chlamydomonas* sp. BR020 e *Monoraphidium* sp. BR023, e a linhagem modelo, *Chlamydomonas reinhardtii* CC503 foram selecionados para avaliar os parâmetros de crescimento, composição bioquímica e perfil metabólico sob diferentes concentrações de N e inibição da via TOR pela rapamicina. Além de respostas típicas que a privação de N leva como redução do crescimento celular, níveis de clorofilas e proteínas, o acúmulo diferencial de compostos primários, composição lipídica da membrana e ácidos graxos também foram observados. A análise metabólica e fisiológica sugere que as linhagens não apresentam apenas sensibilidade diferencial à ausência de N em relação aos tratamentos repleto e saturado de N, mas também diferenças entre as linhagens observadas em condições ótimas de cultivo. A dinâmica de resposta de crescimento, perfil metabólico e lipídico à inibição de TOR pela rapamicina mostrou uma diminuição no crescimento celular e fortes mudanças na partição de carbono e N por meio de um acúmulo de amido, triacilglicerol e aminoácidos. As linhagens apresentaram diferentes níveis de metabólitos, confirmando a diferença no acúmulo de composto de reserva de cada linhagem. Com base nos resultados, este estudo indica que, devido ao seu perfil metabólico, as microalgas apresentam diferentes respostas no crescimento celular

e metabolismo sendo necessária uma melhor compreensão das vias metabólicas em diferentes linhagens para o uso biotecnológico.

Palavras-chave: Clorófitas. *Chlamydomonas reinhardtii*. Metabolismo primário e lipídico. Rapamicina. Privação de nitrogênio.

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GENERAL INTRODUCTION

During evolution microalgae species developed many protection mechanisms to survive under unfavorable conditions (e.g., variable salinity, temperature, nutrients, UV irradiation) that ultimately led them to produce a wide range of fascinating chemical compounds with novel structures and biological activities (Machado et al., 2016; Rocha et al., 2019). Microalgae are biological resources for a vast array of high-value compounds, are a rich source of protein, carbohydrates, lipids (especially essential fatty acids) and diversity of pigments (such as chlorophylls, carotenoids and phycobiliproteins) (Moreno-Garcia et al., 2017; Mobin and Chowdhury, 2019). The cultivation of these photosynthetic microorganisms represents an attractive process for obtaining biochemical components with high potential applications in the food, pharmaceutical, cosmetic, animal feed and biodiesel industries (Rizwan et al., 2018; Nagappan et al., 2020; Machado et al., 2021; Silva et al., 2021). Generally, the production of these bioproducts varies from species to species and even in the same species of algae (Mobin and Alam, 2017). Microalgae combine properties typical of plants (efficient oxygenic photosynthesis and simplicity of nutritional requirements) with biotechnological attributes properties of microbial cells (fast growth in liquid culture and ability to accumulate or secrete some metabolites) (Herrero et al., 2013; Rizwan et al., 2018; Kumar et al., 2020). This particular combination represents the basis of microalgal biotechnology for the use of these microorganisms on high-valued metabolites production (Rizwan et al., 2018; Kumar et al., 2020).

Microalgal metabolic pathways are heavily influenced by the environment (Gupta et al., 2019). Some stress strategies in culture have been studied for enhancing biomass and high-value compounds yields, like salinity, high temperature, nitrogen sources and other factors (Tan and Lee, 2016; Barsanti and Gualtieri, 2018; Choi et al., 2019). These conditions are defined as a significant deviation from the optimal conditions for the normal development and growth of microalgae and cause changes in all functional levels of the organism.

Limitation and starvation of nitrogen (N) are primary factors that influence cell growth and metabolism, and these conditions may be used to enhance lipids/starch biosynthesis with an optimal growth in microalgae (Machado et al., 2016; Soares et al., 2018; Batista et al., 2019). On the other hand, the regulation of metabolic homeostasis is critical to biosynthetic processes and cellular functions in microalgae (Ford et al., 2019). In this context, an emerging route is the TOR (Target of Rapamycin) kinase pathway (Wullschleger, 2006; Soulard et al., 2009). This

pathway is conserved in eukaryotes and acts as a center for the regulation of numerous cell cycle processes linking the perception of both nutrient availability and favorable external conditions to cell anabolic metabolism, growth, and division by recruiting and regulating the diverse TOR kinase substrates (Díaz-Troya et al., 2008b; Prioretti et al., 2017; Werth et al., 2019).

Nitrogen (N) is one of the most important nutrients for microalgae (Paskuliakova et al., 2018). Numerous studies have suggested that N limitation or starvation is one of the most effective approaches in inducing the accumulation of reserve compounds in microalgae (Arumugam et al., 2013; Singh et al., 2016). When N is insufficient to support protein synthesis, excess carbon (C) from photosynthesis is diverted into storage molecules such as triglycerides and starch (Zhu et al., 2014; Himanshu et al., 2016; Juergens et al., 2016; Zhu et al., 2019).

The TOR kinase plays an essential and conserved role in integrating nutritional and energy inputs for the proper regulation of cell growth in eukaryotes (Díaz-Troya et al., 2008b). TOR controls cell growth by promoting a number of anabolic processes, including translation, ribosome biogenesis, and transcription, and by antagonizing catabolic processes such as autophagy and mRNA degradation (Caldana et al., 2013; Shemi et al., 2015). The first experimental evidence of TOR conservation in these organisms was reported in the model green alga *Chlamydomonas reinhardtii* (Crespo et al., 2005). It has been reported that the growth of Chlamydomonas cells is inhibited by rapamycin and this sensitivity of Chlamydomonas cells, together with the easy manipulation of this organism, its simple life cycle, and a growing array of genetic and molecular tools (Menand et al., 2002), have positioned this green alga as a convenient model system to investigate the TOR network in photosynthetic eukaryotes. However, the changes in cell morphology, growth, biochemical composition, and photosynthetic performances of different microalgae during the process of N limitation or starvation and inhibition of the TOR signaling pathway still require more detailed studies. The optimization of biomass yield is an important parameter in cultivation of microalgae. Thus, it is essential to understand the behavior of selected microalgal species under different biotic and abiotic conditions.

The present study aimed at the cultivation of freshwater native Brazilian strains under various culture conditions to induce N-depletion and/or TOR pathway inhibition that are likely to influence algal growth and production of value-added products. The species were selected based on its growth rate and different accumulation of storage compounds ability (Rocha et al., 2017). The growth and biochemical and physiological parameters under different culture

conditions were evaluated. The thesis is organized into three independent chapters. The first chapter aimed to understand the adjustments in C/N metabolism in strains that accumulate in distinct amounts, the storage compounds starch/lipid under N depletion conditions. In the second chapter, the objective was to evaluate changes in metabolic compounds by the TOR signaling pathway inhibited by rapamycin in distinct strains. The last chapter presents a critical review of the current state of knowledge of TOR signaling pathway in microalgae.

REFERENCES

Arumugam M, Agarwal A, Arya MC, Ahmed Z (2013) Influence of nitrogen sources on biomass productivity of microalgae *Scenedesmus bijugatus*. Bioresour Technol **131**: 246–249

Barsanti L, Gualtieri P (2018) Is exploitation of microalgae economically and energetically sustainable? Algal Res **31**: 107–115

Batista AD, Rosa RM, Machado M, Magalhães AS, Shalaguti BA, Gomes PF, Covell L, Vaz MGMV, Araújo WL, Nunes-Nesi A (2019) Increased urea availability promotes adjustments in C/N metabolism and lipid content without impacting growth in *Chlamydomonas reinhardtii*. Metabolomics. doi: 10.1007/s11306-019-1496-3

Caldana C, Li Y, Leisse A, Zhang Y, Bartholomaeus L, Fernie AR, Willmitzer L, Giavalisco P (2013) Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in Arabidopsis thaliana. Plant J **73**: 897–909

Choi WJ, Chae AN, Song KG, Park J, Lee BC (2019) Effect of trophic conditions on microalga growth, nutrient removal, algal organic matter, and energy storage products in *Scenedesmus* (Acutodesmus) *obliquus* KGE-17 cultivation. Bioprocess Biosyst Eng **42**: 1225–1234

Crespo JL, Díaz-Troya S, Florencio FJ (2005) Inhibition of target of rapamycin signaling by rapamycin in the unicellular green alga *Chlamydomonas reinhardtii*. Plant Physiol **139**: 1736–1749

Díaz-Troya S, Pérez-Pérez ME, Florencio FJ, Crespo JL (2008) The role of TOR in autophagy regulation from yeast to plants and mammals. Autophagy **4**: 851–865

Ford MM, Smythers AL, McConnell EW, Lowery SC, Kolling DRJ, Hicks LM (2019) Inhibition of TOR in *Chlamydomonas reinhardtii* Leads to Rapid Cysteine Oxidation Reflecting Sustained Physiological Changes. Cells. doi: 10.3390/cells8101171

Gupta S, Pawar SB, Pandey RA, Kanade GS, Lokhande SK (2019) Outdoor microalgae cultivation in airlift photobioreactor at high irradiance and temperature conditions: effect of

batch and fed-batch strategies, photoinhibition, and temperature stress. Bioprocess Biosyst Eng **42**: 331–344

Herrero M, Mendiola JA, Plaza M, Ibañez E (2013) Screening for Bioactive Compounds from Algae. Screen Bioact Compd from Algae. doi: 10.1007/978-1-4614-3348-4_35

Himanshu S, Manish RS, Basuthkar JR, Kandala VRC (2016) Regulation of starch, lipids and amino acids upon nitrogen sensing in *Chlamydomonas reinhardtii*. Algal Res **18**: 33–44

Juergens MT, Disbrow B, Shachar-Hill Y (2016) The relationship of triacylglycerol and starch accumulation to carbon and energy flows during nutrient deprivation in *Chlamydomonas reinhardtii*. Plant Physiol **171**: 2445–2457

Kumar G, Shekh A, Jakhu S, Sharma Y, Kapoor R, Sharma TR (2020) Bioengineering of Microalgae: Recent Advances, Perspectives, and Regulatory Challenges for Industrial Application. Front Bioeng Biotechnol. doi: 10.3389/fbioe.2020.00914

Machado M, Bromke M, Domingues Júnior AP, Marçal Vieira Vaz MG, Rosa RM, Vinson CC, Sabir JS, Rocha DI, Martins MA, Araújo WL, et al (2016) Comprehensive metabolic reprograming in freshwater Nitzschia palea strains undergoing nitrogen starvation is likely associated with its ecological origin. Algal Res. doi: 10.1016/j.algal.2016.06.003

Machado M, Vaz MGMV, Bromke MA, Rosa RM, Covell L, Souza LP de, Rocha DI, Martins MA, Araújo WL, Szymański J, et al (2021) Metabolic stability of freshwater Nitzschia palea strains under silicon stress associated with triacylglycerol accumulation. Algal Res **60**: 102554

Menand B, Desnos T, Nussaume L, Bergert F, Bouchez D, Meyer C, Robaglia C (2002) Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. Proc Natl Acad Sci U S A **99**: 6422–6427

Mobin A, Chowdhury H (2019) Commercially important bioproducts from microalgae and their current applications – A review. Energy Procedia **160**: 752–760

Mobin S, Alam F (2017) Some Promising Microalgal Species for Commercial Applications: A review. Energy Procedia **110**: 510–517

Moreno-Garcia L, Adjallé K, Barnabé S, Raghavan GSV (2017) Microalgae biomass production for a biorefinery system: Recent advances and the way towards sustainability. Renew Sustain Energy Rev **76**: 493–506

Nagappan S, Devendran S, Tsai PC, Jayaraman H, Alagarsamy V, Pugazhendhi A, Ponnusamy VK (2020) Metabolomics integrated with transcriptomics and proteomics:

Evaluation of systems reaction to nitrogen deficiency stress in microalgae. Process Biochem **91**: 1–14

Paskuliakova A, McGowan T, Tonry S, Touzet N (2018) Microalgal bioremediation of nitrogenous compounds in landfill leachate – The importance of micronutrient balance in the treatment of leachates of variable composition. Algal Res **32**: 162–171

Prioretti L, Avilan L, Carrière F, Montané MH, Field B, Grégori G, Menand B, Gontero B (2017) The inhibition of TOR in the model diatom Phaeodactylum tricornutum promotes a get-fat growth regime. Algal Res **26**: 265–274

Rizwan M, Mujtaba G, Memon SA, Lee K, Rashid N (2018) Exploring the potential of microalgae for new biotechnology applications and beyond: A review. Renew Sustain Energy Rev **92**: 394–404

Rocha DN, Martins MA, Soares J, Vaz MGMV, de Oliveira Leite M, Covell L, Mendes LBB (2019) Combination of trace elements and salt stress in different cultivation modes improves the lipid productivity of *Scenedesmus* spp. Bioresour Technol. doi: 10.1016/j.biortech.2019.121644

Rocha RP, Machado M, Vaz MGMV, Vinson CC, Leite M, Richard R, Mendes LBB, Araujo WL, Caldana C, Martins MA, et al (2017) Exploring the metabolic and physiological diversity of native microalgal strains (Chlorophyta) isolated from tropical freshwater reservoirs. Algal Res **28**: 139–150

Shemi A, Ben-Dor S, Vardi A (2015) Elucidating the composition and conservation of the autophagy pathway in photosynthetic eukaryotes. Autophagy **11**: 701–715

Silva MET da, Leal MA, Resende M de O, Martins MA, Coimbra JS dos R (2021) *Scenedesmus obliquus* protein concentrate: A sustainable alternative emulsifier for the food industry. Algal Res. doi: 10.1016/j.algal.2021.102468

Singh P, Kumari S, Guldhe A, Misra R, Rawat I, Bux F (2016) Trends and novel strategies for enhancing lipid accumulation and quality in microalgae. Renew Sustain Energy Rev **55**: 1–16

Soares J, Kriiger Loterio R, Rosa RM, Santos MO, Nascimento AG, Santos NT, Williams TCR, Nunes-Nesi A, Arêdes Martins M (2018) *Scenedesmus* sp. cultivation using commercial-grade ammonium sources. Ann Microbiol **68**: 35–45

Soulard A, Cohen A, Hall MN (2009) TOR signaling in invertebrates. Curr Opin Cell Biol **21**: 825–836

Tan KWM, Lee YK (2016) The dilemma for lipid productivity in green microalgae: Importance of substrate provision in improving oil yield without sacrificing growth. Biotechnol Biofuels **9**: 1–14

Werth EG, McConnell EW, Couso Lianez I, Perrine Z, Crespo JL, Umen JG, Hicks LM (2019) Investigating the effect of target of rapamycin kinase inhibition on the *Chlamydomonas reinhardtii* phosphoproteome: from known homologs to new targets. New Phytol **221**: 247–260

Wullschleger S (2006) TOR signaling in growth and metabolism. Cell. doi: 10.1016/j.cell.2006.01.016

Zhu L, Li S, Hu T, Nugroho YK, Yin Z, Hu D, Chu R, Mo F, Liu C, Hiltunen E (2019) Effects of nitrogen source heterogeneity on nutrient removal and biodiesel production of mono- and mix-cultured microalgae. Energy Convers Manag. doi: 10.1016/j.enconman.2019.112144

Zhu S, Huang W, Xu J, Wang Z, Xu J, Yuan Z (2014) Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga *Chlorella zofingiensis*. Bioresour Technol **152**: 292–298

CHAPTER 1

TITLE: Metabolite reprograming of freshwater microalgae strains during nitrogen depletion

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ABSTRACT

Nutrient deprivation causes significant stress to microalgae, which responds by altering their metabolic program. In nitrogen (N) deprivation, the accumulation of starch and triacylglycerols (TAGs) are significantly altered following massive reprogramming of cellular metabolism. Thus, a greater understanding of the regulation of metabolic pathways is necessary to understand the physiology and the mechanisms involved in the biosynthesis of storage compounds in microalgae strains. In this study, four uncharacterized microalgae strains, *Scenedesmus obliquus* BR003, *Chlorella vulgaris* BR017, *Chlamydomonas* sp. BR020 and *Monoraphidium* sp.BR023, and the model strain, *Chlamydomonas reinhardtii* CC503, were selected to evaluate the growth parameters, biochemical composition and metabolite profile under N depleted (0 mM), N replete (3.5 mM) and N saturated (7 mM) conditions. Whereas N depletion leads to typical stress-related responses in all strains, including reduction of cell growth, chlorophyll and protein, differential accumulation of primary compounds, membrane lipid composition and fatty acid were also observed. The metabolic and physiological analysis showed not only differential sensitivity to the absence of N in relation to N-replete and N-saturated treatments but also differences between strains. Many studies are driving to the hypothesis of a decrease in biomass accumulation and protein synthesis, with an increase on storage compounds. In this study, it was clearly demonstrated that the highest amount of storage compounds was obtained at the N starvation treatment, in which their growth and protein synthesis was the most affected. In addition, the strains showed different metabolic responses during cultivation. Biomass accumulation and protein content decreased in all strains in N depleted condition. Regarding amino acids, except for the *S. obliquus* BR003, all strains showed a decrease in amino acid content for N depleted treatment at the end of cultivation. It was also observed that only the *Monoraphidium* sp. BR023 did not change the chl *a* content between the treatments. For C contents, as expected, all strains showed an increase in starch and lipid contents for N depleted treatment. Taken together, this study describes an excellent cellular system for probing the N starvation and the consequent changes in carbon flow in relation to both starch/lipid accumulation and their re-mobilization and the changes in free amino acid pools in microalgae.

Keywords: Chlorophytes. *Chlamydomonas reinhardtii*. Metabolomics. Nitrogen nutrition. Biochemical composition. Lipids.

1. INTRODUCTION

Regardless of the intrinsic characteristics of each strain, the growth and metabolism of microalgae are affected by many environmental factors such as light, temperature and nutrient availability, which have a direct influence on the chemical cell composition, thus conditioning the biomass value as raw feedstock (Ikaran et al., 2015; Rocha et al., 2019). Nutrient availability is a vital factor that regulates the development of microalgal production system (Rai and Gupta, 2017). N is one of particularly essential elements for algal biomass production, playing a critical role in the regulation of biomass accumulation and composition (Park et al., 2015; Himanshu et al., 2016; Gargouri et al., 2017). N is an essential macronutrient that supports life in all living beings. This nutrient is an elemental constituent of biomolecules, such as nucleic acids, proteins, chlorophylls, cofactors, and signal molecules, among others (Zhu et al., 2018; Zhu et al., 2019).

N deprivation is a primary factor that influence cell growth and metabolism (Hu et al., 2008; Ikaran et al., 2015; Nagappan et al., 2020). When N is insufficient to support amino acids and protein synthesis, many microalgae are able to adapt their metabolic pathways and excess of carbon (C) from photosynthesis to storage molecules such as triacylglycerols (TAG) and starch (Sarat Chandra et al., 2016; Jagadevan et al., 2018; Mobin and Chowdhury, 2019). In several species, TAG constitute a greater fraction of cell weight, hence being considered the main C and energy storage form in such oleaginous strains (Ikaran et al., 2015; Mobin and Chowdhury, 2019). However, an enhanced TAG accumulation is not the unique metabolic response to N deprivation. Under such circumstances, it has been reported that carbohydrates (mainly starch) reached close to 50% of cell weigh in *Chlorella vulgaris* (Breuer et al., 2012; Chen et al., 2017). Simultaneous accumulation of both compounds has also been reported in some microalgae (Work et al., 2010; Cakmak et al., 2012; Zhu et al., 2014).

From a biological perspective, the accumulation of these C-rich compounds after N depletion occurs when the supply of C and energy through photosynthesis is higher than the metabolic C demand for growth (Hu et al., 2008; Ikaran et al., 2015). It has been also shown that the synthesis of these storage compounds would be mainly as a result of *de novo* C fixation by photosynthesis and its partition between glyoxylate pathways and gluconeogenesis (Work et al., 2010; Blaby et al., 2013). According to Fan et al. (2012), lipid and starch synthesis are competitive pathways in N starved cells of *C. reinhardtii* and C supply seems to be the major factor controlling C partitioning between starch and lipids accumulation in this species. On the other hand, the common physiological behavior associated with N deprivation is the reduction

in the level of nitrogenous compounds (Guerra et al., 2013; Sarat Chandra et al., 2016; Nagappan et al., 2020), mainly, chlorophyll and proteins. The chlorophyll degradation not only provides the cells with valuable N but also protects the cells from oxidative stress (Griffiths et al., 2011; Nama et al., 2015). Protein degradation generates N, which could be remobilized for the synthesis of new enzymes essential for cell viability under stress (López et al., 2010; Nama et al., 2015).

It is important to note that different metabolic responses have been reported in species belonging to the same genus, and that the degree of response can be different even among strains from the same species (Holbrook et al., 2014; Himanshu et al., 2016; Singh et al., 2016; El-Sheekh et al., 2018). Thus, it is essential to understand the behavior of selected microalgal species under stress conditions.

The selection and characterization of microalgal strains with high biomass production and reserve compounds are important to obtain appropriate strains for biotechnological applications and arrive at a better understanding of the relationship between microalgal metabolism and physiology. Therefore, the aim of this work is to evaluate the physiological and metabolic changes associated with different N supply in media containing different microalgae strains. It is known that different microalgae strains, under favorable growth conditions, have different behavior in relationship to biomass accumulation and the reserve compounds. According to Rocha et al., (2017), among the strains, *S. obliquus* BR003 was obtained the greatest biomass levels. However, *Chlamydomonas* sp. BR020 had the highest starch content. In contrast to its high starch content, *Chlamydomonas* sp. BR020 as well as *C. vulgaris* BR017 presented one of the lowest levels of lipids while the highest value was observed for *Monoraphidium* sp. BR023. Therefore, the hypothesis of this work is that the highest amount of reserve compounds will be more accumulated in the N starvation treatment, and of the other hand, growth and protein synthesis will be negatively affected, and that these responses are highly modulated by differential and species/strain-specific metabolic changes. The metabolite reprogramming observed for various pathways in relation to N starvation in microalgae, have been discussed.

2. MATERIAL AND METHODS

2.1 Strains and culture media

The four freshwater microalgae strains (Chlorophyta) used in this study are available at the Collection of Cyanobacteria and Microalgae at the Universidade Federal de Viçosa (CCM-UFV), available in the Laboratory of Phycology and Molecular Biology, Plant Biology

Department at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil (20°45′14″S, 42°52′54″W). *S. obliquus* BR003, *C. vulgaris* BR017, *Chlamydomonas* sp. BR020 and *Monoraphidium* sp. BR023 were isolated from water samples collected from freshwater reservoirs in the city of Viçosa/Brazil (Rocha et al., 2017). The model green microalga, *Chlamydomonas reinhardtii*, is widely selected for diverse experiments, because of its advantages, such as fast growth, short generation time, strong adaptability, easy cultivation and easy isolation of mutants (Harris, 2001).

The strains were cultivated in the mixotrophic Tris-Acetate-Phosphate (TAP) medium (Coleman, 1990). Before the addition of ammonium to the medium, the cultures were incubated for 12 h in TAP medium without N source to exhaust the original ammonium in the medium (Li et al., 2016). To investigate the effects of different N concentrations, we conducted three treatments with initial N-added concentrations of 0 mM (N-depleted), 3.5 mM (N-replete), and 7 mM (N-saturated) during 36 hours. Strains were cultivated under 24±2 ºC, with a 16 h/8 h (light/dark) photoperiod cycle, at a constant irradiance of 100 μmol photons m^{-2} s^{-1}, under continuous shaking at 110 rpm. N-starved cells were then collected in stationary phase, washed twice in N-free media and used to inoculate a new culture at a starting density of around 0.2 OD_{680nm}, which corresponds to a cell dry weight of 100 mg L^{-1}.

2.2. Culture conditions and growth parameters

The experimental cultures were grown in 125 mL Erlenmeyer flasks, filled with 75 mL of culture medium and four repetitions per treatment. The experiments were carried out in a growth room, applying the same conditions described above. For the characterization of cell growth in different media and selection of sampling points we performed growth curves for each strain. The cellular growth was monitored hourly by absorbance on 680 nm and 750 nm in spectrophotometer (UVM 340, AsysHitech) for three days (Griffiths et al., 2011). Based on the obtained growth curves we selected four points to collect samples as indicated in the **Figure 1A**: at initial phase Lag 1 (0h), early logarithmic phase Log 1 (10h), late logarithmic phase Log 2 (18h); and stationary phase Sta 1 (36h).

The dry weight determination was performed by filtration of 10mL in nitrocellulose membranes 0,45 μm (Millipore®) (Griffiths et al., 2011). The ash-free dry weight was determined by subtraction of the dry weight by ash weight after calcination. The result was expressed in grams of ash-free weight per L of culture. These data were used to determine specific growth rate (μ) for all strains in exponential phase (Contois, 1959).

2.3. Extraction of chlorophyll, lipids and primary metabolites

Primary metabolites, chlorophyll and lipids were extracted as described elsewhere (Salem et al., 2016). About 5 mg of freeze-dried biomass cells were used for metabolite profiling. They were re-suspended in 1 mL of pre-cooled (-20°C) methyl tert-butyl ether (MTBE)-extraction buffer [methanol: methyl tert-butyl-ether (1 : 3; v/v) mixture; Biosolve, the Netherlands] (Salem et al., 2016). The resuspended cell pellets were immediately mixed before ultrasonification in an ice-cooled water bath for 10 min. Then the samples were incubated for 1h on an orbital shaker at 4°C. To induce the liquid: liquid-phase separation, 650 µL of UPLC-grade methanol : water (1 : 3; v/v; Biosolve, the Netherlands) was added to the homogenate and mixed, before centrifuging at 20 000 *g* for 5 min at 4°C in a table top centrifuge. The addition of the methanol: water mixture resulted in a separation of an upper MTBE-phase, containing lipids and chlorophyll, and a lower phase, containing the polar and semi-polar metabolites, next to a solid pellet in the bottom of the 1.5 mL plastic tube. The solid pellet contains the precipitated proteins next to starch and other insoluble polymers, such as cell wall fragments (Salem et al., 2016). For further analysis, 500 µL of the MTBE-phase and 650 µL of the lower, aqueous-phase were transferred to fresh 1.5 mL tubes. Samples of the lipid-phases (MTBE-phase) were dried in a vacuum concentrator and re-suspended in the proper resuspension buffer. The polar aliquots were also dried in a vacuum concentrator before storing the pellets at -80°C. The solid pellet was frozen in liquid N and stored at -80°C until further extraction. To measure the impact of contaminations from the extraction process, the whole extraction procedure was additionally performed with two–four empty, sample-free, 1.5 mL tubes (method blank).

2.4. Polar metabolite profiling

The derivatization of the polar-sample (primary metabolites) was performed on a gas chromatographer Agilent 6890 (Agilent) coupled to a Pegasus HT ToF mass spectrometer (LECO Instruments, St. Joseph, MI, USA) as outlined in a previous study (Lisec et al., 2006). Chromatograms were exported from ChromaToF v 3.25 (LECO Corp., St. Joseph, MI, USA) and processed using R. Peak detection, retention time alignment, and library matching were performed using the TargetSearch R package from Bioconductor, as performed by (Bromke et al., 2013). For data normalization, each analyte peak intensity was normalized by the median of all data and dry weight.

2.5. Determination of the chlorophyll and amino acids content

To determine the chlorophyll content, 100 µL of the methyl tert-butyl ether (MTBE)-phase was mixed with 90% methanol and measured using an UV-VIS spectrometer at wavelengths of 665 nm and 652 nm to distinguish between chlorophyll *a* and chlorophyll *b*. The chlorophyll *a*, *b* and total content were determined using the equations described by Bar-Nun (Bar-nun and Ohad, 1980). Total amino acid contents were quantified as reported in Cross et al. (2006).

2.6. Extraction and determination of total water-soluble protein and starch content

Extraction of total protein water-soluble protein content was carried out according to Meijer and Wijffels (1998) and quantification was performed using the Bradford method (Bradford, 1976) at a wavelength of 595 nm in a microplate reader. As a standard, it was used as bovine serum albumin (BSA), and the results were expressed in mg / g of ash free dry mass.

In order to analyze the starch content, we optimized the standard protocol from Smith and Zeeman, 2006. The frozen cell pellet was first washed with 80% ethanol, incubated for 10 min at room temperature. Afterwards the pellet was dissolved in 250 µl sterile water. Then 250 µl 100 mM sodium acetate was added and starch was hydrolyzed by heating for 3h at 99 °C. The dissolved starch was digested into its glucose monomers over night with an enzyme mix of α-amylase (4.2 units per sample) and α-amyloglucosidase (10 units per sample). The digested extract was centrifuged and the supernatant with the starch-derived glucose monomers was dissolved in 100 mM HEPES-buffer (pH 7) that contained 5 mM $MgCl_2$, 60 mg/ml ATP, 36 mg/ml NADP and glucose-6-phophate-dehydrogenase (1 unit per sample). The baseline was measured at 340 nm and the subsequent addition of hexokinase started the reactions and the increase of $NADPH+H^+$ was measured at 340 nm with a plate reader in 96-well formats.

2.7. Determination of neutral lipids

Determination of total neutral lipids was performed according to (De la Hoz Siegler et al., 2012) with minor modifications using the lipophilic fluorescent dye Nile Red. Triolein was used to prepare the standard curve. 300 µl of sample and 1 µl of nile red dissolved in 1 mg/mL acetone were used. Nile red fluorescence intensity was quantified in a microplate reader (Victor X5 – Perkin Elmer) using 485 nm filters for excitation and 595 nm for emission. A black 96-well plate was used to prevent fluorescence scattering. Fluorescence intensity data were normalized by cell density.

2.8. Determination of total lipids

Briefly, 650 µL of the lower aqueous phase of the MTBE extraction, described above, were transferred to fresh 1.5-ml Eppendorf tubes, dried in a SpeedVac and resuspended in a volume of 300 µL of a mixture of acetonitrile: isopropanol (7: 3; Biosolve, the Netherlands). Chromatograms were further processed using Excalibur software version 2.30 (Thermo-Fisher) and Progenesis CoMet software (Version 2.4; Nonlinear, Newcastle upon Tyne, UK). The UPLC/MS lipids data were processed using ToxID (Version 2.1.2; Thermo). To remove noise and contaminants, data for every lipid species with average peak height lower than the average peak height of the method blanks or with 50% of the values below 1,000 arbitrary counts were removed from the dataset. The remaining peaks were then assigned to annotated lipid species using an in-house-generated lipid database for *Arabidopsis* (Hummel et al., 2011). The data were normalized by the median of all data and dry weight. For some lipid species, more than one peak was detected having the same *m*/*z* and identical adducts but different retention times. In these cases, we added the letter A or B to the compound name, depending on their elution order.

2.9. Statistical analysis

The experiments were performed and analyzed following a completely randomized design with four replicates for each treatment. The values obtained for all variables were submitted to analysis of variance (ANOVA, $P < 0.05$) followed by Tukey's test *(*$P \leq 0.05$), and one analyses of the interaction effects, using the software R (Grace and Hudson, 2016). Primary metabolites and lipid profile data were log2 transformed. In addition , data were subjected to a principal component analysis (PCA) using the pcaMethods package in the software R (Stacklies et al., 2007).

3. RESULTS

3.1. Growth and biomass production

The analysis of growth parameters aimed to identify how cells produced biomass over time under different N concentrations. To determine the effect of N on the five strains grown under mixotrophic conditions, absorbance readings of OD_{680nm} were taken over a period of 36 hours **(Figure 1A-E)**. The growth curve was noticeably affected by the differences in N availability. After 10 hours of cultivation, there was no or minor increase in cell growth during N-depleted condition. On the other hand, strains treated with N-saturated and N-replete showed a similar

growth pattern and displayed continuous growth throughout the culture time. Biomass measured as ash free dry weight (AFDW) is shown in four different point of cultivation, in which in the N group reached between 0.75 $g.L^{-1}$ (*Monoraphidium* sp. BR023) and 0.983 $g.L^{-1}$ (*C. reinhardtii* CC503) while the group without N were 0.335 $g.L^{-1}$ (*Chlamydomonas* sp. BR020) and 0.502 $g.L^{-1}$ (*C. reinhardtii* CC503) **(Figure 1F-J)**. Additionally, the impacts of growth, in different culture media, were shown in maximum growth rate (μmax, **Figure 1K**), and demonstrated that there is a difference for the 0 mM treatment when compared to the 7 mM and 3.5 mM treatments. All interactions between strain, time and concentration were statistically significant, even the triple interaction (Table S 1, supplementary material).

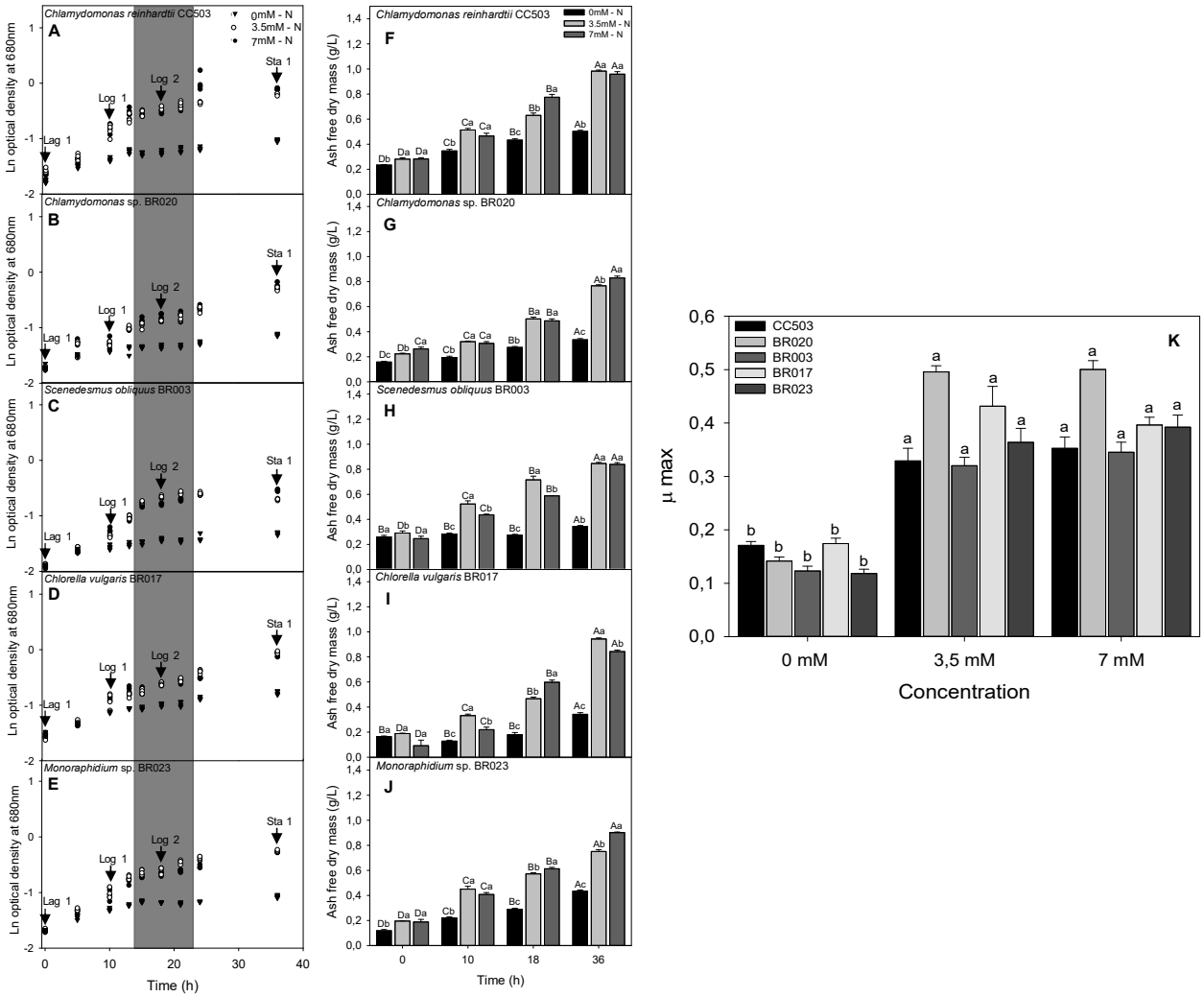


Figure 1. Growth parameters of five strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under tree different nitrogen (N) concentration (0mM, 3.5mM and 7mM). Growth curves based on Optical Density (OD) at 680 nm **(A-E),** ash-free dry mass **(F-J)** and maximum growth rate (μmax) **(K)**. Period of dark highlighted in gray. Values represent the average error of four replications. Means followed by the same letters do not differ by the Tukey test ($P<0.05$), lowercase letters compare different N concentration at the same growth phase and uppercase letters compare each treatment in different growth phases.

3.2.Metabolite levels

The analysis of variance for chlorophyll contents reveled that all interactions between strain, time of cultive and N concentration were statistically significant, with the exception of interaction between strain and time for Chl *a*/*b* ratio (Table S 1). Chlorophyll levels, under N-replete and N-depleted both chlorophyll (Chl) *a* and *b* content were reduced in all strains throughout cultivation **(Figure 2A-J)**. The Chl *a*/*b* ratio for these treatments decreased throughout cultivation in all strains, except for *S. obliquus* BR003 **(Figure 2 K-O)**. For N-saturated, it was observed that at the end of the cultivation all strains had an increase in the chl *a* content, except for *Monoraphidium* sp. BR023 **(Figure 2 A-E)**.

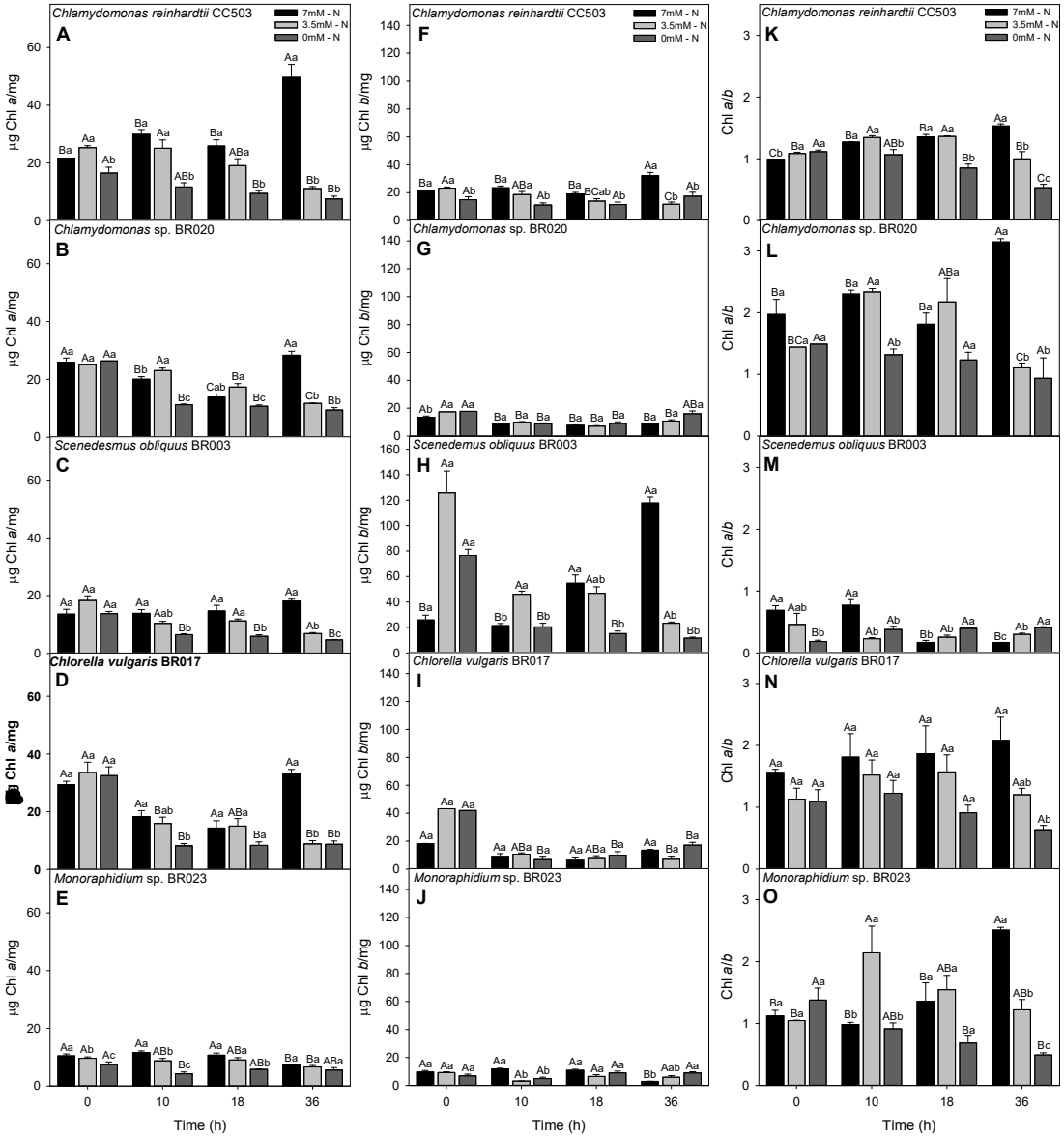


Figure 2. Chlorophyll *a* **(A-E)**, *b* **(F-J)** content and Chlorophyll *a*/*b* ratio **(K-O)** of five strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under tree different nitrogen (N) concentration (0mM, 3.5mM and 7mM). Values represent the average error of four replications. Means followed by the same letters do not differ by the Tukey test (P<0.05), lowercase letters compare different N concentration at the same growth phase and uppercase letters compare each treatment in different growth phases.

Concerning the amount of other N-containing compounds, for all strains, except for *S. obliquus* BR003, the total free amino acids content decreased at the end of cultivation for N-depleted treatment, except *S. obliquus* BR003 that presented similar value during cultivation **(Figure 3 A-E)**. The total protein content, for all strains showed a very similar behavior throughout the 36 hours of cultivation according to the analysis of variance **(Table S1)**, and had a significant increase for N-saturated treatment and the lowest values for N-depleted **(Figure 3 F-J)**.

The protein/amino acids ratio showed higher values for *C. reinhardtii* CC503 and *S. obliquus* BR003 during the cultivation when compared to the other strains **(Figure 3 K-O)**.

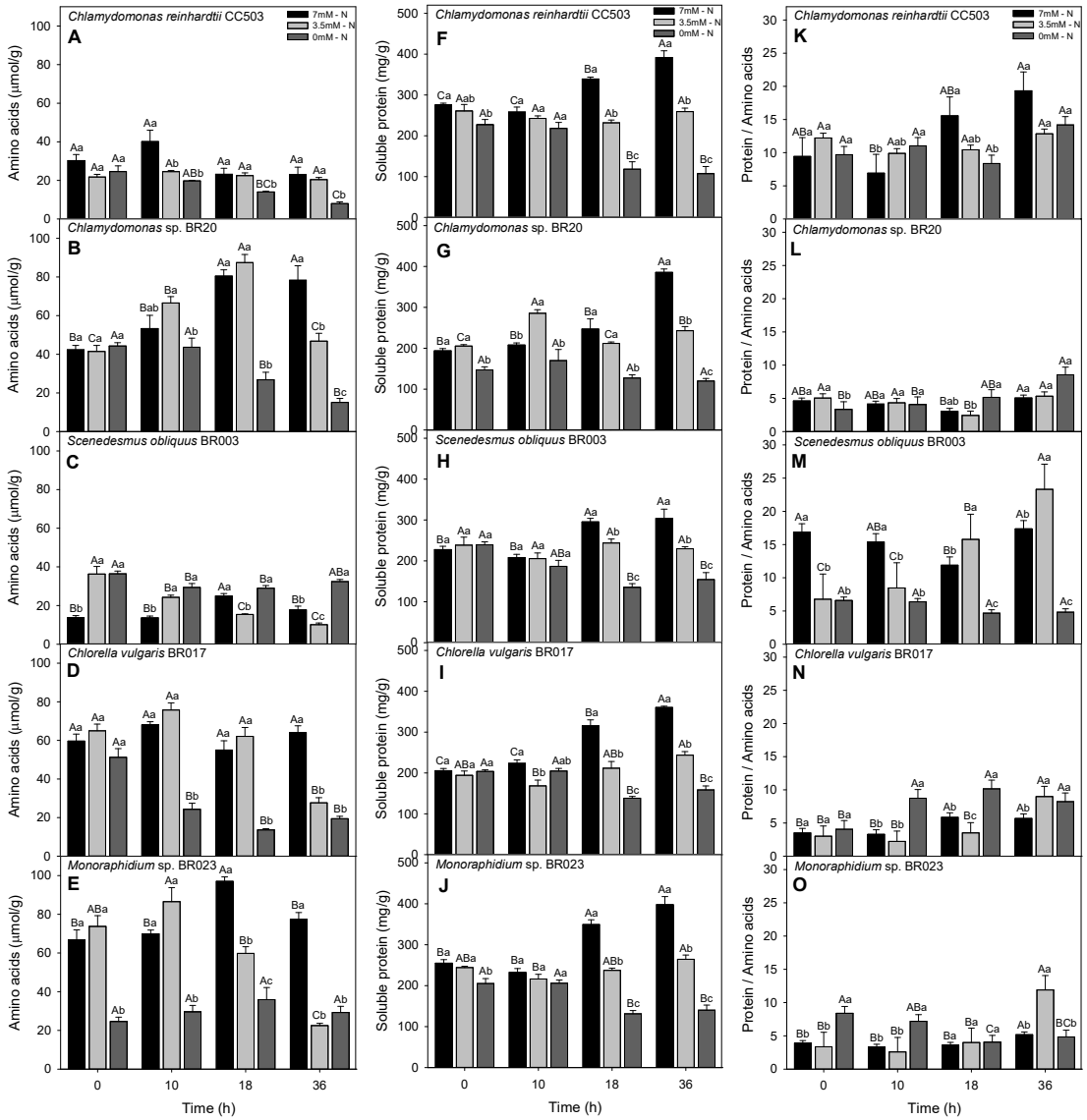


Figure 3: Nitrogen (N) containing metabolites of five strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under tree different nitrogen (N) concentration (0mM, 3.5mM and 7mM). **(A-E)** total free amino acids content, **(F-J)** total protein content and **(K-O)** protein / amino acids ratio. Values represent the average error of four replications. Means followed by the same letters do not differ by the Tukey test ($P<0.05$), lowercase letters compare different N concentration at the same growth phase and uppercase letters compare each treatment in different growth phases.

Regarding the main C containing metabolites, the decrease in nitrogen concentration resulted in an increase in sucrose content for most strains, while for *S. obliquus* BR003 and *C. reinhardtii* CC503 the sucrose content was very low for all treatment **(Figure 4 A-E)**. The increase in the concentration in the starch content is inversely proportional to the concentration of N. Increase in starch levels during different N concentration are shown in **Figure 4 F-J**. Starch levels increased after 10 hours of cultivation for N-depleted treatment for all strains. The highest starch content was for *C. reinhardtti* (944.80 mmol glucose equiv/md DW). Furthermore, N-replete treatment showed a greater increase in starch content at 36 hours when compared to the N- saturated treatment. N-depleted treatment promoted higher neutral lipids production in comparison with the other N concentrations **(Figure 4 K-O)**. Interestingly that for *C. reinhardtii* CC503 the content of neutral lipids was higher during the 36 hours of cultivation for the N-depleted treatment when compared to the N-saturated and N-replete treatments **(Figure 4 K)**. Although the highest value in the neutral lipids content was for *Monoraphidium* sp. BR023 (34.74 %) for N-depleted treatment, this strain also presented a high initial value in the neutral lipid levels (16.18 %) after 10 hours of cultivation **(Figure 4 O)**.

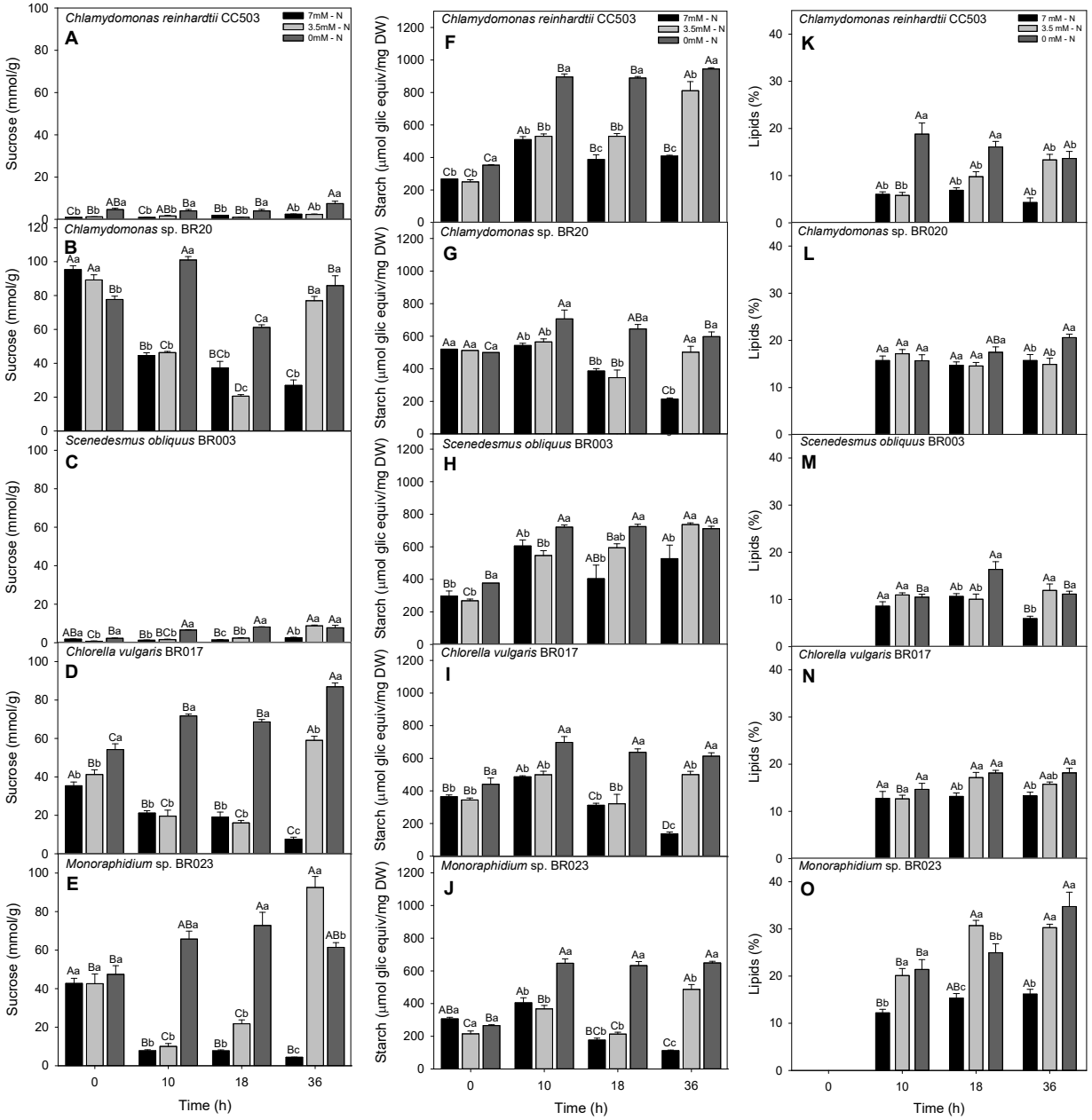


Figure 4: Carbon containing metabolites of five strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under tree different nitrogen (N) concentration (0mM, 3.5mM and 7mM). Sucrose content **(A-E)**, starch content **(F-J)** and neutral lipids content **(K-O)**. Values represent the average error of four replications. Means followed by the same letters do not differ by the Tukey test ($P<0.05$), lowercase letters compare different N concentration at the same growth phase and uppercase letters compare each treatment in different growth phases.

3.3 Metabolic profile

In order to verify the metabolic alterations between different concentrarions of N in the medium, we employed an established GC/TOF–MS based metabolite profiling platform. The metabolite profile is exhibited in a Heat map, wherein the colors red and blue mean increase and decrease, respectively, in relation to N-depleted in each growth phase **(Figure 5, Table S 2-6)**. Heat map exhibited decrease in several amino acids during N-depleted in comparison with N-saturated and N-replete treatments in all strains during the 36 hours of cultivation. Still in relation to amino acid compounds, similar behavior was observed between *Chlamydomonas* sp BR020 and *S. obliquus* BR003, and between *C. reinhardtii* CC503, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023. Remarkably, the levels of alanine, arginine, and glutamine increased for *C. reinhardtii* CC503, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023 strains in the N-saturated and N-replete treatments when compared to *Chlamydomonas* sp. BR020 and *S. obliquus* BR003 strains (**Table S 2-6)**. For ornithine and tryptophan, there was a decrease and an increase, respectively, for *S. obliquus* BR003, different from the other strains. Regarding sugar content, it was possible to observe that *S. obliquus* BR003 strain presented higher values compared to the other strains.

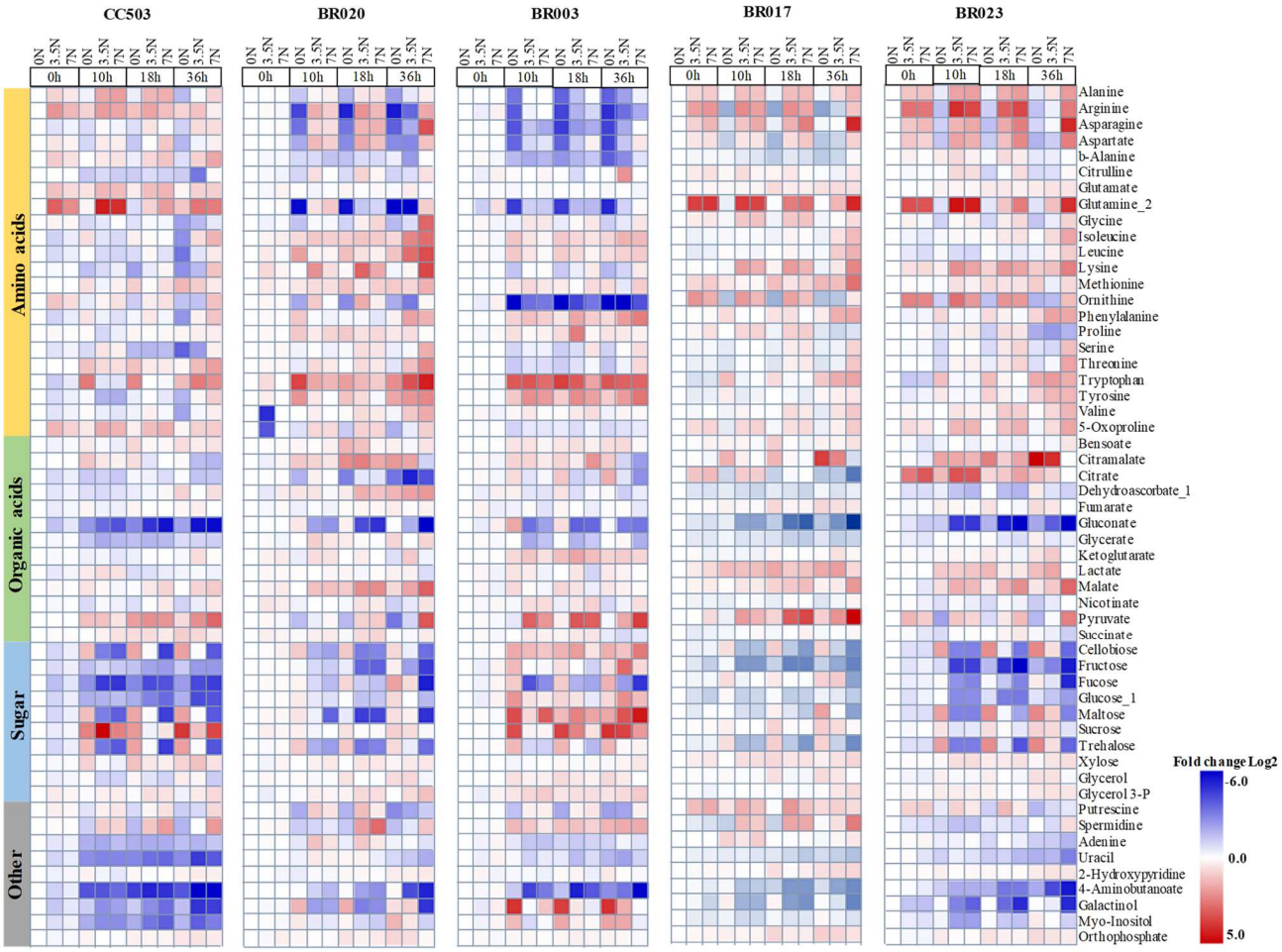


Figure 5. Heat map representing the changes in relative metabolite levels of five strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under tree different nitrogen (N) concentration (0mM, 3.5mM and 7mM). Data are scaled to the mean response measured for N-deplete 0h for each strain; values presented are means of four biological replicates. The average and statistical analysis are presented in supplementary table 2 - 6.

3.4 Lipid profile

It was detected 103 compounds from 8 different lipid classes among the identified metabolites 12 belongs to diacyclglycerols (DAGs), 17 digalactosyldiacylglycerols (DGDGs), 6 Lysophosphatidylcholines (LysoPCs), 9 monogalactosyldiacylglycerols (MGDGs), 20 phosphatidylcholine (PCs), 21 phosphatidylethanolamines (PEs), 7 phosphatidylglycerol (PGs) and 11 sulfoquinovosyl diacylglycerol (SQDGs). The five strains showed different lipid profile (**Figure 6**). Accordingly, changes of lipid levels in BR020, BR017 and BR023 were more pronounced than in CC503 and BR003 during the cultivation **(Figure 6, Table S 7-11)**. Intriguingly, during N-starvation there was a clear decrease in the levels of lipids from thylakoid membranes in BR020, BR017 and BR023 strains. Most of the chloroplast galactolipids (DAGs, MGDGs, DGDGs and SQDGs) increased in BR020, BR017 and BR023 cells under N-saturated conditions. Some phospholipids were not detected in CC503, namely PC 32:0, PC 32:1, PC 32:2, PC 32:3, PC 32:4, PC 34:2, PC 34:4, PC 34:5, PC 34:6, PC 36:3, PC 36:6, PC 38:3, PE 32:1, PE 32:2, PE 34:3, PE 34:4, PE 34:5, PE 36:3, PE 36:4, PE 36:5, PE 36:6, PE 40:1 and PG 32:1. Similarly, in BR017 PE 32:2, PE 34:4, PE 34:6, PE 36:3, PE 36:4, PE 38:3, PE 40:1, PE 40:3, PG 32:0, PG 32:1, PG 34:2, PG 34:3, PG 34:4, PG 36:1 and PG 36:2 were not detected. In the STA phase (36h) the remodeling of thylakoid membranes seems to be more severe in the BR020 strain, followed by CC503, as MGDGs decreased in N-replete and N-saturated treatments. Meanwhile under N-replete conditions these lipids are maintained at stable level in the other strains.

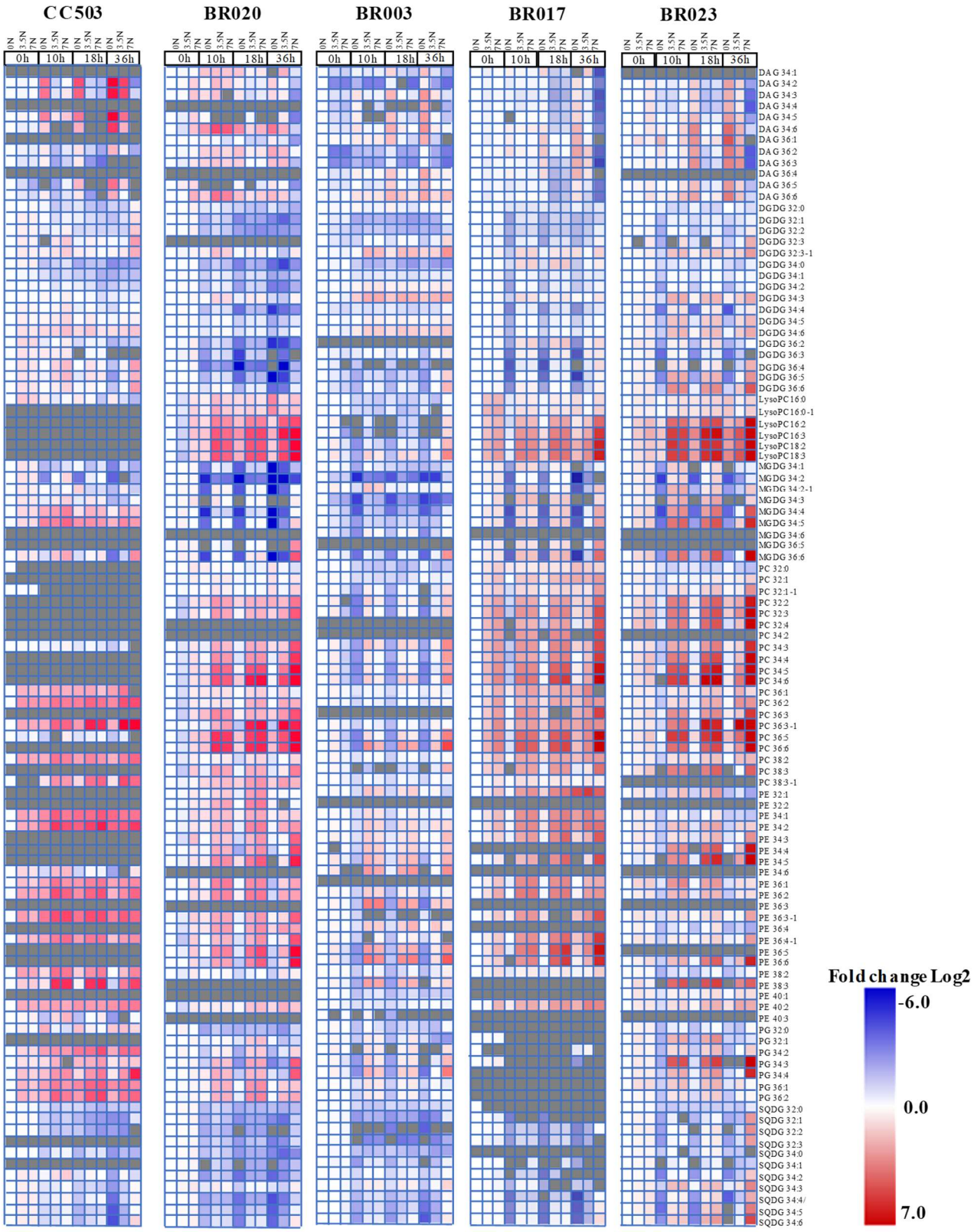


Figure 6. Heat map representing the changes in relative lipid contents of five strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under tree different nitrogen (N) concentration (0mM, 3.5mM and 7mM). Data are scaled to the mean response measured for N-deplete 0h for each strain; values presented are means of four biological replicates. The average and statistical analysis are presented in supplementary table S 7 – 11.

The total fatty acids are presented in **Figure 7** and represent all fatty acids obtained from non-polar extracts. It was 20 fatty acids identified in the lipid samples harvested during LOG and STA phase. Saturated fatty acids (FA16:0, FA18:0, FA20:0 and FA26:0) and unsaturated fatty acid such as FA16:3, FA18:2, FA18:3, FA20:1 and FA22:1 showed higher abundance in BR003 and BR023 strains. While CC503, BR017 and BR020 strains were richer in saturated fatty acids like FA26:0, FA28:0 and FA30:0. Interestingly, BR003 showed a lower detection in the number of fatty acid. In addition, in all strains we observed a significant increase in C18:1 during N starvation.

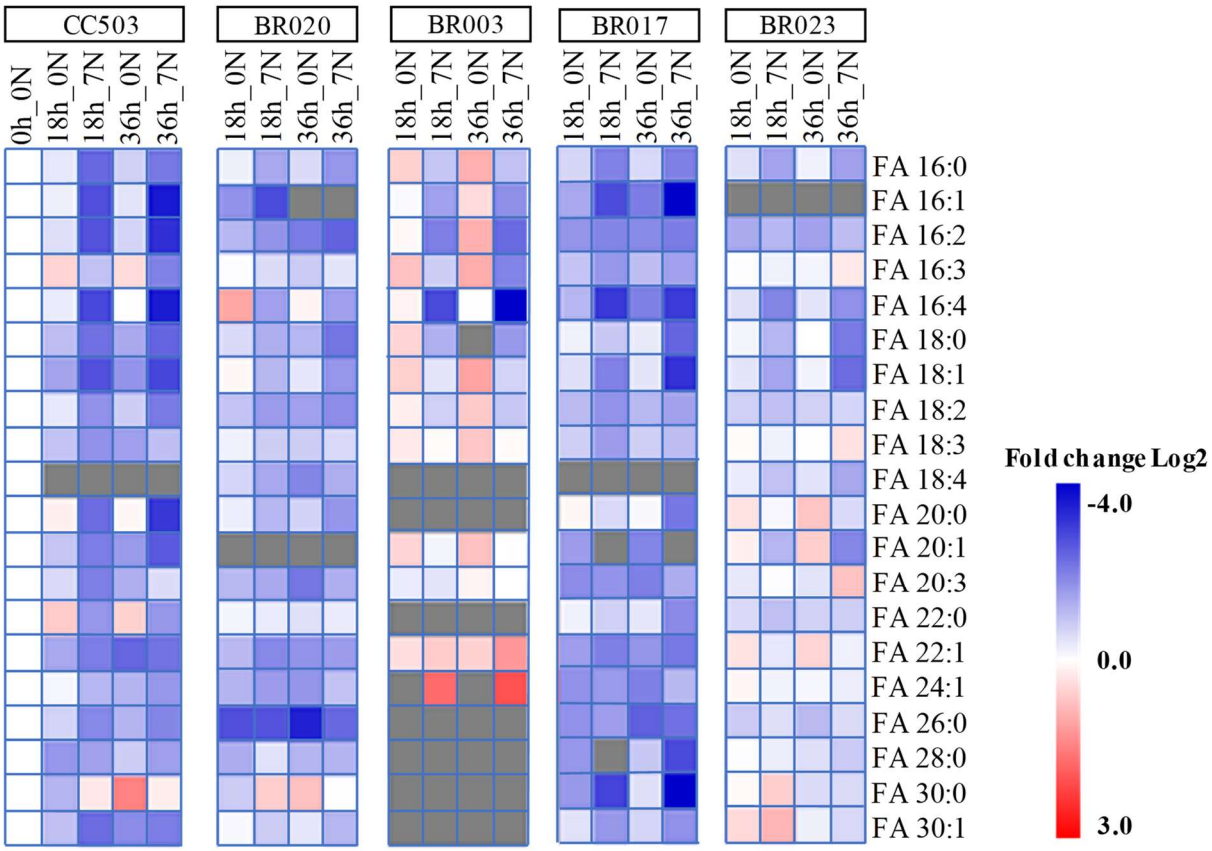


Figure 7. Heat map representing the changes in relative fatty acids contents (FA) of five strains (*C. reinhardtii* CC503, *Chlamydomomas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under two different N concentration (0mM and 7mM) in LOG (18h) and STA (36h) phase. Data are scaled to the mean response measured for N-deplete 0h for each strain; values presented are means of four biological replicates. The average and statistical analysis are presented in the supplementary table S 7 – 11.

In addition, all strains showed a marked accumulation of TAGs in N starvation in LOG and STA phase. Besides the chloroplast membrane lipids, a total of 79 TAGs were identified in the strains **(Figure 8)**. Although all strains showed accumulation of lipid in the N-starvation treatment, BR003 and BR023 strains showed the greatest accumulation of lipid at 18h and 36h (LOG and STA phase), especially TAG 48, TAG 50 and TAG 52. On the other hand, regardless of the N concentration lowest lipid accumulation was observed in CC503 strain, which, in addition to having the lowest accumulation, also had the lowest number of identified TAGs, such as TAG 46:4, TAG 48:3, TAG 48:4, TAG 48:5, TAG 48:6, TAG 48:7, TAG 48:8, TAG 48:9, TAG 50:3, TAG 50:4, TAG 50:7, TAG 50:8, TAG 50:9, TAG 52:5, TAG 58:5 and TAG 58:6 were not detected in this strain.

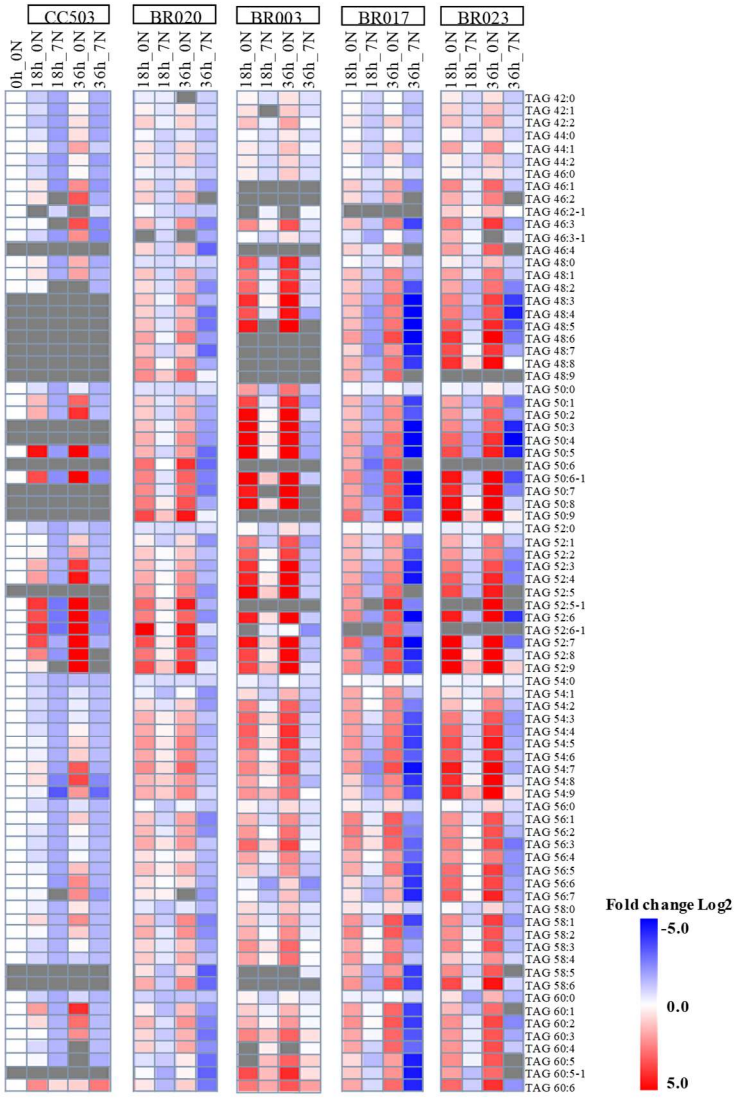
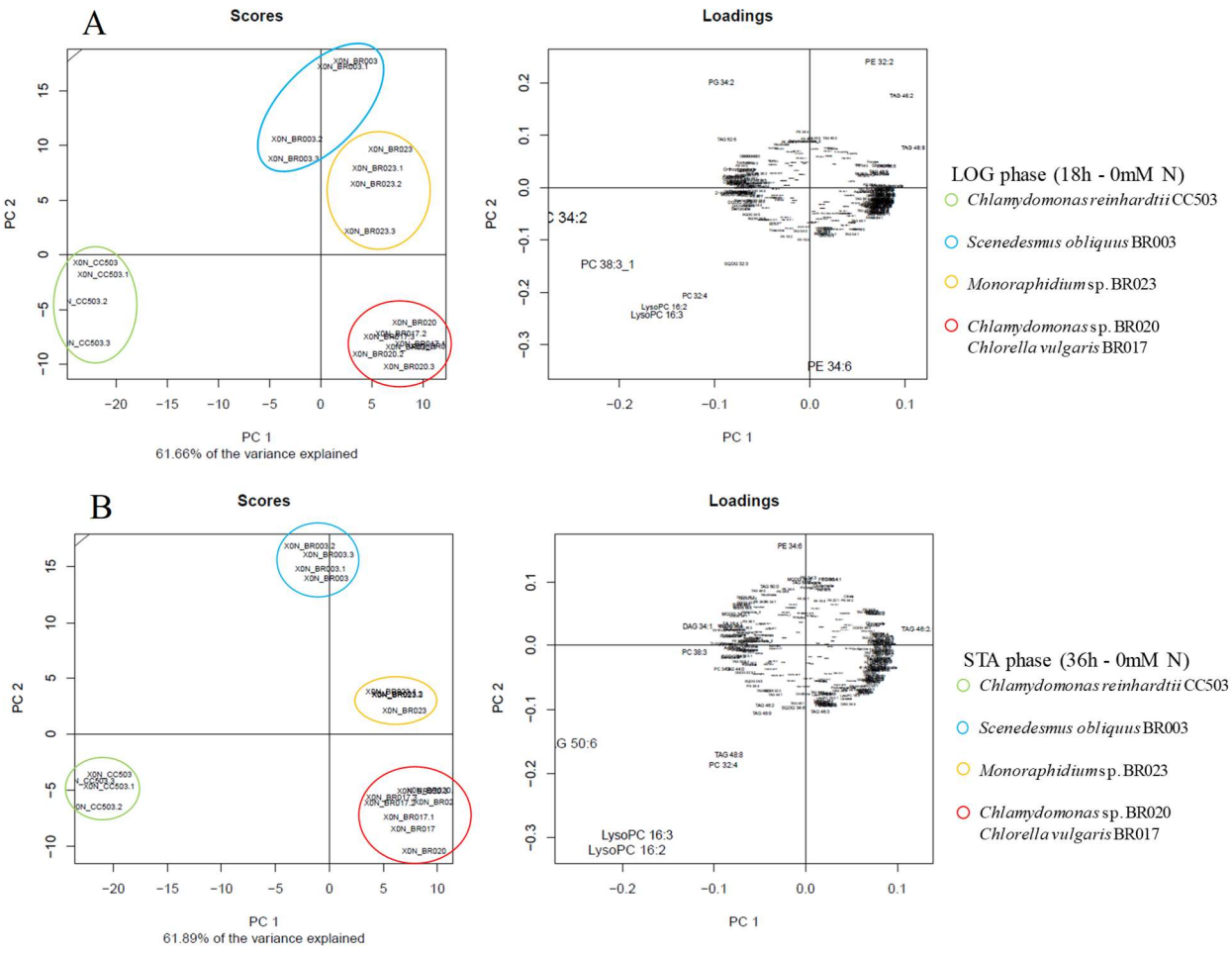


Figure 8. Heat map representing the changes in relative triacylglycerols (TAGs) contents of five strains (*C. reinhardtii* CC503, *Chlamydomomas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under two different nitrogen (N) concentrations (0mM and 7mM) in LOG (18h) and STA (36h) phase. Data are scaled to the mean response measured for N-deplete 0h for each strain; values presented are means of four biological replicates. The average and statistical analysis are presented in the supplementary table S 7 – 11.

3.5 Multivariate analysis

Principal component analysis (PCA) was performed to visualize the similarities between the treatments and thus explore in more detail the effect of the different N concentrations on growth and metabolic responses of the five strains. In the primary metabolite and lipids PCA **(Figure 9 Table S 2-11)** is evident the separation of the strains. N-depleted treatment, the principal components (PC1 and PC2) explained about 61% of the variance, and four groups were formed in the LOG and STA phase: (1) *C. reinhardtii* CC503; (2) *S. obliquus* BR003; (3) *Monoraphidium* sp. BR023 and (4) *Chlamydomonas* sp. BR020 and *C. vulgaris* BR017. Regarding N-saturated treatment, the principal components (PC1 and PC2) explained about 60% of the variance, and four groups were formed in the LOG phase, as in N-depleted treatment, however, in the STA phase five groups were formed: (1) *C. reinhardtii* CC503; (2) *S. obliquus* BR003; (3) *Monoraphidium* sp. BR023; (4) *Chlamydomonas* sp. BR020 and (5) *C. vulgaris* BR017. The analysis results indicated that the strains were clustered especially due to the high abundance of lipid compounds **(Figure 9A - D)**.



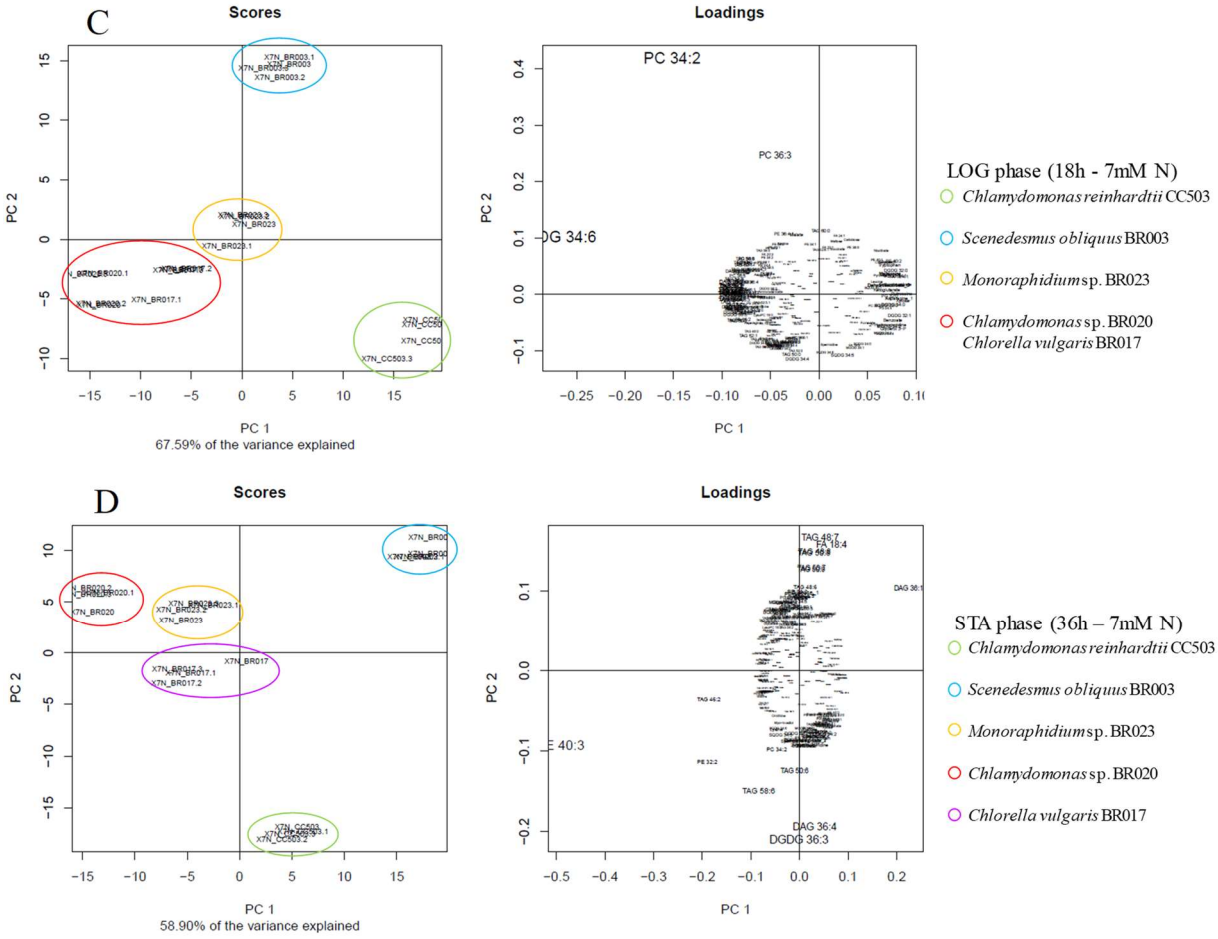


Figure 9. Principal component analysis (PCA) score plot derived data of five strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023)) cultivated under two different nitrogen (N) concentrations (0mM and 7mM) in LOG (18h) and STA (36h) phase. Primary metabolites and lipids profile displayed between 58.90% and 67.59% of the variance explained in the two principal components, PC1 and PC2. Values presented are means of four biological replicates. The average and statistical analysis are presented in the supplementary table S 2 – 11

4. DISCUSSION

Algal cell growth and metabolism can be manipulated by altering environmental conditions to optimize algal biomass production and composition through altering nutrient levels in the medium. Nitrogen limitation or starvation remarkably influences the growth and physiology of microalgae (Sanz-Luque et al., 2015; Dhup, 2016). Breuer et al. (2012) reported that the biomass of nine microalgae, significantly decreased during nitrogen starvation. It has been also demonstrated that nutrient limitation could enhance the accumulation of energy-rich compounds like starch and /or lipid in many microalgae (Zhu et al., 2014; Himanshu et al., 2016; Li-Beisson et al., 2019). Therefore, in this study, four microalgal strains isolated from freshwater reservoirs, and belonging to the phylum Chlorophyta and one model strain, *C. reinhardtii* CC503, were analyzed under different nitrogen concentration.

4.1. Biomass production and cellular components of the strains

As expected, N depletion caused relevant changes on the algal cell growth. Biomass concentration at the end of culture period **(Figure 1 F-J)** is an important factor, given that it indicates the productivity that can be achieved in culture systems (Nagappan and Kumar, 2021). The concentration obtained for the strains considered in this study for N-replete and N-saturated was about 0.8 ± 0.01 g/L while for N-depleted the highest value was 0.5 ± 0.009 g/L. Previous studies, using the same growth media and similar culture conditions (Park et al., 2015; Zhu et al., 2019), obtained similar results to those obtained here. The values obtained here are higher than those previously reported by Rocha et al., 2017, which varied between 0.134 and 0.054 mg mL^{-1} day^{-1} for these same freshwater algal strains. However, in the present study, a mixotrophic medium was used, which was reported to have greater growth potential for some species compared to autotrophic growth (Moon et al., 2013; Girard et al., 2014; Miao et al., 2016).

In this study, upon 10 hours of N starvation, chlorophyll *a* content had been significantly reduced. The degradation of chlorophyll not only provides the cells with valuable N but also protects the cells from oxidative stress (Breuer et al., 2012; Nagappan and Kumar, 2021). Like Yang et al., (2015). Here we demonstrated that the chl *a* content for *Monoraphidium* sp BR023 showed a small variation between treatments throughout the cultivation. Healey, (1978) reported that the ratios of chlorophyll *a* (the main pigment in most microalgal cells) to dry weight (DW) varied more than 30-fold with changes in species composition, temperature and nutrients, and light availability.

Cellular proteins perform the catalytic and structural roles in the form of enzymes, filaments, and tubules. As protein is majorly made up of N, the deprivation of the same has a profound impact on microalgae (Barsanti and Gualtieri, 2018; Nagappan et al., 2020). Five microalgal organisms have been exposed to different N concentration and protein changes have been observed. Protein decrease during N deprivation in all five species tested **(Figure 3 F-J)**. A similar reduction in protein content was found for several microalgae during N deficiency (Li et al., 2016; Sarat Chandra et al., 2016; Kumar et al., 2020).

Starch and lipid are two major C and energy storage compounds of many plants and microalgal cells, particularly in response to stress conditions (Zhu et al., 2018; Batista et al., 2019; Covell et al., 2020). Upon depletion of the N in the growth medium, microalgae cease to synthesize N-rich molecules (e.g., proteins and nucleic acids) but continue to synthesize lipid and carbohydrates (Williams and Laurens, 2010). The first compound known to accumulate to high levels during N deprivation is starch (Klein, 1987) and the upregulation of starch synthesis gene expression has been reported during N deprivation (Ball et al., 1990; Miller et al., 2010; Juergens et al., 2015). Increases in starch levels during N deprivation are shown in Figure 4 F-J.

As mentioned earlier, prolonged N starvation of algae leads to multiple stresses associated with nutritional depletion, which in turn leads to major metabolic reorganization in the cell to form abundant amounts of starch and lipid. In this study, it was clear that the reserve compounds are strain-dependent. Here, *C. reinhardtii* CC503 under N-starvation showed increases in starch content throughout cultivation, which did not occur for lipid contents **(Figure 4F and 4K)**. On the other hand, *Monoraphidium* sp. BR023 under the same conditions, showed an opposite behavior in relation to the accumulation of reserves **(Figure 4J and 4O)**.

4.2. Influence of N starvation on amino acid pools and TAG formation

In recent years, there have been several attempts to study the metabolic, transcriptomic and proteome changes in various cellular systems in relation to their N starvation (Illman et al., 2000; Wu et al., 2013; Kiyota et al., 2014; Himanshu et al., 2016). A Heatmap was exhibited in order to assess whether the N starvation in the current study is accompanied by concomitant changes in cellular free amino acid pools while central C metabolism is readjusting. As expected, N starvation of cells led to overall drop in amino acid pool size **(Figure 5)**. Remarkably, levels of glutamate decreased and methionine increased in the N-starvation treatment during the STA phase only for *C. reinhardtii* CC503. The pool levels of citrulline and

ornithine rise N-saturated treatment, as expected. These N-rich metabolites are not only N-rich but also critical regulatory metabolites that control a wide variety of biosynthetic reactions (Himanshu et al., 2016).

Lipid production in various algae is being explored as an alternate source of fuel and a major source of biofuel. Thus, the focus of the scientific community has been largely on understanding and maximizing the lipid production in *C. reinhardtii* and other algal strains (Huang et al., 2010; James et al., 2011; Li et al., 2015; Cheng et al., 2018). It is important to understand the important roles that fatty acids (FA) play in the interactions of microalgae cells with the environment. The biosynthesis of fatty acids and glycerolipids in plants and eukaryotic algae involves the cooperation of two subcellular organelles, plastids and the endoplasmic reticulum (ER) and for eukaryotic algae, this is also the case (Harwood, 2013). The fatty acids produced in plastids can be incorporated into the plastid pool of phosphatidate which can be subsequently converted into chloroplast membrane lipids, MGDG, DGDG, SQDG and PG (Harwood and Guschina, 2009; Harwood, 2013). Diacylglycerol (DAG) originating from a pool of endoplasmic reticulum PC, may be transferred from ER to plastids and be used there as a substrate for the synthesis of chloroplast lipids (Harwood, 2013; Liu and Benning, 2013). It has been suggested to be important, for normal photosynthetic function, the unique lipid composition in chloroplast membranes, (e.g. high level of fatty acid unsaturation) as well as the galactosylglycerides which are mainly located in these cell organelles (Siegenthaler and Murata, 2004). Investigation of various Chlamydomonas mutants with specific alterations in lipid composition showed to be a powerful tool to study structure-function relationships. Sato et al., (2003) compared the structural and functional properties of photosystem II (PSII) between a mutant of *C. reinhardtii* defective in SQDG (hf-2) and the wild type, to examine the role of SQDG in thylakoid membranes. It has been suggested that SQDG is involved in the maintenance of the normal properties of PSII (Sato et al., 2003).

Cells under N limitation display a rapid stress response, presumably related to the requirements for protein synthesis, whereas more sophisticated lipid-remodeling systems seem to be necessary during N limitation (Machado et al., 2016; Tan and Lee, 2016; Rocha et al., 2019). TAGs are accumulated in many algae species as storage products and the level of TAG accumulation is very variable (Thompson, 1996; John et al., 2002; Rocha et al., 2017; Li-Beisson et al., 2019) and may be stimulated by a number of environmental factors. When algal growth slows down and there is no requirement for the synthesis of new membrane compounds, the cells divert fatty acids into TAG synthesis before conditions improve and there is a need for

further growth (Harwood, 2013; Liu and Benning, 2013; Zienkiewicz et al., 2016). Nitrogen deprivation seems to be a major factor which is important for the stimulation of TAG synthesis (Goncalves et al., 2016; Li-Beisson et al., 2019). Many algae sustain a two- to three-fold increase in lipid content, predominantly TAG, under N limitation (Tan and Lee, 2016; Sivaramakrishnan and Incharoensakdi, 2017; Nagappan and Kumar, 2021).

5. CONCLUSION

In this study, the physiological analysis of the five strains grown in N depleted, N replete and N saturated regimes showed a decrease in biomass accumulation in N depleted conditions.

Regarding the metabolomic analysis, N starvation leads to typical stress-related responses such as reduction of chlorophyll and protein levels.

For C contents, as expected, all strains showed an increase in starch and lipid contents for N depleted treatment.

It seems reasonable to suggest that some microalgae are programmed to accumulate lipids, like *Monoraphidium* sp. BR023 while others, like *Chlamydomonas reinhardtii* CC503, accumulate starch during the stationary phase under nutritional stress as N deplete. All strains also markedly differ in membrane lipid composition, FA saturation and increase in TAGs.

Thus, reinforcing the importance of evolutionary and ecological constraints in the final phenotype of a strain.

6. REFERENCES

Ball SG, Dirick L, Decq A, Martiat JC, Matagne RF (1990) Physiology of starch storage in the monocellular alga *Chlamydomonas reinhardtii*. Plant Sci **66**: 1–9

Bar-nun S, Ohad I (1980) the accuracy of sometimes reported chloroplastic Mn abundance deter- minations based only on a presumed specific activity of Mn in the growth media. **69**: 363–374

Barsanti L, Gualtieri P (2018) Is exploitation of microalgae economically and energetically sustainable? Algal Res **31**: 107–115

Batista AD, Rosa RM, Machado M, Magalhães AS, Shalaguti BA, Gomes PF, Covell L, Vaz MGMV, Araújo WL, Nunes-Nesi A (2019) Increased urea availability promotes adjustments in C/N metabolism and lipid content without impacting growth in *Chlamydomonas reinhardtii*. Metabolomics. doi: 10.1007/s11306-019-1496-3

Blaby IK, Glaesener AG, Mettler T, Fitz-Gibbon ST, Gallaher SD, Liu B, Boyle NR, Kropat J, Stitt M, Johnson S, et al (2013) Systems-level analysis of nitrogen starvation-induced modifications of carbon metabolism in a *Chlamydomonas reinhardtii* starchless mutant. Plant Cell **25**: 4305–4323

Bradford M (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem **72**: 248–254

Breuer G, Lamers PP, Martens DE, Draaisma RB, Wijffels RH (2012) The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. Bioresour Technol **124**: 217–226

Bromke MA, Giavalisco P, Willmitzer L, Hesse H (2013) Metabolic Analysis of Adaptation to Short-Term Changes in Culture Conditions of the Marine Diatom Thalassiosira pseudonana. PLoS One **8**: 1–11

Cakmak T, Angun P, Demiray YE, Ozkan AD, Elibol Z, Tekinay T (2012) Differential effects of nitrogen and sulfur deprivation on growth and biodiesel feedstock production of *Chlamydomonas reinhardtii*. Biotechnol Bioeng **109**: 1947–1957

Chen H, Zheng Y, Zhan J, He C, Wang Q (2017) Comparative metabolic profiling of the lipid-producing green microalga Chlorella reveals that nitrogen and carbon metabolic pathways contribute to lipid metabolism. Biotechnol Biofuels. doi: 10.1186/s13068-017-0839-4

Cheng P, Wang Y, Osei-Wusu D, Wang Y, Liu T (2018) Development of nitrogen supply strategy for *Scenedesmus rubescens* attached cultivation toward growth and lipid accumulation. Bioprocess Biosyst Eng **41**: 435–442

Coleman AW (1990) The Chlamydomonas sourcebook. A comprehensive guide to biology and laboratory use. Trends Genet **6**: 67

Contois DE (1959) Kinetics of Bacterial Growth: Relationship between Population Density and Specific Growth Rate of Continuous Cultures. J Gen Microbiol **21**: 40–50

Covell L, Machado M, Vaz MGMV, Soares J, Batista AD, Araújo WL, Martins MA, Nunes-Nesi A (2020) Alternative fertilizer-based growth media support high lipid contents without growth impairment in *Scenedesmus obliquus* BR003. Bioprocess Biosyst Eng **43**: 1123–1131

Cross JM, Von Korff M, Altmann T, Bartzetko L, Sulpice R, Gibon Y, Palacios N, Stitt M (2006) Variation of enzyme activities and metabolite levels in 24 arabidopsis accessions growing in carbon-limited conditions. Plant Physiol **142**: 1574–1588

Dhup S (2016) Understanding Urea Assimilation and its Effect on Lipid Production and Fatty Acid Composition of *Scenedesmus* Sp. SOJ Biochem **2**: 1–7

El-Sheekh M, Abomohra AEF, Eladel H, Battah M, Mohammed S (2018) Screening of different species of Scenedesmus isolated from Egyptian freshwater habitats for biodiesel production. Renew Energy **129**: 114–120

Fan J, Yan C, Andre C, Shanklin J, Schwender J, Xu C (2012) Oil accumulation is controlled by carbon precursor supply for fatty acid synthesis in *Chlamydomonas reinhardtii*. Plant Cell Physiol **53**: 1380–1390

Gargouri M, Bates PD, Park JJ, Kirchhoff H, Gang DR (2017) Functional photosystem i maintains proper energy balance during nitrogen depletion in *Chlamydomonas reinhardtii*, promoting triacylglycerol accumulation. Biotechnol Biofuels **10**: 1–21

Girard JM, Roy ML, Hafsa M Ben, Gagnon J, Faucheux N, Heitz M, Tremblay R, Deschênes JS (2014) Mixotrophic cultivation of green microalgae *Scenedesmus obliquus* on cheese whey permeate for biodiesel production. Algal Res **5**: 241–248

Goncalves EC, Wilkie AC, Kirst M, Rathinasabapathi B (2016) Metabolic regulation of triacylglycerol accumulation in the green algae: identification of potential targets for engineering to improve oil yield. Plant Biotechnol J **14**: 1649–1660

Grace SC, Hudson DA (2016) Processing and Visualization of Metabolomics Data Using R. Metabolomics - Fundam Appl. doi: 10.5772/65405

Griffiths MJ, Garcin C, van Hille RP, Harrison STL (2011) Interference by pigment in the estimation of microalgal biomass concentration by optical density. J Microbiol Methods **85**: 119–123

Guerra LT, Levitan O, Frada MJ, Sun JS, Falkowski PG, Dismukes GC (2013) Regulatory branch points affecting protein and lipid biosynthesis in the diatom Phaeodactylum tricornutum. Biomass and Bioenergy **59**: 306–315

Harris EH (2001) Chlamydomonas as a model organism. Mol Biol **52**: 363–406

Harwood IAG ad JL (2013) Algae for biofuels and energy. Algae for Biofuels and Energy 1–288

Harwood JL, Guschina IA (2009) The versatility of algae and their lipid metabolism. Biochimie **91**: 679–684

Healey FP (1978) Physiological indicators of nutrient deficiency in algae. SIL Commun 1953-1996 **21**: 34–41

Himanshu S, Manish RS, Basuthkar JR, Kandala VRC (2016) Regulation of starch, lipids and amino acids upon nitrogen sensing in *Chlamydomonas reinhardtii*. Algal Res **18**: 33–44

Holbrook GP, Davidson Z, Tatara RA, Ziemer NL, Rosentrater KA, Scott Grayburn W (2014) Use of the microalga *Monoraphidium* sp. grown in wastewater as a feedstock for biodiesel: Cultivation and fuel characteristics. Appl Energy **131**: 386–393

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. Plant J **54**: 621–639

Huang GH, Chen F, Wei D, Zhang XW, Chen G (2010) Biodiesel production by microalgal biotechnology. Appl Energy **87**: 38–46

Hummel J, Segu S, Li Y, Irgang S, Jueppner J, Giavalisco P (2011) Ultra performance liquid chromatography and high resolution mass spectrometry for the analysis of plant lipids. Front Plant Sci **2**: 1–17

Ikaran Z, Suárez-Alvarez S, Urreta I, Castañón S (2015) The effect of nitrogen limitation on the physiology and metabolism of *Chlorella vulgaris* var L3. Algal Res **10**: 134–144

Illman AM, Scragg AH, Shales SW (2000) Increase in Chlorella strains calorific values when grown in low nitrogen medium. Enzyme Microb Technol **27**: 631–635

Jagadevan S, Banerjee A, Banerjee C, Guria C, Tiwari R, Baweja M, Shukla P (2018) Recent developments in synthetic biology and metabolic engineering in microalgae towards biofuel production. Biotechnol Biofuels **11**: 1–21

James GO, Hocart CH, Hillier W, Chen H, Kordbacheh F, Price GD, Djordjevic MA (2011) Fatty acid profiling of *Chlamydomonas reinhardtii* under nitrogen deprivation. Bioresour Technol **102**: 3343–3351

John U, Tillmann U, Medlin LK (2002) A comparative approach to study inhibition of grazing and lipid composition of a toxic and non-toxic clone of Chrysochromulina polylepis (Prymnesiophyceae). Harmful Algae **1**: 45–57

Juergens MT, Deshpande RR, Lucker BF, Park JJ, Wang H, Gargouri M, Omar Holguin F, Disbrow B, Schaub T, Skepper JN, et al (2015) The regulation of photosynthetic structure and function during nitrogen deprivation in *Chlamydomonas reinhardtii*. Plant Physiol **167**: 558–573

Kiyota H, Hirai M, Ikeuchi M (2014) NblA1/A2-Dependent Homeostasis of Amino Acid Pools during Nitrogen Starvation in Synechocystis sp. PCC 6803. Metabolites **4**: 517–531

Klein U (1987) Intracellular Carbon Partitioning in *Chlamydomonas reinhardtii*. Plant Physiol **85**: 892–897

Kumar G, Shekh A, Jakhu S, Sharma Y, Kapoor R, Sharma TR (2020) Bioengineering of Microalgae: Recent Advances, Perspectives, and Regulatory Challenges for Industrial Application. Front Bioeng Biotechnol. doi: 10.3389/fbioe.2020.00914

De la Hoz Siegler H, Ayidzoe W, Ben-Zvi A, Burrell RE, McCaffrey WC (2012) Improving the reliability of fluorescence-based neutral lipid content measurements in microalgal cultures. Algal Res **1**: 176–184

Li-Beisson Y, Thelen JJ, Fedosejevs E, Harwood JL (2019) The lipid biochemistry of eukaryotic algae. Prog Lipid Res **74**: 31–68

Li T, Gargouri M, Feng J, Park JJ, Gao D, Miao C, Dong T, Gang DR, Chen S (2015) Regulation of starch and lipid accumulation in a microalga *Chlorella sorokiniana*. Bioresour Technol **180**: 250–257

Li T, Xu J, Gao B, Xiang W, Li A, Zhang C (2016) Morphology, growth, biochemical composition and photosynthetic performance of *Chlorella vulgaris* (Trebouxiophyceae) under low and high nitrogen supplies. Algal Res **16**: 481–491

Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry–based metabolite profiling in plants. Nat Protoc **1**: 387–396

Liu B, Benning C (2013) Lipid metabolism in microalgae distinguishes itself. Curr Opin Biotechnol **24**: 300–309

López CVG, del Carmen Cerón García M, Fernández FGA, Bustos CS, Chisti Y, Sevilla JMF (2010) Protein measurements of microalgal and cyanobacterial biomass. Bioresour Technol **101**: 7587–7591

Machado M, Bromke M, Domingues Júnior AP, Marçal Vieira Vaz MG, Rosa RM, Vinson CC, Sabir JS, Rocha DI, Martins MA, Araújo WL, et al (2016) Comprehensive metabolic reprograming in freshwater Nitzschia palea strains undergoing nitrogen starvation is likely associated with its ecological origin. Algal Res. doi: 10.1016/j.algal.2016.06.003

Miao M sheng, Yao X dong, Shu L, Yan Y jie, Wang Z, Li N, Cui X tong, Lin Y min, Kong Q (2016) Mixotrophic growth and biochemical analysis of *Chlorella vulgaris* cultivated with synthetic domestic wastewater. Int Biodeterior Biodegrad **113**: 120–125

Miller R, Wu G, Deshpande RR, Vieler A, Gartner K, Li X, Moellering ER, Zauner S, Cornish AJ, Liu B, et al (2010) Changes in transcript abundance in *Chlamydomonas*

reinhardtii following nitrogen deprivation predict diversion of metabolism. Plant Physiol **154**: 1737–1752

Mobin A, Chowdhury H (2019) Commercially important bioproducts from microalgae and their current applications – A review. Energy Procedia **160**: 752–760

Moon M, Kim CW, Park WK, Yoo G, Choi YE, Yang JW (2013) Mixotrophic growth with acetate or volatile fatty acids maximizes growth and lipid production in *Chlamydomonas reinhardtii*. Algal Res **2**: 352–357

Nagappan S, Devendran S, Tsai P, Jayaraman H, Alagarsamy V, Pugazhendhi A (2020) Metabolomics integrated with transcriptomics and proteomics: Evaluation of systems reaction to nitrogen deficiency stress in microalgae. Process Biochem 143393

Nagappan S, Kumar G (2021) Investigation of four microalgae in nitrogen deficient synthetic wastewater for biorefinery based biofuel production. Environ Technol Innov **23**: 101572

Nama S, Madireddi SK, Devadasu ER, Subramanyam R (2015) High light induced changes in organization, protein profile and function of photosynthetic machinery in *Chlamydomonas reinhardtii*. J Photochem Photobiol B Biol **152**: 367–376

Park JJ, Wang H, Gargouri M, Deshpande RR, Skepper JN, Holguin FO, Juergens MT, Shachar-Hill Y, Hicks LM, Gang DR (2015) The response of *Chlamydomonas reinhardtii* to nitrogen deprivation: A systems biology analysis. Plant J **81**: 611–624

Rai MP, Gupta S (2017) Effect of media composition and light supply on biomass, lipid content and FAME profile for quality biofuel production from Scenedesmus abundans. Energy Convers Manag **141**: 85–92

Rocha DN, Martins MA, Soares J, Vaz MGMV, de Oliveira Leite M, Covell L, Mendes LBB (2019) Combination of trace elements and salt stress in different cultivation modes improves the lipid productivity of *Scenedesmus* spp. Bioresour Technol. doi: 10.1016/j.biortech.2019.121644

Rocha RP, Machado M, Vaz MGMV, Vinson CC, Leite M, Richard R, Mendes LBB, Araujo WL, Caldana C, Martins MA, et al (2017) Exploring the metabolic and physiological diversity of native microalgal strains (Chlorophyta) isolated from tropical freshwater reservoirs. Algal Res **28**: 139–150

Salem MA, Jüppner J, Bajdzienko K, Giavalisco P (2016) Protocol: A fast, comprehensive and reproducible one-step extraction method for the rapid preparation of polar and semi-polar metabolites, lipids, proteins, starch and cell wall polymers from a single sample. Plant Methods **12**: 1–15

Sanz-Luque E, Chamizo-Ampudia A, Llamas A, Galvan A, Fernandez E (2015) Understanding nitrate assimilation and its regulation in microalgae. Front Plant Sci. doi: 10.3389/fpls.2015.00899

Sarat Chandra T, Deepak RS, Maneesh Kumar M, Mukherji S, Chauhan VS, Sarada R, Mudliar SN (2016) Evaluation of indigenous fresh water microalga *Scenedesmus obtusus* for feed and fuel applications: Effect of carbon dioxide, light and nutrient sources on growth and biochemical characteristics. Bioresour Technol **207**: 430–439

Sato N, Aoki M, Maru Y, Sonoike K, Minoda A, Tsuzuki M (2003) Involvement of sulfoquinovosyl diacylglycerol in the structural integrity and heat-tolerance of photosystem II. Planta **217**: 245–251

Siegenthaler P-A, Murata N (2004) Chapter 1: Lipids in photosynthesis - an overview. Lipids Photosynth Struct Funct Genet 1–20

Singh P, Kumari S, Guldhe A, Misra R, Rawat I, Bux F (2016) Trends and novel strategies for enhancing lipid accumulation and quality in microalgae. Renew Sustain Energy Rev **55**: 1–16

Sivaramakrishnan R, Incharoensakdi A (2017) Enhancement of total lipid yield by nitrogen, carbon, and iron supplementation in isolated microalgae. J Phycol **53**: 855–868

Smith AM, Zeeman SC (2006) Quantification of starch in plant tissues. Nat Protoc **1**: 1342–1345

Stacklies W, Redestig H, Scholz M, Walther D, Selbig J (2007) pcaMethods - A bioconductor package providing PCA methods for incomplete data. Bioinformatics **23**: 1164–1167

Tan KWM, Lee YK (2016) The dilemma for lipid productivity in green microalgae: Importance of substrate provision in improving oil yield without sacrificing growth. Biotechnol Biofuels **9**: 1–14

Thompson GA (1996) Lipids and membrane function in green algae. Biochim Biophys Acta - Lipids Lipid Metab **1302**: 17–45

Williams PJLB, Laurens LML (2010) Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetics & economics. Energy Environ Sci **3**: 554–590

Work VH, Radakovits R, Jinkerson RE, Meuser JE, Elliott LG, Vinyard DJ, Laurens LML, Dismukes GC, Posewitz MC (2010) Increased lipid accumulation in the *Chlamydomonas reinhardtii* sta7-10 starchless isoamylase mutant and increased carbohydrate synthesis in complemented strains. Eukaryot Cell **9**: 1251–1261

Wu LF, Chen PC, Lee CM (2013) The effects of nitrogen sources and temperature on cell growth and lipid accumulation of microalgae. Int Biodeterior Biodegrad **85**: 506–510

Yang H, He Q, Hu C (2015) Lipid accumulation by NaCl induction at different growth stages and concentrations in photoautotrophic two-step cultivation of *Monoraphidium dybowskii* LB50. Bioresour Technol **187**: 221–227

Zhu FY, Chen MX, Chan WL, Yang F, Tian Y, Song T, Xie LJ, Zhou Y, Xiao S, Zhang J, et al (2018) SWATH-MS quantitative proteomic investigation of nitrogen starvation in Arabidopsis reveals new aspects of plant nitrogen stress responses. J Proteomics **187**: 161–170

Zhu L, Li S, Hu T, Nugroho YK, Yin Z, Hu D, Chu R, Mo F, Liu C, Hiltunen E (2019) Effects of nitrogen source heterogeneity on nutrient removal and biodiesel production of mono- and mix-cultured microalgae. Energy Convers Manag. doi: 10.1016/j.enconman.2019.112144

Zhu S, Huang W, Xu J, Wang Z, Xu J, Yuan Z (2014) Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga *Chlorella zofingiensis*. Bioresour Technol **152**: 292–298

Zienkiewicz K, Du ZY, Ma W, Vollheyde K, Benning C (2016) Stress-induced neutral lipid biosynthesis in microalgae — Molecular, cellular and physiological insights. Biochim Biophys Acta - Mol Cell Biol Lipids **1861**: 1269–1281

CHAPTER 2

TITLE: On the role of TOR signaling networks in freshwater microalgae strains

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ABSTRACT

Responses of cell growth to environmental changes are essential for the survival of biological systems. The evolutionary conserved Ser/Thr protein kinase "Target of Rapamycin" (TOR) has emerged as a signaling node that integrates the sensing of numerous growth signals to the coordinated regulation of cellular metabolism and growth. The TOR pathway controls cell growth by promoting anabolic processes, such as protein synthesis and ribosome biogenesis, and inhibiting catabolic processes such as autophagy. The TOR kinase has been identified in plants and microalgae, indicating that this pathway is conserved in photosynthetic eukaryotes. A better understanding of the regulation of the metabolic pathways in freshwater microalgae with a different accumulation of reserve compounds is needed to understand the mechanisms involved in the regulation of biosynthesis and catabolism of storage compounds. In this study, two freshwater microalgae, *Chlamydomonas* sp. BR020 and *Chlorella vulgaris* BR017 and a model, *Chlamydomonas reinhardtii* CC503 were used to assess the effects of rapamycin-inhibited TOR signaling pathway on growth cell, biochemical composition and metabolic profile. We analyzed the dynamics responses of the strain metabolome and lipidome to TOR-inhibition by rapamycin. Consistent with the growth phenotype we observed strong changes in carbon (C) and nitrogen (N) partitioning in the direction of storage through an accumulation of starch, triacylglycerol and amino acids. Interestingly, the strains showed different metabolite levels, confirming the difference in reserve compound accumulation of each strain. This study clearly shows the complex picture of metabolic and lipidomic alterations in uncharacterized strains in TOR pathway affected by rapamycin. Furthermore, it reveals a complex regulation and adjustment of metabolite pools and lipid composition in response to TOR inhibition. This study show that TOR inhibition causes a severely reduced growth, leading to a reduction by 50% on the cell growth and TOR is involved in the regulation of cell growth in different strains. Next to the direct cell growth related results our data reveals that the obtained growth-phenotype was accompanied by strong accumulation of carbon (starch and TAGs) reserves. The amino acids profile exhibited a diverse pattern in relationship to the strains, which will aid in specifying the role and mechanism of individual compounds in the response to the rapamycin treatment. Taken together, the present data reveals a clear metabolite reprograming in response to TOR inhibition in a strain specific mode.

Keywords: Chlorophytes. *Chlamydomonas reinhardtii*. Primary- and lipid- metabolism. Rapamycin.

1. INTRODUCTION

The modulation of growth and development is a central process in all organisms, and an intimate relationship exists between nutrient availability, energy status, and cell growth rate, which are influenced by dynamic and diverse environmental stresses (Pérez-Pérez et al., 2017; Pancha et al., 2020). Unicellular organisms, such as yeasts and algae, are in direct contact with environmental nutritional changes (Crespo and Hall, 2002). Multicellular organisms, such as plants and animals, must also sense and coordinate these cell-autonomous processes at the organism level via signals (Ren et al., 2013a). Those factors are reached over signaling networks that compose the sensing of local and systemic nutrient and energy sources and transmit this information to metabolic regulators and enzymes to control cellular anabolic and catabolic processes (Wullschleger, 2006; Dobrenel et al., 2016a).

One of the prominent and conserved nutrient sensing is performed by the Serine/Threonine protein kinase Target of Rapamycin (TOR) (Crespo and Hall, 2002; Soulard et al., 2009; Laplante and Sabatini, 2012). This protein kinase is a conserved and essential regulator of eukaryotic cell growth that is thought to integrate external and internal metabolic cues to balance cell growth with energy and nutrient supplies (Menand et al., 2002; Loewith and Hall, 2011; Xiong and Sheen, 2012; Rexin et al., 2015). As indicated by its name, TOR is the target of a molecule named rapamycin, which is a compound with antifungal and immunosupressive properties that was first isolated in the 1970s from the soil bacterium *Strepomyces hygroscopius* (Sehgal et al., 1975; Vézina and Kudelski, 1975). The elucidation of TOR as the target of rapamycin occurred in the budding yeast *Saccharomyces cerevisiae*, where two independent TOR genes were in the genome (Heitman et al., 1991). The presence of these two expressed genes opened the question of their functions and led to the discovery of two independent TOR complexes (TORC) in yeast (Loewith et al., 2002), but also in mammalina cell cultures (Hara et al., 2002; Kim et al., 2002). Both complexes, termed TOR complex 1 (TORC1) and Tor complex 2 (TORC2), were different in their composition, localization and functionality (Betz and Hall, 2013; Saxton and Sabatini, 2017). While TORC1 emerged as a highly conserved protein complex, composed of TOR, RAPTOR and LST8 whose homologs have been identified in all thus far studied eukaryotes, including fungi, mammals, worms, flies, plants and algae (Loewith et al., 2002; Wullschleger et al., 2005; Soulard et al., 2009; González and Hall, 2017), TORC2 seems to be less conserved. Studies performed in yeasts uncovered the unique mechanism of action of rapamycin. Rapamycin first binds the 12-kD FK506-binding protein

(FKBP12) and this complex inhibits the TOR Ser/Thr kinase. FKBP12 is a member of the FK506- and rapamycin-binding protein (FKBP) family.

The TOR kinase has been described in plants and algae, indicating that this signaling pathway is also conserved in photosynthetic organisms (Loewith et al., 2002; Menand et al., 2002; Wullschleger, 2006). However, in contrast to yeasts, mammals, or flies, the vegetative growth of Arabidopsis and other plants such as *Oryza sativa*, *Nicotiana tabacum*, or *Brassica napus*, is not sensitive to rapamycin (Menand et al., 2002). A feasible explanation to rapamycin resistance of land plants might be the inability of plant FKBP12 to bind this drug. Indeed, studies previously reported that plant FKBP12 has evolved structural changes that hamper this protein to mediate the action of its drug ligands against the functional targets (Xu et al., 1998).

Several photosynthetic red and green algae, including the green model alga species *Chlamydomonas reinhardtii* have been proven to be sensitive to the TOR inhibiting drug rapamycin (Pérez-Pérez et al., 2017). TOR inhibition in Chlamydomonas was not only globally affecting growth and development (Crespo et al., 2005; Jüppner et al., 2018), but its function was also shown to negatively control catabolic process like autophagy (Pérez-Pérez and Crespo, 2010a; Couso et al., 2018), while anabolic process like protein translation were positively controlled by active TORC1 (Díaz-Troya et al., 2011). Still, even though evidence was accumulating that TOR is regulating several metabolic pathways on species like yeast and mammals (Jewell and Guan, 2013; Ben-Sahra and Manning, 2017; González and Hall, 2017), only few studies have been analyzing the impact of TOR inhibiton on primary (Lee and Fiehn, 2013; Kleessen et al., 2015) or lipid metabolism (Imamura et al., 2015; Couso et al., 2016; Werth et al., 2019) in Chlamydomonas or other green alga.

Since most studies involving inhibition of the TOR signaling pathway by rapamycin are conducted with the model strain *C. reinhardtii*, the hypothesis of this study is that other strains with diferent growth rates, accumulation of different storage compounds following N starvation have distinct metabolic responses and signaling pathway mechanisms. Therefore, we decided to conduct an experiment to better understand the function and response of the model algae *C. reinhardtii* CC503 and two Brazilian freshwater strains, *Chlamydomonas* sp. BR020 and *Chlorella vulgaris* BR017 induced by rapamycin to TOR inhibition. According to Rocha et al., 2017, under favorable growth conditions, BR020 and BR017 are characterized by different behavior in relationship to biomass accumulation and the reserve compounds. While the BR020 strain had the highest starch content, BR017 showed high levels of amino acids, protein and sucrose. Although all strains in this study showed a decrease in cell growth, when submitted to

rapamycin treatment, *C. reinhardtii* CC503 and *C. vulgaris* BR017 had the highest starch content when compared to *Chlamydomonas* sp. BR020. However, *C. reinhardtii* CC503 and *Chlamydomonas* sp. BR020 presented higher TAG contents compared to *C. vulgaris* BR017.

2. MATERIAL AND METHODS

2.1 Strains and culture media

Two freshwater microalgae strains used in this study Chlorophyceae are available at the Collection of Cyanobacteria and Microalgae (CCM-UFV), available in the Laboratory of Phycology and Molecular Biology, Plant Biology Department at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil (20°45′14″S, 42°52′54″W). Both strains, *Chlamydomonas* sp. BR020 and *C. vulgaris* BR017 were isolated from water samples collected from freshwater reservoirs on the university campus. The model green microalga, *C. reinhardtii*, is widely selected for diverse experiments, because of its advantages, such as fast growth, short generation time, strong adaptability, easy cultivation and easy isolation of mutants (Harris, 2001a).

The strains were cultivated in the mixotrophic medium Tris-Acetate-Phosphate (TAP) (Coleman, 1990). Strains were cultivated under 24±2 ºC, with a 16 h/8 h (light/dark) photoperiod cycle, at a constant irradiance of 100 µmol photons m^{-2} s^{-1}, under continuous shaking at 110 rpm.

2.2 Culture conditions and growth parameters

2.2.1 Cultivation in microplate

For the determination of a suitable growth inhibitory concentration, various dilutions of rapamycin, re-suspended in dimethyl sulfoxide (DMSO), were applied to liquid cultures. A first set of growth curves in three different concentrations (2.5, 5.0, and 10.0 µM) of rapamycin were carried out aiming to evaluate the maximum growth rate at logarithmic phase (µmax), a selection of sampling points as well as the cell concentration after 70 hours of cultivation, which was obtained by means of absorbance at 680nm (Optic density – OD680nm) (Griffiths et al., 2011).

The rapamycin screening assays were conducted in two steps. Firstly, growth curves were carried out in microplate (96 wells) in order to access the effect of the rapamycin concentration on the growth of the strains. For that, the experiment was carried out in a growth room, applying the same conditions described above (item 2.1). Each microplate well was filled with 280 µL

of TAP medium, 10 µL of strain inoculum (initial cell density of 1.5×10^6 cells mL^{-1}) and a final concentration of 2.5, 5.0 and 10.0 µM rapamycin was applied to each of the treatments, while the same concentrations of drug vehicle (DMSO) were added to the control cultures for the comparison. The treatments were applied at the beginning of experiment and cellular growth was monitored hourly by absorbance measurements at 680 nm (OD680nm) in spectrophotometer (UVM 340, AsysHitech) (Griffiths et al., 2011). The experiment was performed with eight repetitions during 71 hours of growth cultivation.

2.2.2 Cultivation in Erlenmeyer flasks

Subsequently, the concentration of 5 µM of rapamycin was chosen for experiments carried out in Erlenmeyer flasks (Jüppner et al., 2018; Mubeen et al., 2018). Accordingly, in this second round of experiments, besides the cell growth, physiological and biochemical parameters were also evaluated. This experiment was carried out in a growth room, applying the same conditions described in item 2.1. Cultivations were carried out in 125 mL Erlenmeyer flasks filled with 75 mL useful volume. The flasks were filled with 60 mL of TAP medium, 15 mL of strain inoculum (initial cell density of 1.5×10^6 cells mL^{-1}) and a final concentration of 5 µM rapamycin was applied to each of the treatments, while similar concentrations of drug vehicle were added to the control cultures for the comparison. The treatments were applied at the beginning of cultivation and it was performed in quadruplicates. Based on the obtained growth curves we selected three points to collect samples, 0h, 24h and 48h.

2.2.3 Determination of growth parameters

A total of 2 mL homogenous cell suspension was removed from each culture at the 0h, 24h and 48h and fixed with Transeau solution (1:1 sample: fixator) for use in the determination of cell density. Cell counts were carried out using a light microscope with a bright-line hemocytometer (Neubauer's chamber hemocytometer). These data were used to determine specific growth rate (µ), generation time (G2), mass per cell and specific growth rate at each growth phase as previously described (Contois, 1959). And at the same time cellular growth was monitored by absorbance measurements at 680 nm in spectrophotometer (UVM 340, AsysHitech) (Griffiths et al., 2011).

2.3 Extraction of chlorophyll, lipids and primary metabolites

Primary metabolites, chlorophyll and lipids were extracted with minor changes as described elsewhere (Salem et al., 2016). Snap-frozen cell pellets, harvested by centrifugation were re-suspended in 1mL of pre-cooled (-20°C) methyl tert-butyl ether (MTBE)-extraction buffer [methanol: methyl tert-butyl-ether (1 : 3; v/v) mixture; Biosolve, the Netherlands] as mentioned previously (Salem et al., 2016). The resuspended cell pellets were immediately mixed before ultrasonification in an ice-cooled water bath for 10 min. Then the samples were incubated for 1h on an orbital shaker at 4°C. To induce the liquid: liquid-phase separation, 650 µL of UPLC-grade methanol : water (1 : 3; v : v; Biosolve, the Netherlands) was added to the homogenate and mixed, before centrifuging at 20 000 *g* for 5 min at 4°C in a table top centrifuge. The addition of the methanol: water mixture resulted in a separation of an upper MTBE-phase, containing lipids and chlorophyll, and a lower phase, containing the polar and semi-polar metabolites, next to a solid pellet in the bottom of the 1.5 mL plastic tube. The solid pellet contains the precipitated proteins next to starch and other insoluble polymers, such as cell wall fragments (Salem et al., 2016). For further analysis, 500 µL of the MTBE-phase and 650 µL of the lower, aqueous-phase were transferred to fresh 1.5 mL tubes. Samples of the lipid-phases were dried in a vacuum concentrator and re-suspended in the proper resuspension buffer. The polar aliquots were also dried in a vacuum concentrator before storing the pellets at -80°C. The solid pellet was frozen in liquid N and stored at -80°C until further extraction. To measure the impact of contaminations from the extraction process, the whole extraction procedure was additionally performed with two–four empty, sample-free, 1.5 mL tubes (method blank).

2.4 Polar metabolite profiling

The derivatization of the polar-sample (primary metabolites) was performed on a gas chromatographer Agilent 6890 (Agilent) coupled to a Pegasus HT ToF mass spectrometer (LECO Instruments, St. Joseph, MI, USA) as outlined in a previous study (Lisec et al., 2006). Chromatograms were exported from ChromaToF v 3.25 (LECO Corp., St. Joseph, MI, USA) and processed using R. Peak detection, retention time alignment, and library matching were performed using the TargetSearch R package from Bioconductor, as performed by (Bromke et al., 2013). For data normalization, each analyte peak intensity was normalized by the median of all data and dry weight.

2.5 Determination of the chlorophyll content

To determine the chlorophyll content, 100 µL of the MTBE-phase was mixed 1 : 10 (v : v) with 90% methanol and measured using an UV-VIS spectrometer at wavelengths of 665 nm and 652 nm to distinguish between chlorophyll *a* and chlorophyll *b*. The chlorophyll *a*, *b* and total content were determined using the equations described by Bar-Nun (Bar-nun and Ohad, 1980).

2.6 Extraction and determination of starch content

In order to analyze the starch content, we used the standard protocol from Smith and Zeeman, 2006. The frozen cell pellet was first washed with 80% ethanol, incubated for 10 min at room temperature. Afterwards the pellet was dissolved in 250 µl sterile water. Then 250 µl 100 mM sodium acetate was added and starch was hydrolyzed by heating for 3h at 99 °C. The dissolved starch was digested into its glucose monomers over night with an enzyme mix of α-amylase (4.2 units per sample) and α-amyloglucosidase (10 units per sample). The digested extract was centrifuged and the supernatant with the starch-derived glucose monomers was dissolved in 100 mM HEPES-buffer (pH 7) that contained 5 mM $MgCl_2$, 60 mg/ml ATP, 36 mg/ml NADP and glucose-6-phophate-dehydrogenase (1 unit per sample). The baseline was measured at 340 nm and the subsequent addition of hexokinase started the reactions and the increase of $NADPH+H^+$ was measured at 340 nm with a plate reader in 96-well formats.

2.7 Determination of lipids

Briefly, 650 µL of the lower aqueous phase of the MTBE extraction, described above, were transferred to fresh 1.5-ml Eppendorf tubes, dried in a SpeedVac and resuspended in a volume of 300 µL of a mixture of acetonitrile: isopropanol (7: 3; Biosolve, the Netherlands). Chromatograms were further processed using Excalibur software version 2.30 (Thermo-Fisher) and Progenesis CoMet software (Version 2.4; Nonlinear, Newcastle upon Tyne, UK). The UPLC/MS lipids data were processed using ToxID (Version 2.1.2; Thermo). To remove noise and contaminants, data for every lipid species with average peak height lower than the average peak height of the method blanks or with 50% of the values below 1,000 arbitrary counts were removed from the dataset. The remaining peaks were then assigned to annotated lipid species using an in-house-generated lipid database for *Arabidopsis* (Hummel et al., 2011). The data were normalized to sample dry weight. For some lipid species, more than one peak was detected

having the same *m*/*z* and identical adducts but different retention times. In these cases, we added the letter A or B to the compound name, depending on their elution order.

2.8 Statistical analysis

The experiments were performed and analyzed following a completely randomized design with four replicates for each treatment. The values obtained for all variables were submitted to analysis of variance (ANOVA, $P < 0.05$) followed by Tukey's test ($P \leq 0.05$), and one analyses of the interaction effects, using the software R (Grace and Hudson, 2016). In addition, data were subjected to a principal component analysis (PCA) performed using the pcaMethods package in the software R (Stacklies et al., 2007), therefore primary metabolites and lipid profile data were log2 transformed.

3. RESULTS

3.1 Growth curve and maximum growth rate (µmax) of the selected strains with different rapamycin concentration

The analysis of growth parameters aimed to identify how cells grew and accumulated biomass over time. From these data, the growth parameters were calculated allowing other inferences about the behavior of the culture over time. Growth curves based on optical density (OD) at 680nm indicated a similar pattern for *Chlamydomonas* sp. BR020 and *C. vulgaris* BR017 which a decreased in growth of rapamycin treatments compared to the control between 10 and 50 hours **(Figure 1 B and C)**. In *C. reinhardtii* CC503 rapamycin treatments seem to have a more evident effect during all the cultivation **(Figure 1 A)**. Regarding the specific growth rate (µ), only for *Chlamydomonas* sp. BR 020 there was no statistical difference between control and treatments, however for *C. reinhardtii* CC503 and *C. vulgaris* BR017 showed lowest growth rate for the treatments with rapamycin, thus differing statistically from the control **(Figure 1 D)**. The value for control was 0.38 d^{-1}, 0.20 d^{-1} and 023 d^{-1} for CC503, BR020 and BR017, respectively. While for 5µM rapamycin these values were 0.31 d^{-1}, 0.18 d^{-1} and 0.20 d^{-1}. According to these results and current literature (Jüppner et al., 2017; Jüppner et al., 2018), we chose 5µM rapamycin and 0h, 24h and 48 h to perform the experiment in Erlenmeyer's and the biochemical analyses.

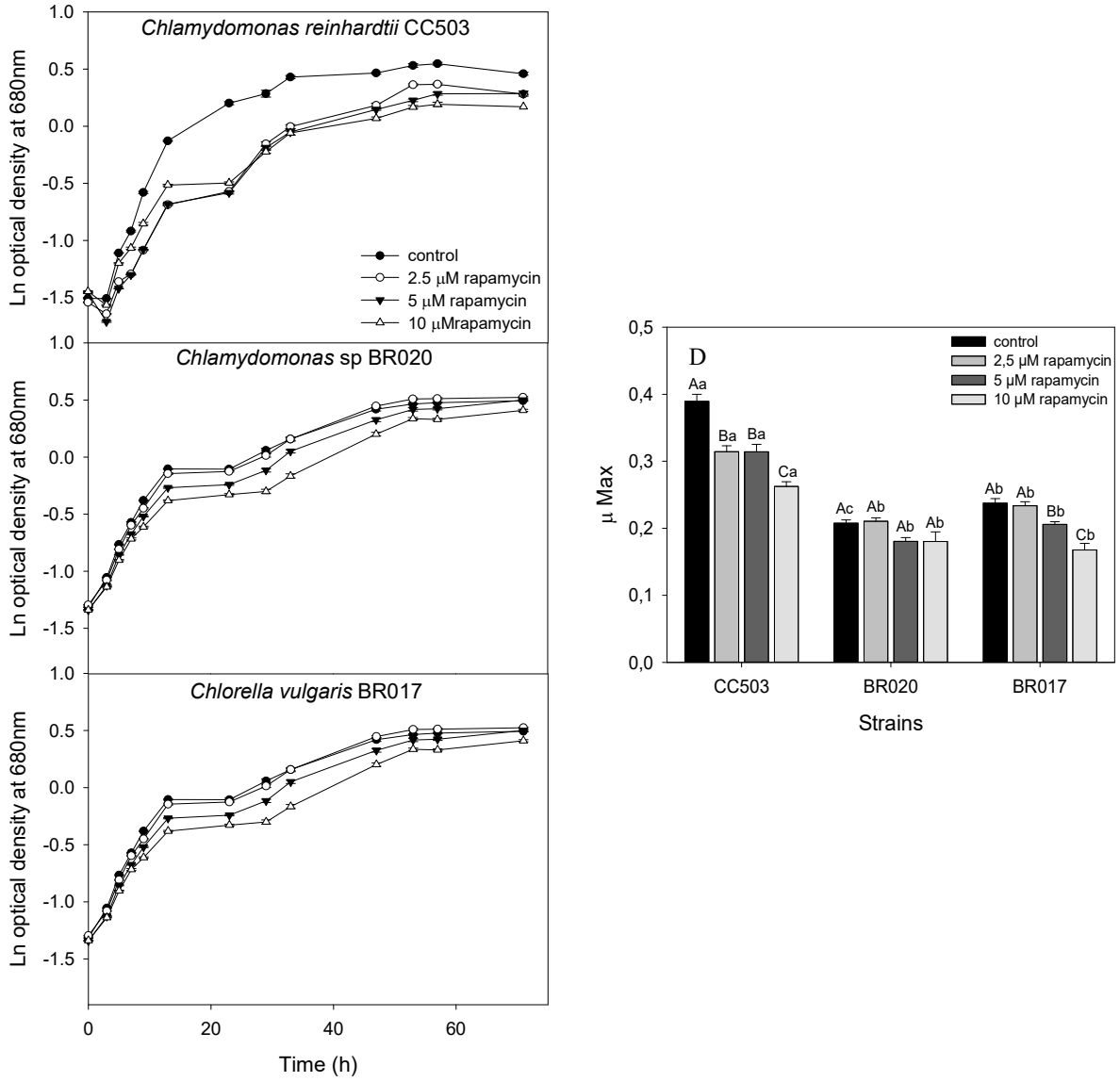


Figure 1. Growth parameters of three freshwater strains (*Chlamydomonas reinhardtii* CC503, *Chlamydomonas* sp. BR020 and *Chlorella vulgaris* BR017)) cultured in TAP medium under three different rapamycin concentrations (2.5; 5 and 10 μM) and control; Growth curves based on Ln Optical Density (OD) at 680 nm **(A-C)** and maximum growth rate (μmax) **(D)**. Values represent the average error of eight replications. Means followed by the same letters do not differ by the Tukey test (P<0.05), uppercase letters compare the rapamycin concentration in each strain and lowercase letters compare each treatment in all strains.

3.2 TOR inhibition results in reduced cell number

The cell number was monitored at three different times of strains growth **(Figure 1)**. The treatment of 5 μM rapamycin reduced the cell number at 48h growth period by approximately 50% compared with control conditions **(Figure 2 A - C)**. Also, the three strains exhibited a reduced number of cells in the first hours of culture when cultivated with 5μM of rapamycin. Regarding the specific growth rate (μ), all strains showed a statistical difference between control and 5μM rapamycin treatment, which it is possible to observe a slowdown growth in rapamycin treatment **(Figure 2 D)**. All interactions between strain, time and concentration were statistically significant, even the triple interaction (Table S 12, supplementary material).

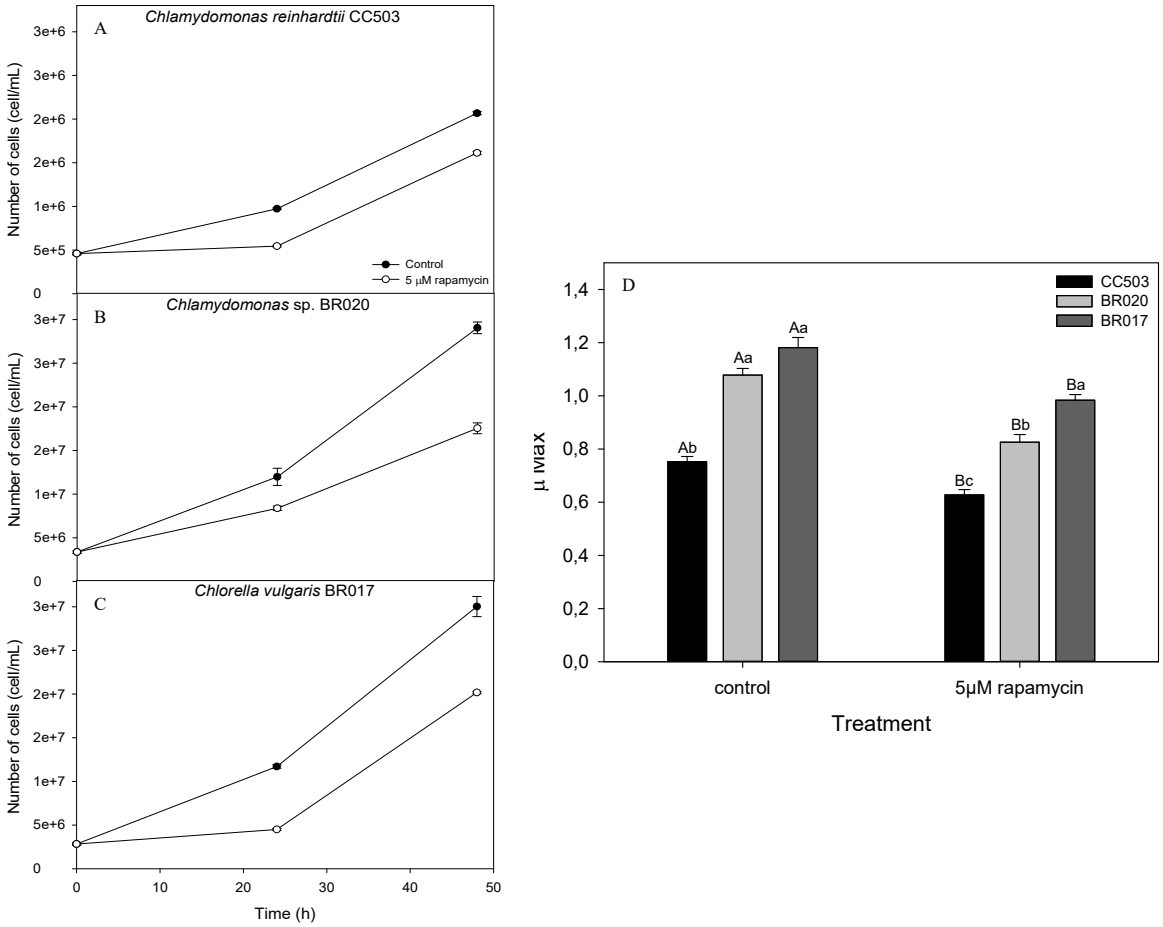


Figure 2. Growth parameters of three freshwater strains (*Chlamydomonas reinhardtii* CC503, *Chlamydomonas* sp. BR020 and *Chlorella vulgaris* BR017) cultured in TAP medium with 5μM rapamycin and control. Growth curves based on cell number **(A – C)** and maximum growth rate (μmax) **(D)**. Values represent the average error of eight replications. Means followed by the same letters do not differ by the Tukey test ($P<0.05$), uppercase letters compare the rapamycin concentration in each strain and lowercase letters compare each treatment in all strains.

3.3 Rapamycin affects differently chlorophyll contents between strains.

Regarding pigments content, all strains exhibited very similar behavior **(Figure 3)**. The analysis of variance for chlorophyll contents reveled that all interaction between strain, time and concentration were statistically significant for Chl *b*. However, for Chl *a* the interaction between strain and concentration, as well as the triple interaction were not significant **(Table S 12)**. The levels of chlorophyll *a* and *b* decreased in rapamycin treatment at the end of cultivation for all strains **(Figure 3 A-B, D-E and G-H)**. The levels of chlorophyll *a*/*b* ratio were also affected by rapamycin treatment **(Figure 3 C, F and I)**, suggesting that the applied treatment was stressful to the microalgae cells.

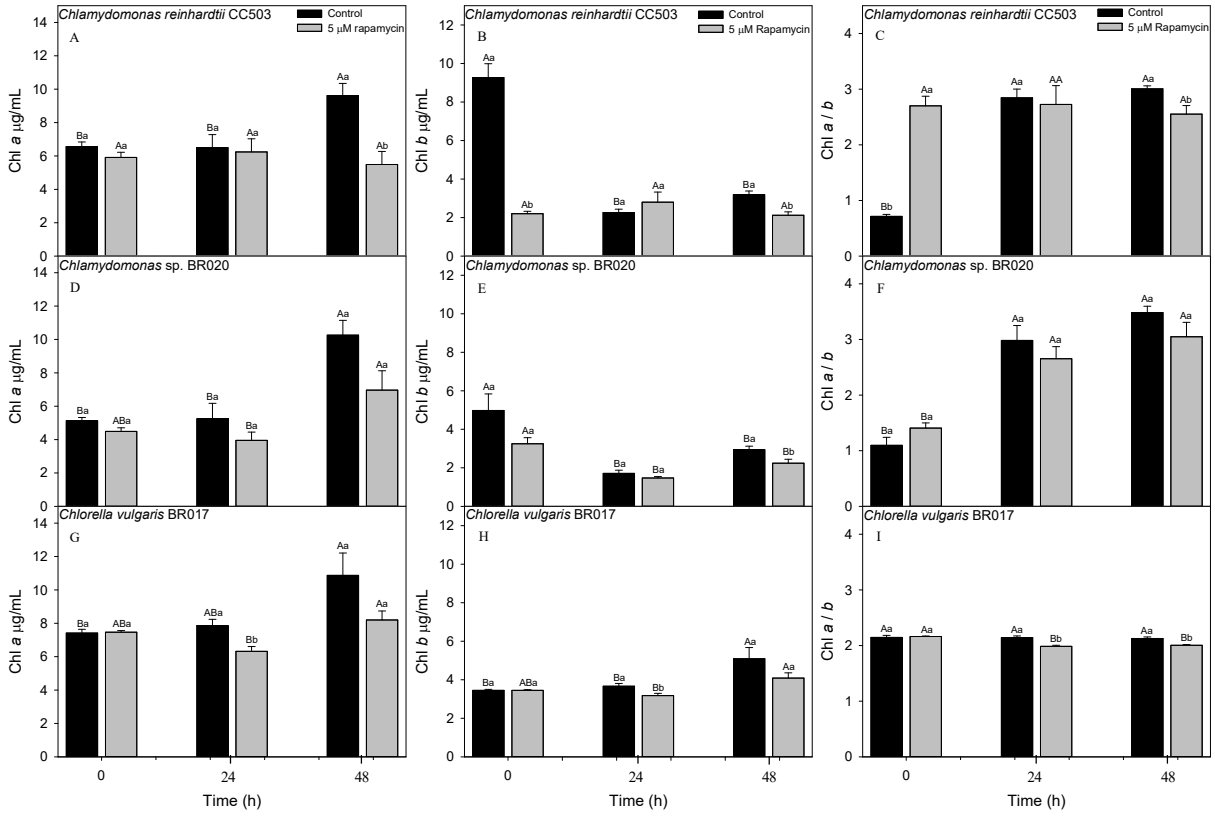


Figure 3. Chlorophyll content of of three freshwater strains (*Chlamydomonas reinhardtii* CC503 **(A – C)**; *Chlamydomonas* sp. BR020 **(D – F)** and *Chlorella vulgaris* BR017 **(G – I)**) cultured in TAP medium with 5μM rapamycin and control. Values represent the average error of eight replications. Means followed by the same letters do not differ by the Tukey test (P<0.05), uppercase letters compare the rapamycin concentration in each strain and lowercase letters compare each treatment in all strains.

3.4 TOR inhibition alters primary metabolism differentially in three microalgae strains

A possible mechanism contributing to increased amino acid accumulation after TOR inhibition might be the immediate induction of amino acid synthesis. The metabolic analysis in *C. reinhardtii* CC503 revealed that all measured amino acids at 24 h exhibited an increase after rapamycin application. Most of these initially elevated amino acids did not maintain the increased concentrations, compared with the control samples **(Figure 4, Table S 13 - 15)**. Similar behavior was also observed in *Chlamydomonas* sp. BR 020 and *C. vulgaris* BR 017 but only for metabolites aspartate, citrulline, glutamate, glutamine, histidine, ornithine, proline, serine, threonine and valine **(Figure 4)**.

The pool sizes of intermediates from glycolysis and tricarboxylic acid (TCA) cycle showed a more variable pattern between control and the rapamycin treated cells for the three strains. The three TCA cycle intermediates citrate, succinate and malate did show consistent differences between the strains. While *C. reinhardtii* CC503 and *C. vulgaris* BR017 showed a citrate and succinate increase at 24h in rapamycin treatments, malate did not differ between control and rapamycin treatments for any of the strains **(Figure 4)**. Surprisingly, these data also show that fructose-6-phosphate and glucose-6-phosphate, which are products of glycolysis, were differentially regulated after TOR inhibition only for *C. vulgaris* BR017, which increased in the TOR-inhibited cultures at 24 h, while *C. reinhardtii* CC503 and *Chlamydomonas* sp. BR020 did not show consistent differences between the control and rapamycin treatments **(Figure 4)**.

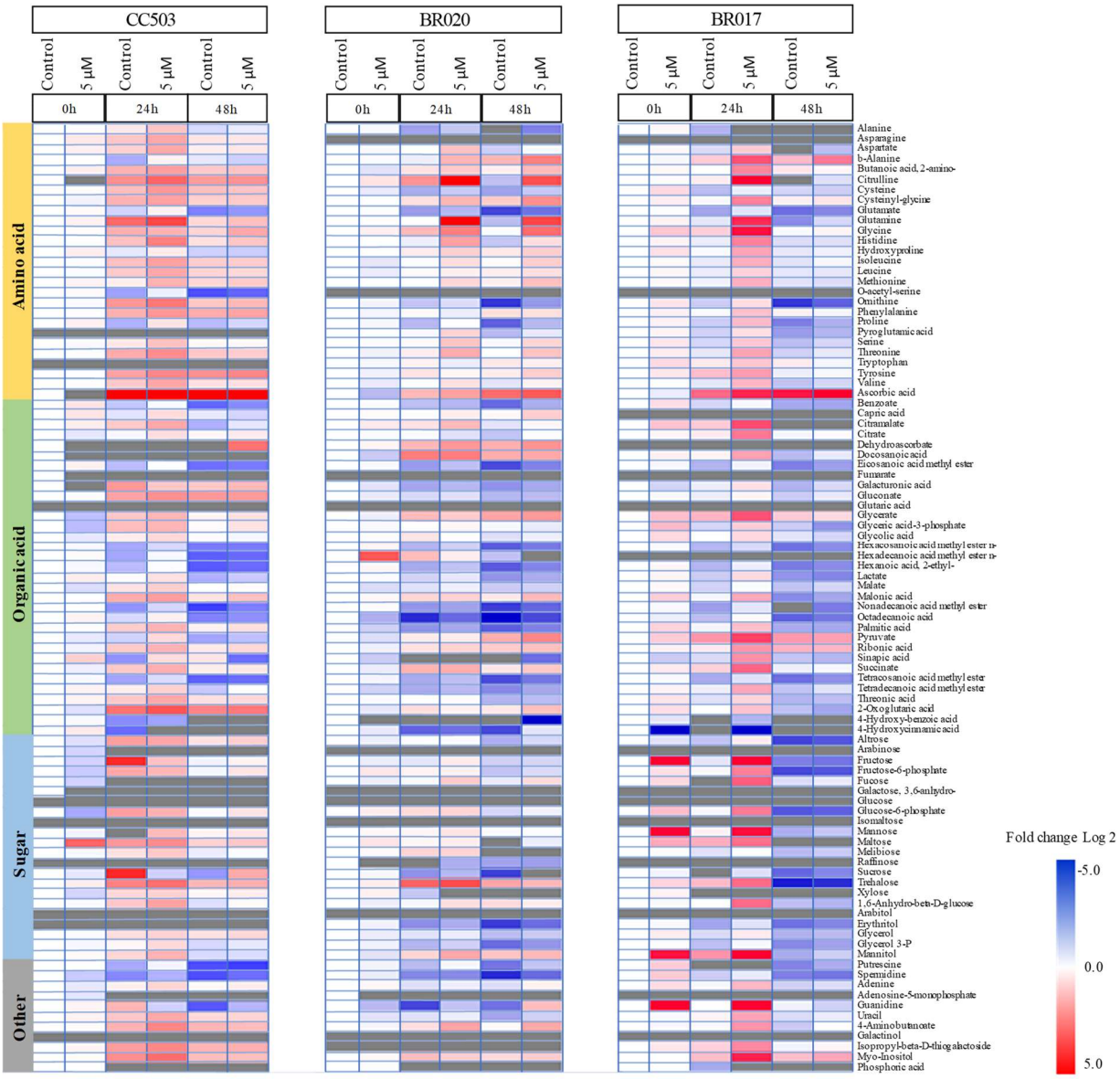


Figure 4. Heat map representing the changes in relative levels of primary metabolites of three freshwater strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, and *C. vulgaris* BR017) cultured in TAP medium with 5µM rapamycin and control. Data are scaled to the mean response measured for control 0h for each strain, values presented are means of four biological replicates. The complete dataset and statistics are presented in the supplementary table S13 – 15.

3.5 Repression of the TOR kinase leads to higher levels of storage compounds per cell

To determine if increased levels of stored carbon occur after inhibition of TOR, we extracted starch and lipids from the cultures and analyzed these compounds during three cultivation time. The two main storage carbon pools, TAGs and starch, clearly follow the expectation that growth and carbon storage are inversely proportional in *C. reinhardtii* CC503 and *C. vulgaris* BR017. While we detected a twofold decrease in the cell number of the rapamycin-treated cultures **(Figure 2 A – C)**, there was an increase in starch content at the end of 24 h in the rapamycin-treated cultures for *C. reinhardtii* CC503 and *C. vulgaris* BR017 **(Figure 5 A and C)**, followed by a decrease at 48 h. Surprisingly, *Chlamydomonas* sp. BR020 strain had the lowest starch content when treated with rapamycin, both in 24 h and 48 h of cultivation **(Figure 5 B)**.

The relationship between growth and accumulation of TAGs was different of starch, all three strains showed an increase in TAG levels, especially at 24 h of treatment. The highest levels of TAG were in 24 h of cultivation, however, BR020 strain was the one that showed the greatest increase when compared to the control, followed by BR017 and CC503 strains, respectively **(Figure 6)**. Although strain CC503 had the lowest composition of TAGs when compared to the other two strains analyzed, it was possible to observe an increase in DAGs at the end of cultivation, which leads to an increase in the content of TAGs **(Figure 6)**. It has been demonstrated that *C. reinhardtii* may employ a distinct pathway that uses DAG derived almost exclusively from the chloroplast to produce TAG (J. Fan, 2011).

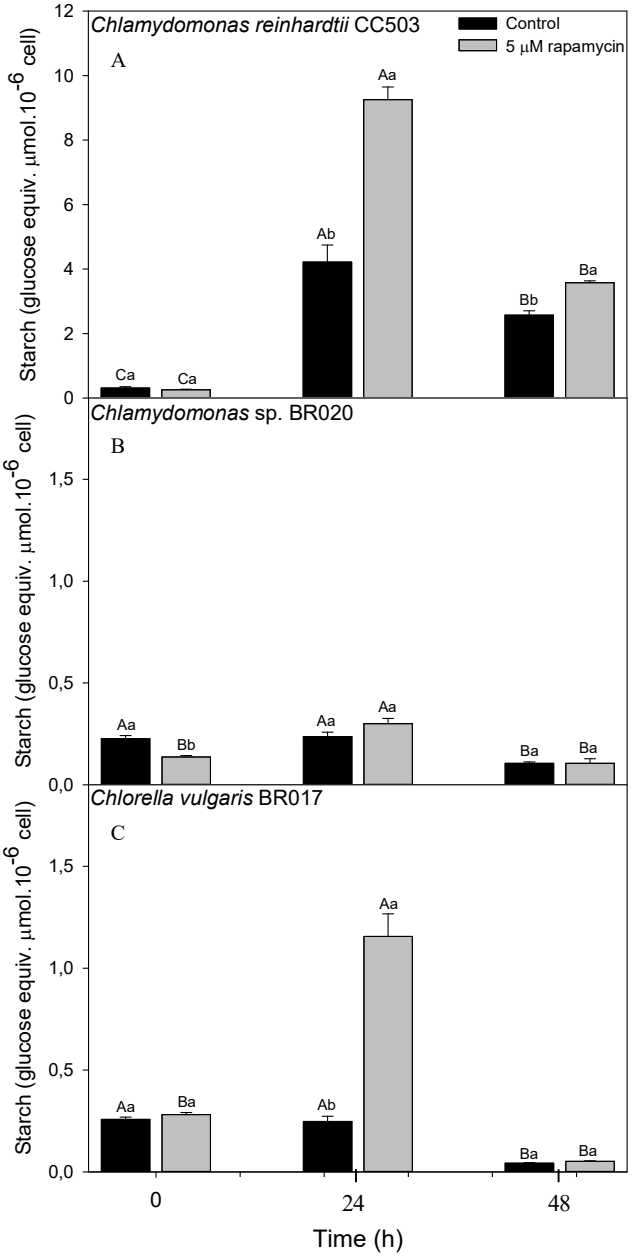


Figure 5. Starch content of three freshwater strains cultured in TAP medium with 5μM rapamycin and control. *Chlamydomonas reinhardtii* CC503 **(A)**; *Chlamydomonas* sp. BR020 **(B)** and *Chlorella vulgaris* BR017 **(C)**. Values represent the average error of eight replications. Means followed by the same letters do not differ by the Tukey test (P<0.05), uppercase letters compare the rapamycin concentration in each strain and lowercase letters compare each treatment in all strains.

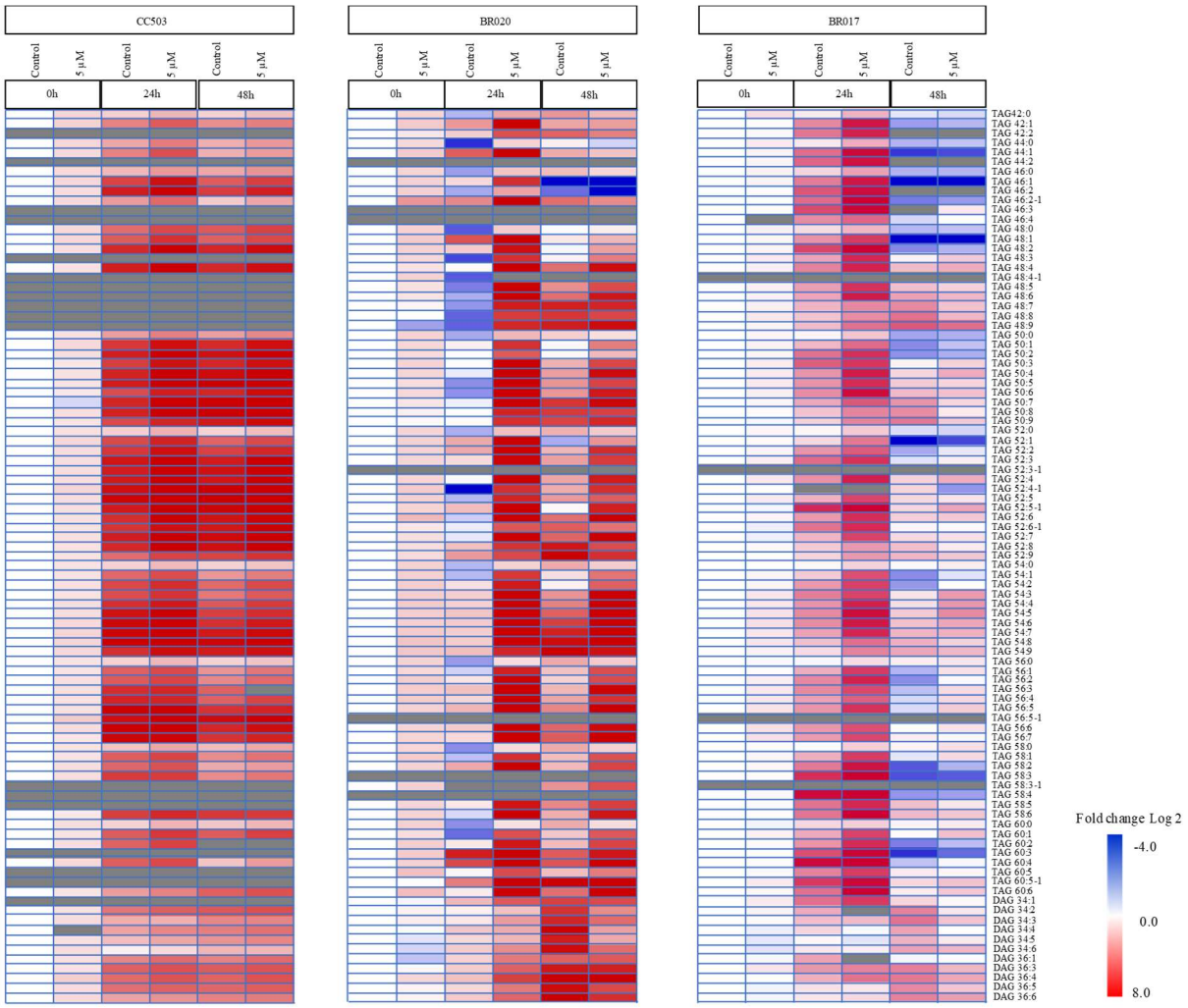


Figure 6. Heat map representing the changes in relative triacylglycerols (TAGs) of three freshwater strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, and *C. vulgaris* BR017) cultured in TAP medium with 5µM rapamycin and control. Data are scaled to the mean response measured for control 0h for each strain, values presented are means of four biological replicates. The complete dataset and statistics are presented in the supplementary table S 16 – 18).

3.6 Lipidomic analysis reveals changes between all strains in the composition of structural lipids

As there were severe changes in the composition of the storage compounds, especially the storage lipids, we hypothesized if the other lipid classes were also affected after rapamycin treatment. To obtain an overview of the data we prepared a heat map, where the colors blue and red mean decrease and increase in relation to control in each growth phase **(Figure 7)**. In total 106 compounds from 8 different lipid classes were determined. Of the identified metabolites 16 belongs to digalactosyldiacylglycerols (DGDGs), 12 monogalactosyldiacylglycerols (MGDGs), 10 sulfoquinovosyl diacylglycerol (SQDGs), 10 Lysophosphatidylcholines (LysoPCs), 20 phosphatidylcholine (PCs), 16 phosphatidylethanolamines (PEs), 5 phosphatidylglycerol (PGs) and 17 Fatty acids (FAs) and lipid profile for the three strains were different **(Figure 7, Table S 16 - 18)**. In keeping with the diverse structure of different algae, the quantitative and qualitative compositions of lipids vary considerably (Harwood and Guschina, 2009; Li-Beisson et al., 2019). In the present study, CC503 strain showed a lower number of lipids compositions (173) in relationship to BR020 (194) and BR017 (195) strains **(Figure 7)**. For CC503 strain, the lipid compositions TAG (10), PC (6), LysoPC (2) and DGDG, MGDG and DAG (1) were not finded.

Most of the chloroplast galactolipids (MGDG, DGDG and SQDG) increased in BR017 and BR020 cells under control conditions at the end of cultivation. Under rapamycin-treatment, only CC503 cells increased at 48 h of cultivation. Three phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), are the major phosphoglycerides identified in most algae species (Borowitzka and Moheimani, 2013). In the present study, we observed that rapamycin treatment did not alter the levels of PC, PE, and PG in CC503 and BR020, but reduced levels of PE were pesent in BR017 cells.

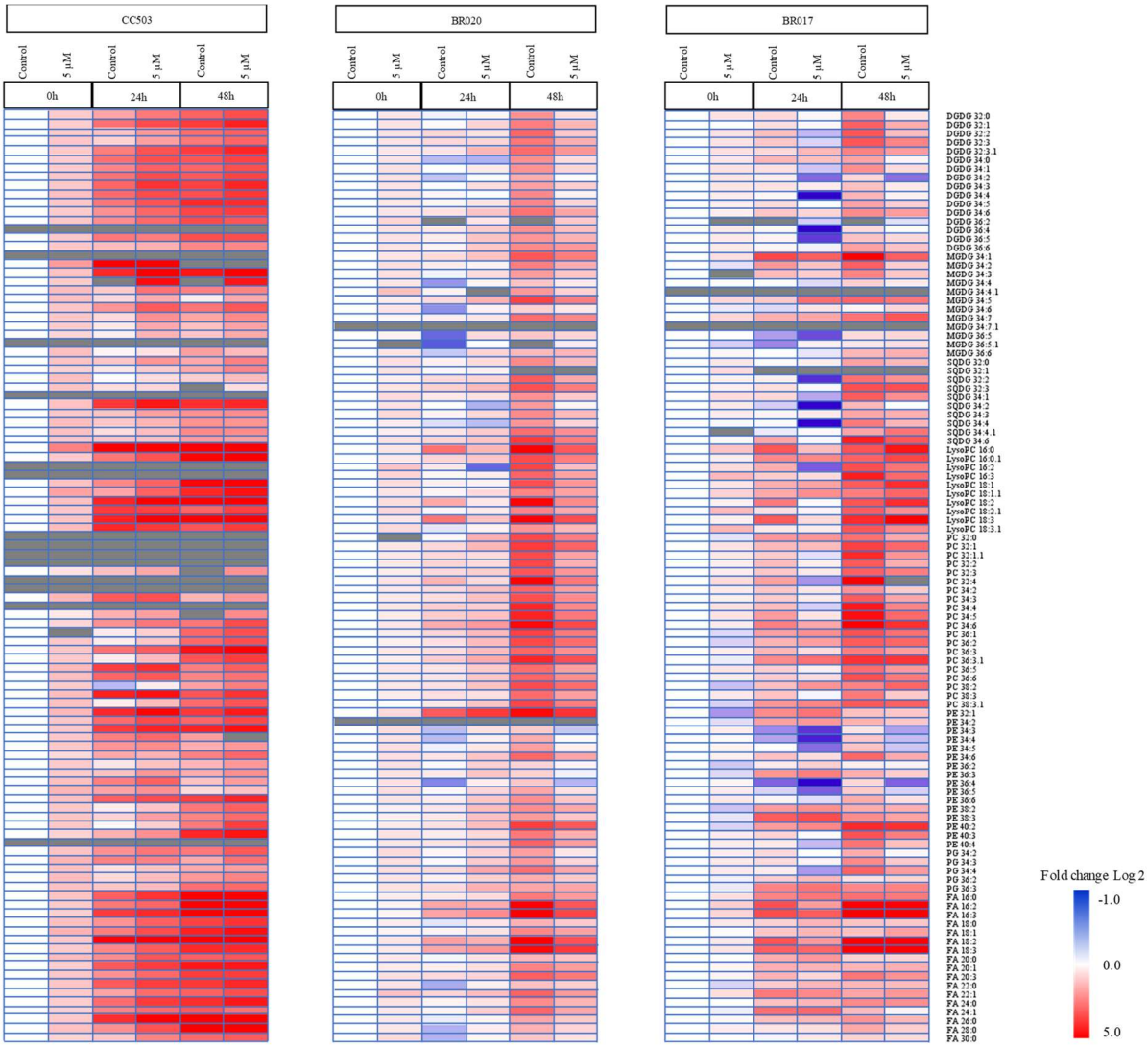
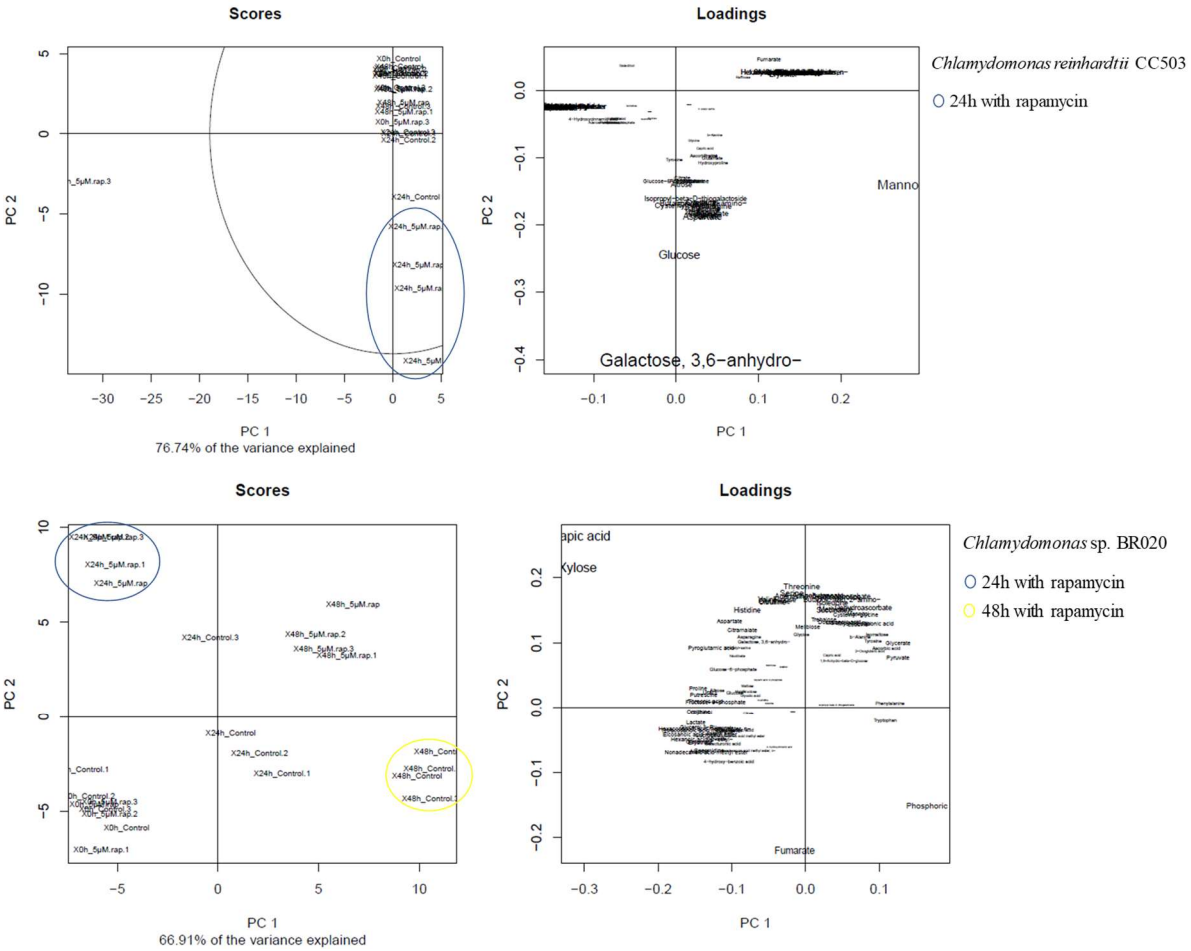


Figure 7. Heat map representing the changes in relative lipid levels contents of three freshwater strains (*C. reinhardtii* CC503, *Chlamydomomas* sp. BR020, and *C. vulgaris* BR017) cultured in TAP medium with 5µM rapamycin and control. Data are scaled to the mean response measured for control 0h for each strain, values presented are means of four biological replicates. The complete dataset and statistics are presented in the supplementary table S 16 – 18).

3.7 Multivariate analysis

Principal component analysis (PCA) was performed to visualize the results between control and rapamycin treatment and thus explore the effect of rapamycin inhibition of the TOR pathway on growth and metabolism of three strains. The primary metabolite PCA **(Figure 8)** shows the difference between the strains. For *C. reinhardtii* CC503 and *C. vulgaris* BR017, the principal components (PC1 and PC2) explained 76.74% and 71.82% of the variance, respectively. The analysis results indicated that the 5µM rapamycin treatment at 24h was clustered because of glucose and galactose, 3, 6-anhydro for *C. reinhardtti* CC503 **(Figure 8A)** while for *C. vulgaris* BR017 was clustered by glutaric acid **(Figure 8C)**. Regarding *Chlamydomonas* sp. BR020, the principal components (PC1 and PC2) explained 66.91% of the variance and two groups were formed. The 5µM rapamycin treatment at 24h was clustered because of sinapic acid and Xylose, while 5µM rapamycin treatment at 48h was clustered by phosphoric acid **(Figure 8B)**.



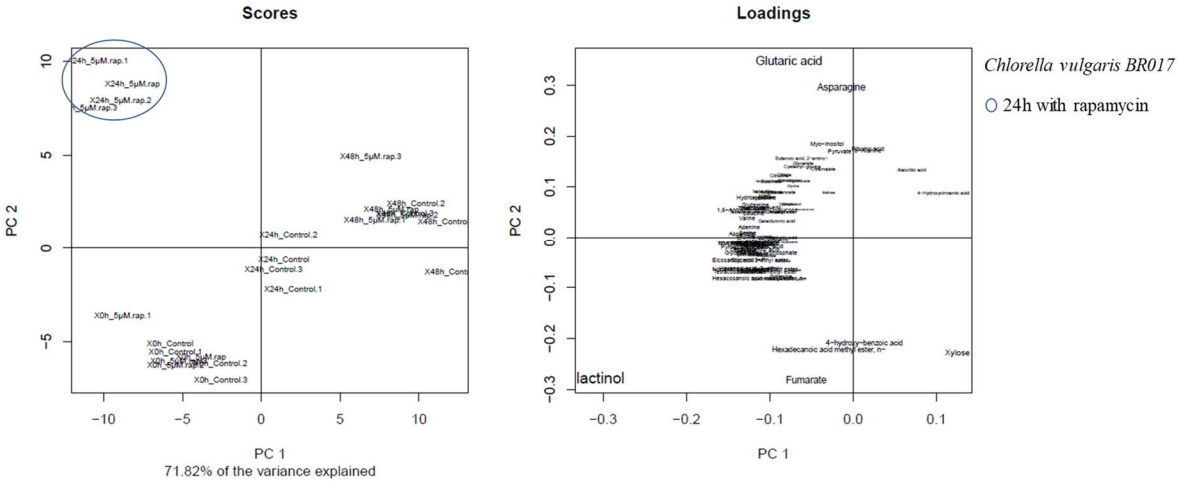


Figure 8. Principal component analysis (PCA) score plot derived data of three freshwater strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, and *C. vulgaris* BR017) cultured in TAP medium with 5µM rapamycin and control in three collect points (0h, 24h and 48h). Primary metabolites profile displayed between 66.91% and 76.74% of the variance explained in the two principal components, PC1 and PC2.

4. DISCUSSION

4.1. TOR inhibition decreases differentialy the cell number in three strains of phylum Chlorophyta

The phenotype of the rapamycin treatment was characterized by a 50% reduction in the total cell number when compared to control **(Figure 2 A - C)**. This partial repression of the cell number is in full agreement with previous studies elucidating the function of TOR in Chlamydomonas (Crespo et al., 2005; Lee and Fiehn, 2013; Kleessen et al., 2015; Couso et al., 2016; Jüppner et al., 2018). Part of the explanation for the partial growth repression was traced back to the fact that the native FKBP12 (FK506 binding protein 12 kDa) protein in Chlamydomonas does not effectively bind rapamycin (Crespo et al., 2005; Pérez-Pérez et al., 2017). The sensitivity of the Chlamydomonas FKBP12 protein to the drug could be increased by two point mutations but, still, contrary to the yeast system, where rapamycin treatment leads to a full growth arrest in G1 cell cycle (Barbet et al., 1996; Oldenhof et al., 2007; Jüppner et al., 2018), Chlamydomonas cells were not fully inhibited in growth (Pancha et al., 2020). To overcome, at least in part, this problem, we used the rapamycin concentration by 10-fold to 5µM, to make sure that we obtained maximal inhibition of the TOR complex **(Figure 2 A - C)**. Our results show that rapamycin inhibits the growth of a photosynthetic organism and strongly suggests the presence of a TOR signaling cascade, not only in the model strain, *C. reinhardtii*, but also in other strains belonging to the same phylum. According to the strain, concentration and time interaction results, *C. reinhardtii* CC503, *Chlamydomonas* sp BR020 and *C. vulgaris* BR017 showed a reduced number of cells between the control and the treatment with rapamycin

(**Table S 12)**. All strains exhibited reduced growth at the end of 48 hours of cultivation with the use of rapamycin **(Figure 2 A - C)**.

4.2. Effect of TOR inhibition on primary metabolism of microalgae cells

Transition from a growing state to a stationary state or vice versa profoundly changes the metabolism of cells. Broadly speaking, there is a metabolic shift from anabolic processes for the *de novo* synthesis of macromolecules (growing or proliferating cells) towards catabolic processes for breakdown of macromolecules to release energy (stationary phase cells) (Mubeen et al., 2018). The TOR kinase has emerged as a signaling node that promotes cell growth and proliferation by the activation of key anabolic processes (Howell et al., 2013). The aim of this study was to describe the metabolic changes within the cell growth of three strains and compare these results to cells where the TOR signaling pathway was inhibited by rapamycin.

The sensitivity of Chlamydomonas to rapamycin has been considered an experimental advantage to explore the function of TOR in a photosynthetic organism. Transcriptomic and metabolomic analyses of Chlamydomonas cells treated with this drug have been recently reported (Lee and Fiehn, 2013; Li et al., 2014; Mubeen et al., 2018). Metabolomic studies revealed that the TCA cycle is largely affected by the inhibition of TOR, and some TCA cycle intermediates including malate, succinate, and citrate accumulated in rapamycin-treated cells (Lee and Fiehn, 2013; Kleessen et al., 2015; Pérez-Pérez et al., 2017). As in the present study **(Figure 4)**, these metabolites had similar behavior and increased in rapamycin treatment. The negative effect on the TCA cycle intermediates is accompanied by the simultaneous down-regulation of some amino acids such as glutamic acid, asparagine, or glutamine in *C. reinhardtii* (Lee and Fiehn, 2013; Pérez-Pérez et al., 2017), which can be observed for the results in figure 4. It has been proposed that the decrease of these amino acids in rapamycin-treated cells can be associated with the activation of nutrient recycling processes such as senescence or autophagy (Lee and Fiehn, 2013; Pérez-Pérez and Crespo, 2017), which are activated by rapamycin. Thus, TOR seems to play a prominent role in the control of primary metabolism in Chlamydomonas, as previously reported in Arabidopsis mutant plants with decreased TOR expression (Xiong and Sheen, 2015; Dobrenel et al., 2016). Metabolomics data also indicated that cysteine pool was strongly affected in CC503, BR020 and BR017 strains cells treated with rapamycin **(Figure 4).** A cysteine and methionine metabolism is needed for the assimilation of sulfur and the synthesis of glutathione, a highly abundant free soluble thiol that maintains the intracellular redox balance in the cell (Foyer and Noctor, 2011). The altered metabolism of cysteine and

methionine in rapamycin-treated cells suggests that proper TOR function might be required to maintain redox homeostasis. In close agreement with this hypothesis, it has been shown that the down-regulation of TOR in Arabidopsis results in the enhanced synthesis of glutathione (Caldana et al., 2013; Ren et al., 2013). Furthermore, pyruvate can be produced by the degradation of the amino acids glycine, alanine, tryptophan, threonine, serine and cysteine (Bhatnagar et al., 2011). As the amino acids are strongly accumulated after rapamycin treatment, they might support the accumulation of pyruvate **(Figure 4)**. Ketogenic amino acids, as leucine, lysine, isoleucine, phenylalanine, tryptophan, tyrosine and threonine can be converted to acetyl-CoA (Johnson and Alric, 2013; Mubeen et al., 2018). Among these, leucine, lysine and the aromatic amino acids (phenylalanine, tryptophan, tyrosine) show a weaker accumulation after rapamycin treatment compared to other amino acids **(Figure 4)**. This might indicate that breakdown of ketogenic amino acids contributes to pools for fatty acid synthesis.

4.3. TOR inhibition by rapamycin changes the carbon partitioning

Starch is one of the main products of photosynthetic carbon fixation and serves as a transient carbon reserve for the night. It has already been shown that Chlamydomonas cells, grown under a light-dark regime, accumulate starch within the chloroplast during the light and degrade it in the dark (Klein, 1987; Batista et al., 2019; Covell et al., 2020). The accumulation of starch was accompanied by a decrease of hexoses phosphate, as glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), during the cultivation **(Figure 4)**. This is in agreement with observation made by Lee and Fiehn in Chlamydomonas after TOR inhibition (Lee and Fiehn, 2013) and was also reported for artificial micro RNA lines of TOR (amiR-tor) in Arabidopsis (Caldana et al., 2013). After starch, non-membrane forming neutral lipids as the TAGs represent a major storage for highly reduced carbon and energy (Johnson and Alric, 2013). It has been shown that plant leaves typically do not accumulate high amounts of storage lipids, but they do in fact synthesize significant amounts of TAGs during the day and degrade them during the night (Lin and Oliver, 2008; Caldana et al., 2013). The results show that similar to starch TAGs can increase during the light phase **(Figure 6)**.

In agreement with results from yeast (Barbet et al., 1996; Schmelzle et al., 2004), fruit fly (Teleman et al., 2005; Bjedov et al., 2010) and Arabidopsis (Caldana et al., 2013; Mubeen et al., 2019), we found that the inhibition of TOR in the all strains leads to an increased accumulation of starch **(Figure 5)** and TAGs **(Figure 6)**. It has been already shown in Chlamydomonas cells subjected to N starvation that the TAG accumulation lags behind that of

starch (Fan et al., 2012; Himanshu et al., 2016). Interestingly, it was also reported that rapid TAG synthesis occurs only when carbon supply exceeds the capacity of starch synthesis (Smith and Gilmour, 2018). Therefore, our results might imply that TOR has a dominant role in the regulation of TAG biosynthesis and in the regulation of starch.

4.4. TOR inhibition affects membrane lipid composition

Studies published over the last few years have shown that TOR controls lipogenesis through the regulation of the transcription factor sterol regulatory element-binding protein1 (SREBP1) and the phosphatidic acid phosphatase LIPIN1 (Porstmann et al., 2008; Peterson et al., 2011; Lamming and Sabatini, 2013). Beside the strong accumulation of TAG, the analysis of lipids revealed that TOR inhibition in Chlamydomonas strongly affects the composition of membrane lipids on the level of saturation and fatty acid chain length (Jüppner et al., 2017; Pancha et al., 2020). Several studies mainly focused on lipid content as a way to evaluate the nutritional value of different microalgal species (Phong et al., 2018; Silva et al., 2019; Kumar et al., 2020; Amorim et al., 2021; Silva et al., 2021).

The major plastid lipids, galactosylglycerides, are polar lipids. In plants, MGDG and DGDG account for 40–55% and 15–35% of the total lipids in thylakoid membranes, respectively (Harwood, 2006; Borowitzka and Moheimani, 2013). The identified lipid classes in this study are presented in a heat map **(Figure 6 and 7)**, and the largest number of annotated lipid species belong to the class of structural polar glycosylglycerides MGDGs, DGDGs and SQDGs and the neutral storage, glycerolipids of the TAG class. Furthermore, we found that with minor exceptions the most abundant lipid species within the membrane lipids consists of 34 carbons in their two fatty acids, most of which are polyunsaturated. This was not surprising as it has already been shown that the dominant chain lengths of the total fatty acid composition in Chlamydomonas were C16 and C18 (Giroud et al., 1988; Harwood, 2006; Huerlimann et al., 2010; Li-Beisson et al., 2019). Plastid galactolipids are characterized by a very high content of polyunsaturated fatty acids (Giroud et al., 1988; Harwood, 2006). The glycolipids from some algal species, e.g. green algae *Trebouxia* spp., *Coccomyxa* spp., *Chlamydomonas* spp., *Scenedesmus* spp., may also be esterified with unsaturated C16 acids, such as hexadecatrienoic (C16:3n-3/C16:3n-2) and hexadecatetraenoic (C16:4) (Harwood and Guschina, 2009; Huerlimann et al., 2010; Li-Beisson et al., 2019). Three phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), are the major phosphoglycerides identified in most algae species (Borowitzka and Moheimani, 2013). In the

present work, it was possible to find 41 of those phospholipids **(Figure 7)**, practically half of the total lipids. The phospholipids are located in the extrachloroplast membranes with the exception of PG. This phospholipid is present in substantial quantities in thylakoid membranes. PG accounts for around 10 and 20% of the total polar glycerolipids in eukaryotic green algae (Harwood and Guschina, 2009; Borowitzka and Moheimani, 2013).

5. CONCLUSION

Cells of *C. reinhardtii* CC503 and native Brazilian strains, *Chlamydomonas* sp. BR020 and *C. vulgaris* BR017, were used to analyze the impact of TOR inhibition on cell growth. The results from this study clearly show that TOR inhibition causes a severely reduced growth, leading to a reduction by 50% on the cell growth. The present data therefore reveals that TOR is involved in the regulation of cell growth in different strains.

Next to the direct cell growth related results our data reveals that the obtained growth-phenotype was accompanied by strong accumulation of carbon (starch and TAGs) reserves. The amino acids profile exhibited a diverse pattern in relationship to the strains, which will aid in specifying the role and mechanism of individual compounds in the response to the rapamycin treatment. Taken together, the present data reveals a clear metabolite reprograming in response to TOR inhibition in a strain specific mode **(Figure 9)**.

Therefore, further analyses using *C. reinhardtii*, together with other algae, will provide important information on the regulatory mechanism of reserve compounds accumulation and TOR signaling pathway. Furthermore, such data will be useful for the construction of algae being targeted to specific goals.

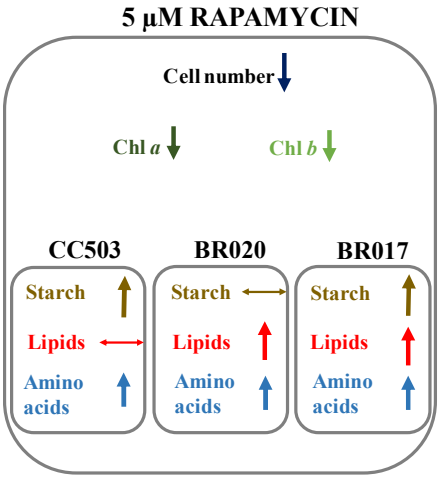


Figure 9. Summary representation of principal responses in *Chlamydomonas reinhardtii* CC503, *Chlamydomonas* sp. BR020 and *Chlorella vulgaris* BR017 according to the rapamycin treatment compared to control. Up arrows indicate increase, down arrows indicate decrease and vertical double arrows indicate there were no difference. The thickness of the arrows was related to the values of compounds or parameters evaluated.

6. REFERENCES:

Amorim ML, Soares J, Coimbra JS dos R, Leite M de O, Albino LFT, Martins MA (2021) Microalgae proteins: production, separation, isolation, quantification, and application in food and feed. Crit Rev Food Sci Nutr **61**: 1976–2002

Bar-nun S, Ohad I (1980) the accuracy of sometimes reported chloroplastic Mn abundance deter- minations based only on a presumed specific activity of Mn in the growth media. **69**: 363–374

Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF, Hall MN (1996) TOR controls translation initiation and early G1 progression in yeast. Mol Biol Cell **7**: 25–42

Batista AD, Rosa RM, Machado M, Magalhães AS, Shalaguti BA, Gomes PF, Covell L, Vaz MGMV, Araújo WL, Nunes-Nesi A (2019) Increased urea availability promotes adjustments in C/N metabolism and lipid content without impacting growth in *Chlamydomonas reinhardtii*. Metabolomics. doi: 10.1007/s11306-019-1496-3

Bhatnagar A, Chinnasamy S, Singh M, Das KC (2011) Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. Appl Energy **88**: 3425–3431

Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, Partridge L (2010) Mechanisms of Life Span Extension by Rapamycin in the Fruit Fly Drosophila melanogaster. Cell Metab **11**: 35–46

Borowitzka MA, Moheimani NR (2013) Algae for biofuels and energy. Algae for Biofuels and Energy 1–288

Bromke MA, Giavalisco P, Willmitzer L, Hesse H (2013) Metabolic Analysis of Adaptation to Short-Term Changes in Culture Conditions of the Marine Diatom Thalassiosira pseudonana. PLoS One **8**: 1–11

Caldana C, Li Y, Leisse A, Zhang Y, Bartholomaeus L, Fernie AR, Willmitzer L, Giavalisco P (2013) Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in Arabidopsis thaliana. Plant J **73**: 897–909

Contois DE (1959) Kinetics of Bacterial Growth: Relationship between Population Density and Specific Growth Rate of Continuous Cultures. J Gen Microbiol **21**: 40–50

Couso I, Evans BS, Li J, Liu Y, Ma F, Diamond S, Allen DK, Umen JG (2016) Synergism between inositol polyphosphates and TOR kinase signaling in nutrient sensing, growth control, and lipid metabolism in Chlamydomonas. Plant Cell **28**: 2026–2042

Covell L, Machado M, Vaz MGMV, Soares J, Batista AD, Araújo WL, Martins MA, Nunes-Nesi A (2020) Alternative fertilizer-based growth media support high lipid contents without growth impairment in *Scenedesmus obliquus* BR003. Bioprocess Biosyst Eng **43**: 1123–1131

Crespo JL, Díaz-Troya S, Florencio FJ (2005) Inhibition of target of rapamycin signaling by rapamycin in the unicellular green alga *Chlamydomonas reinhardtii*. Plant Physiol **139**: 1736–1749

Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, Veit B, Meyer C (2016) TOR Signaling and Nutrient Sensing. Annu Rev Plant Biol **67**: 261–285

Fan J, Yan C, Andre C, Shanklin J, Schwender J, Xu C (2012) Oil accumulation is controlled by carbon precursor supply for fatty acid synthesis in *Chlamydomonas reinhardtii*. Plant Cell Physiol **53**: 1380–1390

Foyer CH, Noctor G (2011) Ascorbate and glutathione: The heart of the redox hub. Plant Physiol **155**: 2–18

Giroud C, Gerber A, Eichenberger W (1988) Lipids of *Chlamydomonas reinhardtii*. Analysis of molecular species and intracellular site(s) of biosynthesis. Plant Cell Physiol **29**: 587–595

Grace SC, Hudson DA (2016) Processing and Visualization of Metabolomics Data Using R. Metabolomics - Fundam Appl. doi: 10.5772/65405

Griffiths MJ, Garcin C, van Hille RP, Harrison STL (2011) Interference by pigment in the estimation of microalgal biomass concentration by optical density. J Microbiol Methods **85**: 119–123

Harwood JL (2006) Membrane Lipids in Algae. Lipids Photosynth Struct Funct Genet 53–64

Harwood JL, Guschina IA (2009) The versatility of algae and their lipid metabolism. Biochimie **91**: 679–684

Himanshu S, Manish RS, Basuthkar JR, Kandala VRC (2016) Regulation of starch, lipids and amino acids upon nitrogen sensing in *Chlamydomonas reinhardtii*. Algal Res **18**: 33–44

Howell JJ, Ricoult SJH, Ben-Sahra I, Manning BD (2013) A growing role for mTOR in promoting anabolic metabolism. Biochem Soc Trans **41**: 906–912

Huerlimann R, de Nys R, Heimann K (2010) Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnol Bioeng **107**: 245–257

Hummel J, Segu S, Li Y, Irgang S, Jueppner J, Giavalisco P (2011) Ultra performance liquid chromatography and high resolution mass spectrometry for the analysis of plant lipids. Front Plant Sci **2**: 1–17

J. Fan CA (2011) A Chloroplast pathway for the de novo biosynthesis of triacylglycerol in *Chlamydomonas reinhardtii*. 1985–1991

Johnson X, Alric J (2013) Central carbon metabolism and electron transport in *Chlamydomonas reinhardtii*: Metabolic constraints for carbon partitioning between oil and starch. Eukaryot Cell **12**: 776–793

Jüppner J, Mubeen U, Leisse A, Caldana C, Brust H, Steup M, Herrmann M, Steinhauser D, Giavalisco P (2017) Dynamics of lipids and metabolites during the cell cycle of *Chlamydomonas reinhardtii*. Plant J **92**: 331–343

Jüppner J, Mubeen U, Leisse A, Caldana C, Wiszniewski A, Steinhauser D, Giavalisco P (2018) The target of rapamycin kinase affects biomass accumulation and cell cycle progression by altering carbon/nitrogen balance in synchronized *Chlamydomonas reinhardtii* cells. Plant J **93**: 355–376

Kleessen S, Irgang S, Klie S, Giavalisco P, Nikoloski Z (2015) Integration of transcriptomics and metabolomics data specifies the metabolic response of Chlamydomonas to rapamycin treatment. Plant J **81**: 822–835

Klein U (1987) Intracellular Carbon Partitioning in *Chlamydomonas reinhardtii* . Plant Physiol **85**: 892–897

Kumar G, Shekh A, Jakhu S, Sharma Y, Kapoor R, Sharma TR (2020) Bioengineering of Microalgae: Recent Advances, Perspectives, and Regulatory Challenges for Industrial Application. Front Bioeng Biotechnol. doi: 10.3389/fbioe.2020.00914

Lamming DW, Sabatini DM (2013) A central role for mTOR in lipid homeostasis. Cell Metab **18**: 465–469

Lee DY, Fiehn O (2013) Metabolomic response of *Chlamydomonas reinhardtii* to the s inhibition of target of rapamycin (tor) by rapamycin. J Microbiol Biotechnol **23**: 923–931

Li-Beisson Y, Thelen JJ, Fedosejevs E, Harwood JL (2019) The lipid biochemistry of eukaryotic algae. Prog Lipid Res **74**: 31–68

Li J, Kim SG, Blenis J (2014) Rapamycin: One drug, many effects. Cell Metab **19**: 373–379

Lin W, Oliver DJ (2008) Role of triacylglycerols in leaves. Plant Sci **175**: 233–237

Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry–based metabolite profiling in plants. Nat Protoc **1**: 387–396

Mubeen U, Giavalisco P, Caldana C (2019) TOR inhibition interrupts the metabolic homeostasis by shifting the carbon-nitrogen balance in *Chlamydomonas reinhardtii*. Plant Signal Behav **14**: 1670595

Mubeen U, Jüppner J, Alpers J, Hincha DK, Giavalisco P (2018) Target of rapamycin inhibition in *Chlamydomonas reinhardtii* triggers de novo amino acid synthesis by enhancing nitrogen assimilation[open]. Plant Cell **30**: 2240–2254

Oldenhof H, Zachleder V, Van Den Ende H (2007) The cell cycle of *Chlamydomonas reinhardtii*: The role of the commitment point. Folia Microbiol (Praha) **52**: 53–60

Pancha I, Chokshi K, Tanaka K, Imamura S (2020) Microalgal Target of Rapamycin (TOR): A Central Regulatory Hub for Growth, Stress Response and Biomass Production. Plant Cell Physiol **61**: 675–684

Pérez-Pérez ME, Couso I, Crespo JL (2017) The TOR signaling network in the model unicellular green alga *Chlamydomonas reinhardtii*. Biomolecules **7**: 1–13

Pérez-Pérez ME, Crespo JL (2017) Monitoring Autophagy in the Model Green Microalga *Chlamydomonas reinhardtii*. Cells **6**: 36

Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, Balderas E, Guertin DA, Madden KL, Carpenter AE, Finck BN, et al (2011) MTOR complex 1 regulates lipin 1 localization to control the srebp pathway. Cell **146**: 408–420

Phong WN, Show PL, Ling TC, Juan JC, Ng EP, Chang JS (2018) Mild cell disruption methods for bio-functional proteins recovery from microalgae—Recent developments and future perspectives. Algal Res **31**: 506–516

Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung YL, Schulze A (2008) SREBP Activity Is Regulated by mTORC1 and Contributes to Akt-Dependent Cell Growth. Cell Metab **8**: 224–236

Ren M, Venglat P, Qiu S, Feng L, Cao Y, Wang E, Xiang D, Wang J, Alexander D, Chalivendra S, et al (2013) Target of rapamycin signaling regulates metabolism, growth, and life Span in arabidopsis. Plant Cell **24**: 4850–4874

Salem MA, Jüppner J, Bajdzienko K, Giavalisco P (2016) Protocol: A fast, comprehensive and reproducible one-step extraction method for the rapid preparation of polar and semi-polar metabolites, lipids, proteins, starch and cell wall polymers from a single sample. Plant Methods **12**: 1–15

Schmelzle T, Beck T, Martin DE, Hall MN (2004) Activation of the RAS/Cyclic AMP Pathway Suppresses a TOR Deficiency in Yeast. Mol Cell Biol **24**: 338–351

Silva MET da, Correa K de P, Martins MA, da Matta SLP, Martino HSD, Coimbra JS dos R (2019) Food safety, hypolipidemic and hypoglycemic activities, and in vivo protein quality of microalga *Scenedesmus obliquus* in Wistar rats. J Funct Foods 103711

Silva MET da, Leal MA, Resende M de O, Martins MA, Coimbra JS dos R (2021) *Scenedesmus obliquus* protein concentrate: A sustainable alternative emulsifier for the food industry. Algal Res. doi: 10.1016/j.algal.2021.102468

Smith AM, Zeeman SC (2006) Quantification of starch in plant tissues. Nat Protoc **1**: 1342–1345

Smith RT, Gilmour DJ (2018) The influence of exogenous organic carbon assimilation and photoperiod on the carbon and lipid metabolism of *Chlamydomonas reinhardtii*. Algal Res **31**: 122–137

Stacklies W, Redestig H, Scholz M, Walther D, Selbig J (2007) pcaMethods - A bioconductor package providing PCA methods for incomplete data. Bioinformatics **23**: 1164–1167

Teleman AA, Chen YW, Cohen SM (2005) Drosophila melted modulates FOXO and TOR activity. Dev Cell **9**: 271–281

Xiong Y, Sheen J (2015) Novel links in the plant TOR kinase signaling network. Curr Opin Plant Biol **28**: 83–91

CHAPTER 3

TITLE: The Target of Rapamycin (TOR) – a central growth regulator in microalgae

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ABSTRACT

The central role that microalgae play in global biomass production, together with the high biotechnological potential of these organisms in industrial sectors like agriculture or biofuel production has drawn attention to the study of proteins that regulate its cell growth including the Target of rapamycin (TOR) kinase. This protein is an evolutionarily conserved protein kinase which plays an important role in the regulation of cell growth and the sensing of nutrient and energy status in eukaryotes. TOR functions in two distinct multiprotein complexes termed TOR complex 1 (TORC1) and TOR complex 2 (TORC2). While the structure and functions of TORC1 are highly conserved in all eukaryotes, including algae and plants, TORC2 core proteins seems to be missing in photosynthetic organisms. In yeasts and mammals, the roles of TOR have been already well described. Several functions of TOR signaling in plant lineages have also been revealed during the last years. In microalgae, most of functions of TOR have been primarily studied in the model green alga *Chlamydomonas reinhardtii*. Indeed, the development of tools for the functional analysis of TOR have helped to reveal the involvement of TOR in microalgae in many functions, including transcription, translation, accumulation of energy storage molecules, autophagy, etc. In this review, we discuss recent findings relating to TOR signaling and its roles in microalgae.

Keywords: Microalgae. Cell growth. Metabolism. Signaling pathway.

1 INTROCUCTION

The amount and quality of nutrients regulate cell growth and development in all living organisms (Dobrenel et al., 2016a; Pérez-Pérez et al., 2017). This modulation is a central process in organisms and there is a specific relationship between nutrient availability, energy state and cell growth rate, influenced by stresses and various environmental challenges (Wullschleger, 2006; Dobrenel et al., 2013; Xiong and Sheen, 2014). Availability of nutrients, energy, oxygen and stress signals determine the transition and balance between anabolic and catabolic processes (Dibble and Manning, 2013; Howell et al., 2013; Shimobayashi and Hall, 2014). In eukaryotes, nutrient availability and growth output have evolved different signaling pathways (Chantranupong et al., 2015; Efeyan et al., 2015). One of such highly conserved signaling cascades is Target of Rapamycin (TOR) kinase (Crespo and Hall, 2002; Jewell and Guan, 2013; Yuan et al., 2013).

The TOR kinase related pathway controls cell growth by promoting anabolic processes, including protein synthesis, transcription and ribosome biogenesis, and inhibiting catabolic processes such as autophagy and mRNA degradation (Díaz-Troya et al., 2011; Loewith and Hall, 2011; Ingargiola et al., 2020). In yeasts and mammals, TOR is composed of two structurally and functionally different protein complexes: TOR complex 1 (TORC1) and TOR complex 2 (TORC2), and core components of these complexes are, like TOR, evolutionarily conserved. TORC1 mainly responds to the nutrient and energy status of cells and accordingly alters their growth and metabolism (Wullschleger, 2006; Hall, 2016), while TORC2 is mainly responsible for the regulation of the cytoskeleton and other cellular processes through phosphorylation of several targets (Maegawa et al., 2015; Gaubitz et al., 2016; Saxton and Sabatini, 2017). TORC1 is sensitive to the drug rapamycin, while TORC2 is not (Loewith et al., 2002; Pérez-Pérez and Crespo, 2017). The macrolide antibiotic rapamycin is produced by the bacterium *Streptomyces hygroscopicus* and was originally identified as a potent antifungal agent with immunosuppressive activity and anticancer properties (Vézina and Kudelski, 1975; Pérez-Pérez and Crespo, 2010b).

Target of Rapamycin (TOR) is a conserved serine/threonine-protein kinase, member of the phosphatidylinositol 3-kinase-related kinase family (Heitman et al., 1991) and was originally identified by mutations, TOR1-1 and TOR2-1, that confer resistance to the growth inhibitory properties of rapamycin, in the budding yeast *Saccharomyces cerevisiae* (Heitman et al., 1991).

Rapamycin first binds the FKBP12 (FK506-binding protein of 12 kDa) immunophilin, and this complex specifically inhibits TOR function by restricting the access of substrates to the (FRB) domain in TOR, adjacent to the catalytic kinase domain (Wullschleger, 2006; Ren et al., 2013b).

Every eukaryote genome examined (including yeasts, algae, slime mold, plants, worms, flies, and mammals) contains a TOR gene. Unlike yeast, which in some cases possess two TOR genes, eukaryotes possess only a single TOR gene (Loewith et al., 2002; Laplante and Sabatini, 2012; Gaubitz et al., 2015). Heitman et al. (1991) succeeded to identify two target genes, namely TOR1 and TOR2 by employing a genetic mutant screening in the budding yeast *S. cerevisiae*. Whereas rapamycin treatment leads to a growth arrest at G1 state in growing yeast, the two mutants affected in either TOR1 or TOR2 did not show any growth phenotype. This result provided the starting point for the investigation and elucidation of the function and regulation of TOR and leads to the identification of TOR homologues in all eukaryotic organisms, including fungi, mammals, worms, flies, plants, and algae (Crespo and Hall, 2002; Menand et al., 2002; Porstmann et al., 2008; Shimobayashi and Hall, 2014).

Although rapamycin potently inhibits cell growth in most eukaryotes, the vegetative growth of Arabidopsis and other plants such as *Oryza sativa*, *Nicotiana tabacum*, or *Brassica napus* is not sensitive to rapamycin (Menand et al., 2002) due to the inability of plant FKBP12 to form a stable complex with this drug (Xu et al., 1998; Sormani et al., 2007; Xiong and Sheen, 2012). Unlike plants, the growth of the unicellular green alga *Chlamydomonas reinhardtii* is sensitive to rapamycin, indicating the presence of a rapamycin-sensitive TOR signaling cascade in this photosynthetic organism (Crespo et al., 2005; Prioretti et al., 2019). The sensitivity of Chlamydomonas cells to rapamycin, together with the easy manipulation of this organism, its simple life cycle, and a growing array of genetic and molecular tools (Harris, 2001b; Cross and Umen, 2015; Imamura et al., 2016), have positioned this green alga as a convenient model system to investigate the TOR network in photosynthetic eukaryotes.

Several new TOR functions and signaling pathways have since been revealed in various microalgae by a few research groups using different comprehensive omics tools and other analyses (Mukaida et al., 2016; Imamura et al., 2018; Pancha et al., 2020; Prioretti et al., 2020). The central role that this microalga and native Brazilian strains, play in global biomass production, together with their high biotechnological potential in biofuel generation, animal food, and pharmacology, has boosted our research focused on improving cell growth and better understanding of metabolic pathways.

2 TOR COMPLEXES

Unlike most eukaryotes, *S. cerevisiae* has two TOR genes named TOR1 and TOR2. The TOR kinase resides in two distinct multiprotein complexes, termed TORC1 and TORC2 (Loewith et al., 2002). TORC1 and TORC2 differ in protein composition, although some proteins are common in both complexes (Helliwell et al., 1994; Pérez-Pérez and Crespo, 2010b). In addition to TOR kinase, a TOR-interacting protein, LST8 protein, is present in all TORC1 and TORC2 complexes described (Loewith et al., 2002; Kim et al., 2003; Wedaman et al., 2003; Hayashi et al., 2007; Matsuo et al., 2007). Together with TOR kinase and LST8 is composed of regulatory-associated protein of TOR (RAPTOR) or also known as Kontroller of Growth 1 (KOG1) (Moreau et al., 2012; Saxton and Sabatini, 2017), whereas the core of TORC2 contains AVO1/hSIN1 and AVO3/RICTOR, the rapamycin-insensitive companion of TOR complex (Gaubitz et al., 2016; Burkart and Brandizzi, 2021).

Mammals also have two TOR-containing complexes but only one gene encoding mTOR. mTOR complex 1 (mTORC1) consists of raptor, mLST8, PRAS40, and Deptor (Laplante and Sabatini, 2012; Tulin et al., 2021). mTOR is a serine/threonine protein kinase that responds to a variety of environmental cues, including nutrient, energy, and growth factor levels, as well as diverse forms of stress, to regulate many anabolic and catabolic processes (Howell et al., 2013; Kim et al., 2013; Prioretti et al., 2019).

In both yeasts and mammals, the FKBP12-rapamycin complex targets and inhibits TORC1 but curiously not TORC2 (Hara et al., 2002; Kim et al., 2002; Schmelzle et al., 2004). A thorough analysis of the molecular architecture of TORC2 revealed that AVO3, a subunit unique of this complex, could mask the FKBP12-rapamycin-binding (FRB) domain of TOR2, thereby conferring insensitivity to inhibition by FKBP12-rapamycin (Figure 1A and B) (Gaubitz et al., 2015; Gaubitz et al., 2016; McCready et al., 2020).

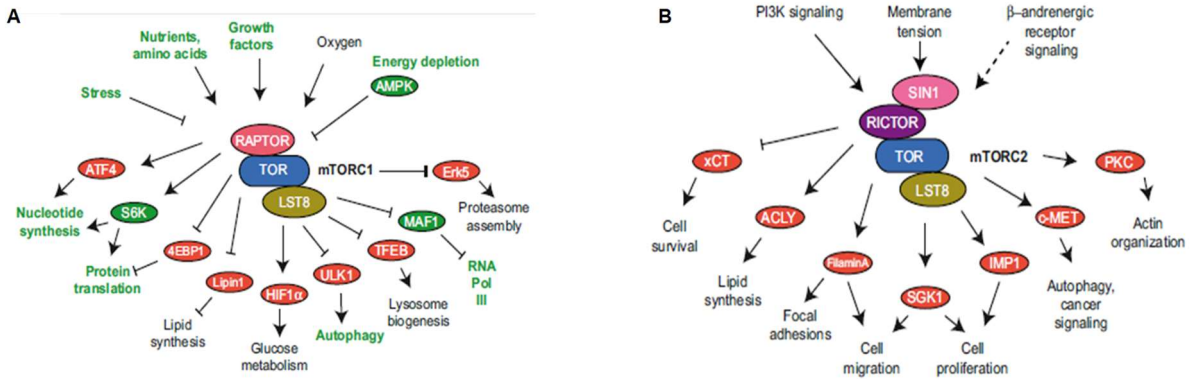

Figure 1. Overview of Mammalian Target of Rapamycin Complex (mTORC) Signaling. **(A)** Upstream activators/repressors and downstream effectors of mTORC1 regulate numerous metabolic and catabolic processes. Activators, effectors, and processes in green are conserved TOR-regulated processes in plants. **(B)** Signaling through mTORC2 modulates diverse cellular functions. Abbreviations: LST8, lethal with SEC13 protein 8; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; RAPTOR, regulatory-associated protein of mTOR; S6K1, S6 KINASE β-1; RICTOR, rapamycin-insensitive companion of mTOR; SIN1, SAPK-interacting protein 1 (Burkart and Brandizzi, 2021).

The current knowledge about control of cell growth by TOR in photosynthetic organisms is limited compared to other eukaryotes. The TOR kinase has been identified in the model photosynthetic systems *Arabidopsis thaliana* and *Chlamydomonas reinhardtii* (Pérez-Pérez and Crespo, 2010a). The high degree of conservation of TOR proteins throughout the eukaryotic kingdom was leading to the identification of a TOR homolog in plants (Menand et al., 2002; Dobrenel et al., 2011) and a subsequent identification of TOR in *C. reinhardtii* (Crespo et al., 2005; Pérez-Pérez and Crespo, 2010b), including freshwater and marine species from the Chlorophyta, Rhodophyta and Chromalveolata clades (Shemi et al., 2015).

The presence of a single RAPTOR gene and a functional LST8 gene also seems to be conserved in algae, LST8 was copurified with TOR and FKBP12 in the presence of rapamycin, suggesting its involvement in the formation of rapamycin-sensitive TORC1 (Díaz-Troya et al., 2008a). In addition, CrTOR and CrLST8 exist in high-molecular-mass complexes that associate with microsomal membranes in high abundance in the peri-basal body region (Loewith et al., 2002; Li et al., 2014). Unlike TORC1 components, the TORC2-specific proteins AVO1/hSIN1 and AVO3/Rictor seem to be lost in algae and plants, it remains unknown whether TORC2 is also structurally conserved in photosynthetic organisms (Figure 2A and B) (Dobrenel et al., 2011; Pérez-Pérez and Crespo, 2017; Brunkard, 2020). However, it is possible that algae and plants may have a TOR complex functionally similar to TORC2 constituted by highly divergent proteins not seen in other eukaryotes (Díaz-Troya et al., 2011; Burkart and Brandizzi, 2021).

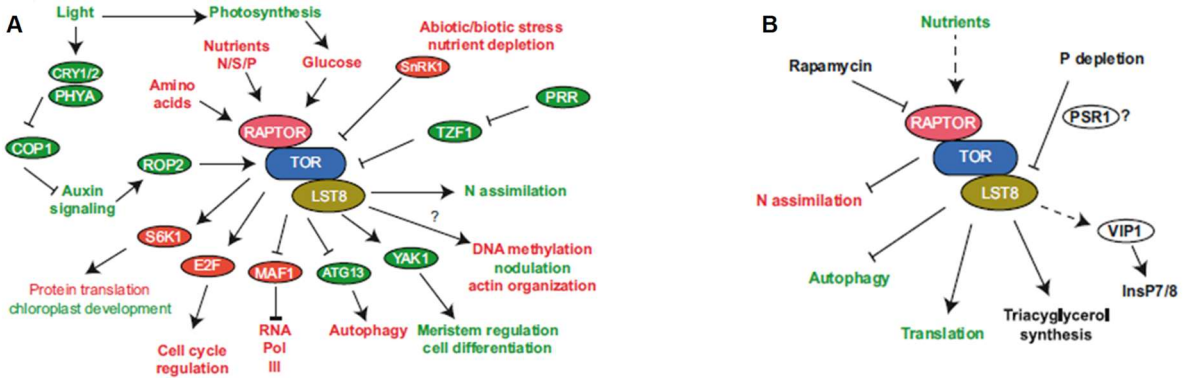

Figure 2. Summary of Plant Target of Rapamycin (TOR) Signaling in *Arabidopsis thaliana and Chlamydomonas reinhardtii.* **(A)** Inputs and mechanisms for the activation/inhibition of TOR signaling and the downstream processes regulated by TOR in *A. thaliana*. Activators/inhibitors, effectors, and cellular processes in red are conserved with mammalian TOR signaling. Those in green are plant specific. **(B)** Inputs and mechanisms for the activation/inhibition of TOR signaling and the downstream processes regulated by TOR in *C. reinhardtii*. Activators/inhibitors, effectors, and cellular processes in green are conserved with arabidopsis TOR signaling. Nitrogen assimilation in *C. reinhardtii* is highlighted in red because TOR appears to have the opposite regulation on this process compared with arabidopsis. Abbreviations: ATG, autophagy-related protein; COP1, CONSTITUTIVE PHOTOMORPHOGENESIS 1; LST8, lethal with sec13 protein 8; N, nitrogen; P, phosphorus; PRR, pseudo response regulators; PSR1, phosphate starvation regulator protein 1; RAPTOR, regulatory-associated protein of mTOR; ROP2, RHO OF PLANTS 2; S, sulfur; S6K1, S6 KINASE β-1; YAK1, yet another kinase 1 (Burkart and Brandizzi, 2021).

3 TOR COMPLEXES – CELULAR LOCALIZATION

Yeast and mammalian TORs have been detected in different cellular compartments, including plasma, vacuolar, and ER membranes, as well as in the nucleus, the Golgi apparatus and the mitochondria, and despite significant discrepancies, these studies indicate that TOR complexes are primarily membrane associated (Wedaman et al., 2003; Walther, 2009; Gaubitz et al., 2016). Biochemical fractionation and immunofluorescence studies of Chlamydomonas cells have revealed that CrTOR and CrLST8 are peripherally associated to internal membranes, suggesting that TOR complexes may localize on these membranous sites (Díaz-Troya et al., 2008a).

Both CrTOR and CrLST8, in Chlamydomonas cells, have a common localization at the peribasal body region, which is enriched in membranes (Díaz-Troya et al., 2008a). However, CrTOR is also detected in discrete punctae that are abundant in regions adjacent to the plasma membrane, while CrLST8 is more abundant around the nucleus (Pérez-Pérez and Crespo, 2010b; Pérez-Pérez et al., 2017). Like in other evolutionarily distant eukaryotes (Drenan et al., 2004; Barquilla et al., 2008), the link of TORC proteins to ER membranes in Chlamydomonas indicates the important role that this cellular compartment may play in TOR signaling.

4 EFFECTS OF RAPAMYCIN ON METABOLISM

The sensitivity of Chlamydomonas to rapamycin has been considered an experimental advantage to explore the function of TOR in a photosynthetic organism (Dobrenel et al., 2016a; Upadhyaya et al., 2018; Mubeen et al., 2019; Prioretti et al., 2019). The mechanism by which TOR regulates transcription is still poorly understood not only in microalgae but also in land plants and is a topic to be addressed in future research.

Light, sugar, and brassinosteroid signaling through TOR maintain the balance between hormone-promoted growth and carbon availability in plants (Xiong et al., 2013; Dong et al., 2015; Zhang et al., 2016; Kajikawa and Fukuzawa, 2020). During photosynthesis, light energy is captured to convert the carbon intake into sugars, that can be used as material for plant growth or stored as starch for long-term reserves (Ingargiola et al., 2020; Pancha et al., 2020). Photosynthesis, the primary source of energy for plants, also appears to be under the control of the TOR pathway (Ren et al., 2013a; Burkart and Brandizzi, 2021). Plants deficient in TOR signaling exhibit abnormalities in thylakoid grana architecture and normal photosynthetic ability (Zhang et al., 2016; Schepetilnikov and Ryabova, 2018; Bakshi et al., 2021).

In terms of metabolomic approaches, analysis conducted using *C. reinhardtii* cells revealed that the TCA cycle intermediates, malate, succinate and citrate, were significantly increased in cells after exposure to 24 h of rapamycin treatment compared to the control culture (Lee and Fiehn, 2013; Jüppner et al., 2018). The negative effect on the TCA cycle is accompanied by the simultaneous down-regulation of some amino acids such as phenylalanine, glutamic acid, aspartic acid, asparagine, tyrosine, or glutamine (Mubeen et al., 2019).

Remarkably, metabolomics data also indicated that cysteine and methionine pools are strongly affected in Chlamydomonas cells treated with rapamycin (Kleessen et al., 2015; Ford et al., 2019). A cysteine and methionine metabolism is needed for the assimilation of sulfur and the synthesis of glutathione, a highly abundant free soluble thiol that maintains the intracellular redox balance in the cell (Foyer and Noctor, 2011). Recent studies using synchronized *C. reinhardtii* cultures indicate that the levels of primary metabolites and carbon storage molecules are higher in rapamycin-treated cultures (Jüppner et al., 2018). A study by Mubeen et al., (2018) on *C. reinhardtii* indicated that in few minutes of rapamycin treatment, the cells accumulate a very high amount of almost all amino acids. When cells were simultaneously exposed to rapamycin and either translation inhibitor cycloheximide or proteasome inhibitor MG132, the amount of amino acids also increased, indicating that translation and proteasome-dependent proteolysis are not major events in this phenomenon. Instead, it was confirmed that the *de novo*

amino acid synthesis mainly depends upon the carbon and nitrogen pull availability (Jüppner et al., 2018; Mubeen et al., 2018). Studies also indicate that the amounts of isomaltotriose and hexose phosphate obtained from the Calvin-Benson cycle are significantly higher during rapamycin treatment in the early-nightphase (Jüppner et al., 2018).

Transcriptomic studies performed on inhibition of TOR signaling, in Chlamydomonas cells treated with rapamycin, demonstrated a profound effect on the expression of several genes (Ramundo et al., 2014; Kleessen et al., 2015; Werth et al., 2019; Busche et al., 2021). Up-regulated genes are implicated in amino acid metabolism, vacuolar function, tetrapyrrole metabolism, autophagy, and the transport of metabolites (Kim and Guan, 2011; Couso et al., 2018; Pancha et al., 2019). Most highly induced genes include small heat shock proteins and chaperones, proteases, proteins involved in autophagy and thylakoid membrane biogenesis, protein kinases, and transporters (Ramundo et al., 2014; Prioretti et al., 2020). Down regulated genes upon rapamycin treatment are involved in cell cycle, DNA replication and repair, nucleotide metabolism, and photosynthesis (Shimobayashi and Hall, 2014; Imamura et al., 2018; Jüppner et al., 2018). Together, these transcriptomic and metabolomic data highlight the central role of TOR in cell growth control by regulating anabolic and catabolic processes in Chlamydomonas.

5 TOR SIGNALING IN THE ACCUMULATION OF ENERGY STORAGE MOLECULES

There is usually in microalgae an inverse relationship between growth and accumulation of energy storage molecules (Jüppner et al., 2018; Covell et al., 2020). Starch and triacylglycerols (TAGs) are accumulated in relatively low amounts under favorable growth conditions and in high amounts under the influence of various stress, such as nitrogen depletion (Siaut et al., 2011; Rocha et al., 2019; Zhu et al., 2019; Covell et al., 2020). Stress-related responses, e.g. inhibition of cell growth, accumulation of starch/TAGS, are also generally observed under rapamycin-induced TOR inactivation (Jüppner et al., 2018; Pancha et al., 2019; Werth et al., 2019), indicating that algal TOR is involved in sensing the nutrient and energy status of cells.

The inhibition of TOR signaling by rapamycin in Chlamydomonas and the distant red alga *Cyanidioschyzon merolae* resulted in the accumulation of lipid bodies containing TAGs and the up-regulation of genes involved in TAG synthesis such as glycerol-3-phosphate acyltransferase and acyl-CoA:diacylglycerol acyltransferase (DGAT) (Imamura et al., 2015; Imamura et al.,

2016). In the green alga *E. gracilis* treated with rapamycin has also been demonstrated a similar effect on lipid body accumulation (Mukaida et al., 2016). However, as the genome sequence of *E. gracilis* is not publicly available, it is not possible to identify the molecular mechanism by which rapamycin affects TAG metabolism in this alga (Mukaida et al., 2016). In the diatom *P. tricornutum* TOR inhibition by AZD8055 also results in a dose-dependent increase in TAG accumulation and inhibition of cell proliferation (Prioretti et al., 2017). The biochemical activity of certain enzymes, e.g. glyceraldehyde 3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase, increased significantly upon AZD8055 treatment. Since these enzymes are important for intracellular levels of ATP and pyruvate, which are involved in lipid production, this indicate a mechanism by which TOR signaling regulates TAG accumulation in diatoms (Prioretti et al., 2017).

During the screening of a *C. reinhardtii* insertion mutant library, it has been identified rapamycin-hypersensitive VIP1 loss-of-function mutant cells (Couso et al., 2016). Even under normal growth conditions, these mutants accumulate a large amount of TAGs. The vip1 gene encodes an inositol polyphosphate kinase, which phosphorylates inositol phosphate (InsP) to produce the signaling metabolites InsP7 and InsP8 (Couso et al., 2016). In addition, vip1-1 mutant has decreased InsP7 and InsP8 levels and increased TAG levels under normal growth conditions. These observations demonstrate that TOR signaling regulates the amount of InsPs in cells and that InsP levels determine intracellular TAG levels (Couso et al., 2016; Prioretti et al., 2020). Based on these observations, it is clear that algal TOR signaling is also involved in TAG accumulation in divergent microalgae, this signaling pathway might also be a biotechnological target to improve TAG productivity in algae.

Apart from TAGs, starch is another important carbon storage molecule in microalgae (Juergens et al., 2016; Zhao and Wang, 2020). Microalgae generally accumulate starch under the same growth conditions that lead to TAG accumulation (Himanshu et al., 2016; Mubeen et al., 2018), but starch accumulation is also induced by TOR inactivation (Fan et al., 2012; Johnson and Alric, 2013). Starch accumulation after TOR inactivation has been observed in *C. reinhardtii* (Pérez-Pérez and Crespo, 2017; Jüppner et al., 2018; Mubeen et al., 2019), indicating that TOR signaling is also involved in starch accumulation in microalgae. Microalgae are a potential feedstock for the production of biofuels (Chisti, 2007), so the enhancement of TAG/starch content in microalgae is necessary for the commercialization of microalgae-based biofuel. Thus, modulation of the TOR signaling pathway(s) and its regulator(s) appear to be a potential method by which the accumulation of TAGs/starch can be enhanced without

compromising the growth of microalgae. Furthermore, accumulation of TAGs and starch after TOR inactivation was also observed in *A. thaliana* (Caldana et al., 2013), indicating that the role of TOR signaling in the accumulation of these energy storage molecules seems to be conserved in plant lineages. Therefore, further research on the effects of TOR on the accumulation of energy storage molecules will be helpful for the improvement of biomass production not only in microalgae but also in plants (Prioretti et al., 2019).

6 CONTROL OF AUTOPHAGY BY TOR

Eukaryotic cells have developed specialized mechanisms to respond and adapt to stresses in the extracellular environment (Couso et al., 2018). Autophagy is a major catabolic pathway by which eukaryotic cells remove damaged or unnecessary cytoplasmic material to the vacuole for degradation and recycling in order to maintain cellular homeostasis (Marshall and Vierstra, 2018; Barros et al., 2020). During this degradative process, cytosolic components, including proteins, membranes, and entire organelles, are engulfed in bulk or selectively within a double-membrane vesicle known as an autophagosome and delivered to the vacuole (Liu and Bassham, 2012; Zeng et al., 2019). Generally, autophagy occurs as a result of stress, such as carbon and/or nitrogen depletion, pathogen attack and senescence (Liu and Bassham, 2012; Marshall and Vierstra, 2018). Autophagy is also carried out, albeit to a lesser extent, under normal growth conditions in all cells to remove waste materials (Mizushima et al., 2011; Ohsumi, 2014; Avin-Wittenberg, 2019).

The genes enconding proteins functioning in the autophagy machinery (autophagy-related genes - ATG genes), which were originally identified in yeast and subsequently in mammals (Elander et al., 2018), have also been identified in photosynthetic eukaryotes, including land plants and various microalgae (Liu and Bassham, 2012; Pérez-Pérez and Crespo, 2017; Couso et al., 2018; Marshall and Vierstra, 2018). In plants, autophagy has been described in the model plant *A. thaliana* as well as the model algae *C. reinhardtii* (Doelling et al., 2002; Pérez-Pérez et al., 2012; Barros et al., 2020).

Autophagy induction can be initiated by multiple signaling pathways, one of which is the negative regulator of TOR protein (Mizushima et al., 2011; Couso et al., 2018). Autophagy is triggered when the cell senses a reduction in the availability of nutrients (Kajikawa and Fukuzawa, 2020). TORC1 has been identified as an essential component in the molecular mechanism that transmits this starvation signal to the autophagic machinery (Elander et al., 2018; Marshall and Vierstra, 2018). TORC1 inhibits autophagy by negatively regulating the

association between the Ser/Thr kinase ATG1 with ATG13, a regulatory subunit of ATG1 (Couso et al., 2018; Pugkaew et al., 2018). Under nutrient-rich conditions, TORC1 promotes phosphorylation of ATG1 and ATG13. When phosphorylated, these proteins cannot interact and consequently, ATG1 kinase remains inactive (Zeng et al., 2019; Barros et al., 2020).

TOR inactivation via RNAi-mediated downregulation, chemical inhibitors such as rapamycin and AZD8055, or genetic elimination of RAPTOR or LST8, causes rapid dephosphorylation of ATG1 and ATG13 (Couso et al., 2018; Zeng et al., 2019), which results in ATG1 activation and autophagy induction (Couso et al., 2018; Avin-Wittenberg, 2019). Whole-genome analysis of *C. reinhardtii* revealed that its nucleus encodes a single copy of almost all ATGs (Díaz-Troya et al., 2008b; Pérez-Pérez and Crespo, 2017). Structural analysis indicates that the structure of CrATG8 is very similar to that of A. thaliana ATG8. Both CrATG8 and AtATG8 proteins possess a long C-terminal extension after a conserved glycine residue (Pérez-Martín et al., 2015; Couso et al., 2018). It is already known that ATG8 is conjugated to phosphatidylethanolamine (PE), in which the glycine residue plays a key role, and this process is required autophagosome formation (Nakatogawa et al., 2007; Avin-Wittenberg, 2019; Barros et al., 2020). In *C. reinhardtii* cells, CrATG8 is present as punctate structures with rapamycin treatment or under other stresses, such as nitrogen depletion or oxidative stress, suggesting that TOR plays an important role in signaling nutritional to proteins involved in autophagy activation and autophagosome formation (Pérez-Pérez and Crespo, 2010a; Couso et al., 2018; Kajikawa and Fukuzawa, 2020).

Overall, the mechanism underlying the TOR-dependent regulation of autophagy in green microalgae is still poorly understood and further research on this topic is needed for clarification.

7 ROLE OF TOR IN PROTEIN SYNTHESIS

When energy or amino acids become limiting, protein production needs to be downregulated, so that cells can use their limited resources to survive (Ma and Blenis, 2009; Guerra et al., 2013; Liang et al., 2019). Protein synthesis is an intricate, energy-demanding, and strictly controlled process that plays a fundamental role in cell growth, proliferation, and differentiation. The control of protein synthesis is one of the best-characterized processes downstream of TOR which regulates various catabolic and anabolic processes in cells. Protein synthesis is one such process and must be tightly regulated according to cell growth to maintain cellular homeostasis (Schepetilnikov and Ryabova, 2018).

In mammalian and yeast cells, ribosomal S6 kinases (S6Ks, SCH9 in yeast) and elF4E-binding proteins (4E-BPs) are major downstream targets of TOR that are involved in the translation process (Albert and Hall, 2015; Dobrenel et al., 2016b). TORC1 promotes the synthesis of proteins through phosphorylation of two main substrates namely ribosomal protein S6kinase – (S6K/Sch9 the functional homolog in yeast) and 4E-BP1 (translation initiation factor 4E-binding protein-1) (Ma and Blenis, 2009; Schepetilnikov and Ryabova, 2018). S6K (Sch9) phosphorylation by TORC1 activates the S6K as well as multiple other substrates including eIF4B (elongation initiation factor-4B). The eIF4B protein regulates cap-dependent translation by forming an active complex with eIF4A (Hay and Sonenberg, 2004; Holz et al., 2005; Dorrello et al., 2006). The 4E-BP1 inhibits the assembly of eIF-4E complex for cap-dependent translation. When the TORC1 is active it negatively regulates 4E-BP1 by phosphorylating at multiple sites to inhibit its interaction with eIF4E (Brunn et al., 1997; Gingras et al., 1999; Howell et al., 2013). As in yeasts and mammals, S6K is well conserved in photosynthetic eukaryotes, although no 4E-BP-related proteins have been identified in plants or algae (Schepetilnikov and Ryabova, 2018).

The Arabidopsis genome encodes two S6K homologs, S6K1 and S6K2 (Zhang et al., 1994), and it has been demonstrated that TOR regulates S6K1 and S6 phosphorylation in Arabidopsis (Schepetilnikov et al., 2011; Dobrenel et al., 2016b). It has been reported that S6Ks play an essential role in the control of the translation process through the phosphorylation of ribosomal protein S6 and eukaryotic elongation factor-2 kinase (Schepetilnikov and Ryabova, 2018). The recent development of S6K antibody, which can be used to detect the specific phosphorylation residue of endogenous *A. thaliana* S6K as well as for phosphoproteomic analysis, has resulted in the discovery that Ser240 of S6K is phosphorylated by TOR (Dobrenel et al., 2016b).

Recently, it was identified a single copy of CrS6K in *C. reinhardtii* and confirmed the presence of the well conserved TOR phosphorylation motif FLGFTYVAP at the C-terminal of the protein (Upadhyaya et al., 2018). It still has not been clear whether this protein is a canonical S6K, although the C-terminus of the protein contains the TOR phosphorylation motif. Still according to Upadhyaya et al., (2018), CrS6K is involved in protein synthesis under light conditions. Therefore, it has been reported no direct evidence relating to the involvement of CrS6K in protein synthesis.

Díaz-Troya et al., (2011) showed that, the function of the ER chaperone BiP is regulated by its phosphorylation status and that its phosphorylation is accelerated by rapamycin-induced

TOR inactivation in *C. reinhardtii*. In addition, rapamycin treatment also results in translation inhibition in this alga. Under ER stress induced by heat shock or tunicamycin, both of which inhibit the glycosylation of protein in the ER, BiP was dephosphorylated. A recent study in *C. reinhardtii* showed that phosphorylation of Ser245, a conserved amino acid of RPS6 in the C-terminus region, is strongly decreased under the treatment of rapamycin, Torin 1 and AZD8055. These results also support that TOR inactivation results in translation inhibition in *C. reinhardtii* (Couso et al., 2020).

8 PHOTOSYNTHETIC ORGANISMS – TOR SIGNALING IN CHLOROPLAST PROCESSES

Chloroplasts are one of the most important cellular compartments of plant and algae cells, as they are responsible for the essential processes of photosynthesis (Couso et al., 2021). The availability of nutrients and other environmental conditions determine the size, number and activity of chloroplasts in plant cells (Li et al., 2013; Upadhyaya et al., 2018; Ingargiola et al., 2020). How the regulation of chloroplast processes occurs via TOR remains largely unknown. The mechanism by which TOR regulates the transcription of chloroplast ribosomal RNA (rRNA) was revealed in *C. merolae* (Imamura et al., 2018). TOR inactivation increased the expression of a nuclear-encoded chloroplast RelA-SpoT homolog (RSH) gene, CmRSH4b, which encodes a homolog of the guanosine 30-diphosphate 50-diphosphate (ppGpp) synthetases. Biochemical analyses showed that ppGpp inhibits bacteria-type RNA polymerase-dependent chloroplast rRNA synthesis and chloroplast guanylate kinase (GK). The inhibition of GK reduces the GTP pool, inhibiting rRNA transcription, for which GTP is the initiating nucleotide (Imamura et al., 2018).

Recent proteomic and phosphoproteomic analyses using *C. reinhardtii* indicated that the levels of tetrapyrrole-related proteins are increased, while the levels of most of the proteins related to the Calvin-Benson cycle are decreased in cells under TOR inactivation (Roustan and Weckwerth, 2018; Couso et al., 2021). This suggests that TOR signaling regulates photosynthesis and other processes in the chloroplast (Couso et al., 2021). Furthermore, two metalloproteases, A8IL08 and A8J6C7, which localize in the thylakoid membrane, are decreased, while the serine protease A8IL21 is increased during rapamycin treatment (Roustan and Weckwerth, 2018). Thus, it seems likely that TOR signaling plays important role in cellular processes in chloroplast. Therefore, further analyses of the functions of TOR in these cellular

processes may better clarify the function of TOR pathway signaling and its association with photosynthetic performance in microalgae.

9 PERSPECTIVES

Compared to animals and yeasts, the current knowledge of TOR functions and signaling in microalgae is very limited. The recent development of rapamycin-sensitive strains and novel TOR inhibitors combined with various "omic" analyses have revealed that algal TOR is involved in many cellular processes in microalgae, as which the accumulation of energy storage molecules, autophagy, rRNA transcription in the chloroplast. However, still little is known about the molecular mechanisms by which TOR promotes photosynthetic cell growth. Efforts should concentrate in the identification of components of this signaling cascade that may act upstream or downstream of TOR, being able to provide valuable information about nutritional or stress inputs to this kinase. This knowledge will help us not only to understand TOR functions in plant and algae lineages but also to develop various tools for the sustainable development of biomass production using microalgae and algal biotechnology to benefits in industrial sectors like agriculture or biofuel production.

10 REFERENCES

Albert V, Hall MN (2015) mTOR signaling in cellular and organismal energetics. Curr Opin Cell Biol **33**: 55–66

Avin-Wittenberg T (2019) Autophagy and its role in plant abiotic stress management. Plant Cell Environ **42**: 1045–1053

Bakshi A, Moin M, Madhav MS, Datla R, Kirti PB (2021) Target of Rapamycin (TOR) negatively regulates chlorophyll degradation and lipid peroxidation and controls responses under abiotic stress in Arabidopsis thaliana. Plant Stress **2**: 100020

Barquilla A, Crespo JL, Navarro M (2008) Rapamycin inhibits trypanosome cell growth by preventing TOR complex 2 formation. Proc Natl Acad Sci U S A **105**: 14579–14584

Barros JAS, Siqueira JAB, Cavalcanti JHF, Araújo WL, Avin-Wittenberg T (2020) Multifaceted Roles of Plant Autophagy in Lipid and Energy Metabolism. Trends Plant Sci **25**: 1141–1153

Brunkard JO (2020) Exaptive Evolution of Target of Rapamycin Signaling in Multicellular Eukaryotes. Dev Cell **54**: 142–155

Burkart GM, Brandizzi F (2021) A Tour of TOR Complex Signaling in Plants. Trends Biochem Sci **46**: 417–428

Busche M, Regina Scarpin M, Hnasko R, Brunkard JO (2021) TOR coordinates nucleotide availability with ribosome biogenesis in plants. Plant Cell **33**: 1615–1632

Caldana C, Li Y, Leisse A, Zhang Y, Bartholomaeus L, Fernie AR, Willmitzer L, Giavalisco P (2013) Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in Arabidopsis thaliana. Plant J **73**: 897–909

Chantranupong L, Wolfson RL, Sabatini DM (2015) Nutrient-sensing mechanisms across evolution. Cell **161**: 67–83

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv **25**: 294–306

Couso I, Evans BS, Li J, Liu Y, Ma F, Diamond S, Allen DK, Umen JG (2016) Synergism between inositol polyphosphates and TOR kinase signaling in nutrient sensing, growth control, and lipid metabolism in Chlamydomonas. Plant Cell **28**: 2026–2042

Couso I, Pérez-Pérez ME, Ford MM, Martínez-Force E, Hicks LM, Umen JG, Crespo JL (2020) Phosphorus Availability Regulates TORC1 Signaling via LST8 in Chlamydomonas. Plant Cell **32**: 69–80

Couso I, Pérez-Pérez ME, Martínez-Force E, Kim HS, He Y, Umen JG, Crespo JL (2018) Autophagic flux is required for the synthesis of triacylglycerols and ribosomal protein turnover in Chlamydomonas. J Exp Bot **69**: 1355–1367

Couso I, Smythers AL, Ford MM, Umen JG, Crespo JL, Hicks LM (2021) Inositol polyphosphates and target of rapamycin kinase signalling govern photosystem II protein phosphorylation and photosynthetic function under light stress in Chlamydomonas. New Phytol **232**: 2011–2025

Covell L, Machado M, Vaz MGMV, Soares J, Batista AD, Araújo WL, Martins MA, Nunes-Nesi A (2020) Alternative fertilizer-based growth media support high lipid contents without growth impairment in *Scenedesmus obliquus* BR003. Bioprocess Biosyst Eng **43**: 1123–1131

Crespo JL, Díaz-Troya S, Florencio FJ (2005) Inhibition of target of rapamycin signaling by rapamycin in the unicellular green alga *Chlamydomonas reinhardtii*. Plant Physiol **139**: 1736–1749

Crespo JL, Hall MN (2002) Elucidating TOR Signaling and Rapamycin Action: Lessons from Saccharomyces cerevisiae . Microbiol Mol Biol Rev **66**: 579–591

Cross FR, Umen JG (2015) The Chlamydomonas cell cycle. Plant J **82**: 370–392

Díaz-Troya S, Florencio FJ, Crespo JL (2008a) Target of rapamycin and LST8 proteins associate with membranes from the endoplasmic reticulum in the unicellular green alga *Chlamydomonas reinhardtii*. Eukaryot Cell **7**: 212–222

Díaz-Troya S, Pérez-Pérez ME, Florencio FJ, Crespo JL (2008b) The role of TOR in autophagy regulation from yeast to plants and mammals. Autophagy **4**: 851–865

Díaz-Troya S, Pérez-Pérez ME, Pérez-Martín M, Moes S, Jeno P, Florencio FJ, Crespo JL (2011) Inhibition of protein synthesis by TOR inactivation revealed a conserved regulatory mechanism of the BiP chaperone in Chlamydomonas. Plant Physiol **157**: 730–741

Dibble CC, Manning BD (2013) Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. Nat Cell Biol **15**: 555–564

Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, Veit B, Meyer C (2016a) TOR Signaling and Nutrient Sensing. Annu Rev Plant Biol **67**: 261–285

Dobrenel T, Mancera-Martínez E, Forzani C, Azzopardi M, Davanture M, Moreau M, Schepetilnikov M, Chicher J, Langella O, Zivy M, et al (2016b) The arabidopsis TOR kinase specifically regulates the expression of nuclear genes coding for plastidic ribosomal proteins and the phosphorylation of the cytosolic ribosomal protein S6. Front Plant Sci **7**: 1–16

Dobrenel T, Marchive C, Azzopardi M, Clément G, Moreau M, Sormani R, Robaglia C, Meyer C (2013) Sugar metabolism and the plant target of rapamycin kinase: A sweet operaTOR? Front Plant Sci **4**: 1–6

Dobrenel T, Marchive C, Sormani R, Moreau M, Mozzo M, Montané MH, Menand B, Robaglia C, Meyer C (2011) Regulation of plant growth and metabolism by the TOR kinase. Biochem Soc Trans **39**: 477–481

Doelling JH, Walker JM, Friedman EM, Thompson AR, Vierstra RD (2002) The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in Arabidopsis thaliana. J Biol Chem **277**: 33105–33114

Dong P, Xiong F, Que Y, Wang K, Yu L, Li Z, Ren M (2015) Expression profiling and functional analysis reveals that TOR is a key player in regulating photosynthesis and phytohormone signaling pathways in Arabidopsis. Front Plant Sci **6**: 1–15

Dorrello NV, Peschiaroli A, Guardavaccaro D, Colburn NH, Sherman NE, Pagano M (2006) S6k1- and βTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. Science (80-) **314**: 467–471

Drenan RM, Liu X, Bertram PG, Zheng XFS (2004) FKBP12-Rapamycin-associated Protein or Mammalian Target of Rapamycin (FRAP/mTOR) Localization in the Endoplasmic Reticulum and the Golgi Apparatus. J Biol Chem **279**: 772–778

Efeyan A, Comb WC, Sabatini DM (2015) Nutrient-sensing mechanisms and pathways. Nature **517**: 302–310

Elander PH, Minina EA, Bozhkov P V. (2018) Autophagy in turnover of lipid stores: Trans-kingdom comparison. J Exp Bot **69**: 1301–1311

Fan J, Yan C, Andre C, Shanklin J, Schwender J, Xu C (2012) Oil accumulation is controlled by carbon precursor supply for fatty acid synthesis in *Chlamydomonas reinhardtii*. Plant Cell Physiol **53**: 1380–1390

Ford MM, Smythers AL, McConnell EW, Lowery SC, Kolling DRJ, Hicks LM (2019) Inhibition of TOR in *Chlamydomonas reinhardtii* Leads to Rapid Cysteine Oxidation Reflecting Sustained Physiological Changes. Cells. doi: 10.3390/cells8101171

Foyer CH, Noctor G (2011) Ascorbate and glutathione: The heart of the redox hub. Plant Physiol **155**: 2–18

Gaubitz C, Oliveira TM, Prouteau M, Leitner A, Karuppasamy M, Konstantinidou G, Rispal D, Eltschinger S, Robinson GC, Thore S, et al (2015) Molecular Basis of the Rapamycin Insensitivity of Target Of Rapamycin Complex 2. Mol Cell **58**: 977–988

Gaubitz C, Prouteau M, Kusmider B, Loewith R (2016) TORC2 Structure and Function. Trends Biochem Sci **41**: 532–545

Gingras A, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R, Sonenberg N (1999) Regulation of 4E-BP1 phosphorylation : a novel two-step mechanism. 1422–1437

Hall MN (2016) TOR and paradigm change: Cell growth is controlled. Mol Biol Cell **27**: 2804–2806

Hara K, Maruki Y, Long X, Yoshino K ichi, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell **110**: 177–189

Hayashi T, Hatanaka M, Nagao K, Nakaseko Y, Kanoh J, Kokubu A, Ebe M, Yanagida M (2007) Rapamycin sensitivity of the Schizosaccharomyces pombe tor2 mutant and organization of two highly phosphorylated TOR complexes by specific and common subunits. Genes to Cells **12**: 1357–1370

Heitman J, Movva NR, Hall MN (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science (80-) **253**: 905–909

Helliwell SB, Wagner P, Kunz J, Deuter-Reinhard M, Henriquez R, Hall MN (1994) TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. Mol Biol Cell **5**: 105–118

Himanshu S, Manish RS, Basuthkar JR, Kandala VRC (2016) Regulation of starch, lipids and amino acids upon nitrogen sensing in *Chlamydomonas reinhardtii*. Algal Res **18**: 33–44

Holz MK, Ballif BA, Gygi SP, Blenis J (2005) mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell **123**: 569–580

Howell JJ, Ricoult SJH, Ben-Sahra I, Manning BD (2013) A growing role for mTOR in promoting anabolic metabolism. Biochem Soc Trans **41**: 906–912

Imamura S, Kawase Y, Kobayashi I, Shimojima M, Ohta H, Tanaka K (2016) TOR (target of rapamycin) is a key regulator of triacylglycerol accumulation in microalgae. Plant Signal Behav. doi: 10.1080/15592324.2016.1149285

Imamura S, Kawase Y, Kobayashi I, Sone T, Era A, Miyagishima S ya, Shimojima M, Ohta H, Tanaka K (2015) Target of rapamycin (TOR) plays a critical role in triacylglycerol accumulation in microalgae. Plant Mol Biol **89**: 309–318

Imamura S, Nomura Y, Takemura T, Pancha I, Taki K, Toguchi K, Tozawa Y, Tanaka K (2018) The checkpoint kinase TOR (target of rapamycin) regulates expression of a nuclear-encoded chloroplast RelA-SpoT homolog (RSH) and modulates chloroplast ribosomal RNA synthesis in a unicellular red alga. Plant J **94**: 327–339

Ingargiola C, Duarte GT, Robaglia C, Leprince A-S, Meyer C (2020) The Plant Target of Rapamycin: A Conduc TOR of Nutrition and Metabolism in Photosunthetic Organisms. doi: doi.org/10.3390/genes11111285

Jewell JL, Guan KL (2013) Nutrient signaling to mTOR and cell growth. Trends Biochem Sci **38**: 233–242

Johnson X, Alric J (2013) Central carbon metabolism and electron transport in *Chlamydomonas reinhardtii*: Metabolic constraints for carbon partitioning between oil and starch. Eukaryot Cell **12**: 776–793

Juergens MT, Disbrow B, Shachar-Hill Y (2016) The relationship of triacylglycerol and starch accumulation to carbon and energy flows during nutrient deprivation in *Chlamydomonas reinhardtii*. Plant Physiol **171**: 2445–2457

Jüppner J, Mubeen U, Leisse A, Caldana C, Wiszniewski A, Steinhauser D, Giavalisco P (2018) The target of rapamycin kinase affects biomass accumulation and cell cycle progression by altering carbon/nitrogen balance in synchronized *Chlamydomonas reinhardtii* cells. Plant J **93**: 355–376

Kajikawa M, Fukuzawa H (2020) Algal Autophagy Is Necessary for the Regulation of Carbon Metabolism Under Nutrient Deficiency. Front Plant Sci **11**: 1–6

Kim D, Sarbassov DD, Ali SM, Latek RR, Guntur KVP, Erdjument-bromage H, Tempst P, Sabatini DM (2003) <1-s2.0-S109727650300114X-main.pdf>. **11**: 895–904

Kim SG, Buel GR, Blenis J (2013) Nutrient regulation of the mTOR complex 1 signaling pathway. Mol Cells **35**: 463–473

Kleessen S, Irgang S, Klie S, Giavalisco P, Nikoloski Z (2015) Integration of transcriptomics and metabolomics data specifies the metabolic response of Chlamydomonas to rapamycin treatment. Plant J **81**: 822–835

Laplante M, Sabatini DM (2012) MTOR signaling in growth control and disease. Cell **149**: 274–293

Li J, Kim SG, Blenis J (2014) Rapamycin: One drug, many effects. Cell Metab **19**: 373–379

Liang Y, Kong F, Torres-Romero I, Burlacot A, Cuine S, Légeret B, Billon E, Brotman Y, Alseekh S, Fernie AR, et al (2019) Branched-chain amino acid catabolism impacts triacylglycerol homeostasis in *Chlamydomonas reinhardtii*. Plant Physiol **179**: 1502–1514

Liu Y, Bassham DC (2012) Autophagy: Pathways for self-eating in plant cells. Annu Rev Plant Biol **63**: 215–237

Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell **10**: 457–468

Ma XM, Blenis J (2009) Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol **10**: 307–318

Marshall RS, Vierstra RD (2018) Autophagy: The Master of Bulk and Selective Recycling. Annu Rev Plant Biol **69**: 173–208

Matsuo T, Otsubo Y, Urano J, Tamanoi F, Yamamoto M (2007) Loss of the TOR Kinase Tor2 Mimics Nitrogen Starvation and Activates the Sexual Development Pathway in Fission Yeast. Mol Cell Biol **27**: 3154–3164

McCready K, Spencer V, Kim M (2020) The Importance of TOR Kinase in Plant Development. Front Plant Sci **11**: 1–7

Menand B, Desnos T, Nussaume L, Bergert F, Bouchez D, Meyer C, Robaglia C (2002) Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. Proc Natl Acad Sci U S A **99**: 6422–6427

Mizushima N, Yoshimori T, Ohsumi Y (2011) The role of atg proteins in autophagosome formation. Annu Rev Cell Dev Biol **27**: 107–132

Moreau M, Azzopardi M, Clé ment G, Dobrenel T, Marchive C, Renne C, Martin-Magniette ML, Taconnat L, Renou JP, Robaglia C, et al (2012) Mutations in the Arabidopsis homolog of LST8/GβL, a partner of the target of Rapamycin kinase, impair plant growth, flowering, and metabolic adaptation to long days. Plant Cell **24**: 463–481

Mubeen U, Giavalisco P, Caldana C (2019) TOR inhibition interrupts the metabolic homeostasis by shifting the carbon-nitrogen balance in *Chlamydomonas reinhardtii*. Plant Signal Behav **14**: 1670595

Mubeen U, Jüppner J, Alpers J, Hincha DK, Giavalisco P (2018) Target of rapamycin inhibition in *Chlamydomonas reinhardtii* triggers de novo amino acid synthesis by enhancing nitrogen assimilation[open]. Plant Cell **30**: 2240–2254

Mukaida S, Ogawa T, Ohishi K, Tanizawa Y, Ohta D, Arita M (2016) The effect of rapamycin on biodiesel-producing protist euglena gracilis. Biosci Biotechnol Biochem **80**: 1223–1229

Ohsumi Y (2014) Historical landmarks of autophagy research. Cell Res **24**: 9–23

Pancha I, Chokshi K, Tanaka K, Imamura S (2020) Microalgal Target of Rapamycin (TOR): A Central Regulatory Hub for Growth, Stress Response and Biomass Production. Plant Cell Physiol **61**: 675–684

Pancha I, Shima H, Higashitani N, Igarashi K, Higashitani A, Tanaka K, Imamura S (2019) Target of rapamycin-signaling modulates starch accumulation via glycogenin phosphorylation status in the unicellular red alga Cyanidioschyzon merolae. Plant J **97**: 485–499

Pérez-Martín M, Blaby-Haas CE, Pérez-Pérez ME, Andrés-Garrido A, Blaby IK, Merchant SS, Crespo JL (2015) Activation of autophagy by metals in *Chlamydomonas reinhardtii*. Eukaryot Cell **14**: 964–973

Pérez-Pérez ME, Couso I, Crespo JL (2017) The TOR signaling network in the model unicellular green alga *Chlamydomonas reinhardtii*. Biomolecules **7**: 1–13

Pérez-Pérez ME, Crespo JL (2010a) Autophagy in the model alga *Chlamydomonas reinhardtii*. Autophagy **6**: 562–563

Pérez-Pérez ME, Crespo JL (2017) Monitoring Autophagy in the Model Green Microalga *Chlamydomonas reinhardtii*. Cells **6**: 36

Pérez-Pérez ME, Crespo JL (2010b) Elucidating TOR signaling in *Chlamydomonas reinhardtii*. Enzymes **28**: 245–261

Pérez-Pérez ME, Lemaire SD, Crespo JL (2012) Reactive oxygen species and autophagy in plants and algae. Plant Physiol **160**: 156–164

Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung YL, Schulze A (2008) SREBP Activity Is Regulated by mTORC1 and Contributes to Akt-Dependent Cell Growth. Cell Metab **8**: 224–236

Prioretti L, Avilan L, Carrière F, Montané MH, Field B, Grégori G, Menand B, Gontero B (2017) The inhibition of TOR in the model diatom Phaeodactylum tricornutum promotes a get-fat growth regime. Algal Res **26**: 265–274

Prioretti L, Carriere F, Field B, Avilan L, Montané M, Menand B, Gontero B (2019) SC.

Prioretti L, Carriere F, Field B, Avilan L, Montané MH, Menand B, Gontero B (2020) Targeting TOR signaling for enhanced lipid productivity in algae. Biochimie **169**: 12–17

Pugkaew W, Meetam M, Ponpuak M, Yokthongwattana K, Pokethitiyook P (2018) Role of autophagy in triacylglycerol biosynthesis in *Chlamydomonas reinhardtii* revealed by chemical inducer and inhibitors. J Appl Phycol **30**: 15–22

Ramundo S, Casero D, Mühlhaus T, Hemme D, Sommer F, Crèvecoeur M, Rahire M, Schroda M, Rusch J, Goodenough U, et al (2014) Conditional depletion of the Chlamydomonas chloroplast ClpP protease activates nuclear genes involved in autophagy and plastid protein quality control. Plant Cell **26**: 2201–2222

Ren M, Venglat P, Qiu S, Feng L, Cao Y, Wang E, Xiang D, Wang J, Alexander D, Chalivendra S, et al (2013a) Target of rapamycin signaling regulates metabolism, growth, and life Span in arabidopsis. Plant Cell **24**: 4850–4874

Ren M, Venglat P, Qiu S, Feng L, Cao Y, Wang E, Xiang D, Wang J, Alexander D, Chalivendra S, et al (2013b) Target of rapamycin signaling regulates metabolism, growth, and life Span in arabidopsis. Plant Cell **24**: 4850–4874

Rocha DN, Martins MA, Soares J, Vaz MGMV, de Oliveira Leite M, Covell L, Mendes LBB (2019) Combination of trace elements and salt stress in different cultivation modes

improves the lipid productivity of *Scenedesmus* spp. Bioresour Technol. doi: 10.1016/j.biortech.2019.121644

Rory J. Flinn,* Ying Yan SG, Peter J. Parker and JMB, *Department (2009) The Late Endosome is Essential for mTORC1 Signaling. Mol Biol Cell **21**: 833–841

Roustan V, Weckwerth W (2018) Quantitative phosphoproteomic and system-level analysis of TOR inhibition unravel distinct organellar acclimation in *Chlamydomonas reinhardtii*. Front Plant Sci **871**: 1–21

Saxton RA, Sabatini DM (2017) mTOR Signaling in Growth, Metabolism, and Disease. Cell **168**: 960–976

Schepetilnikov M, Kobayashi K, Geldreich A, Caranta C, Robaglia C, Keller M, Ryabova LA (2011) Viral factor TAV recruits TOR/S6K1 signalling to activate reinitiation after long ORF translation. EMBO J **30**: 1343–1356

Schepetilnikov M, Ryabova LA (2018) Recent discoveries on the role of tor (Target of rapamycin) signaling in translation in plants. Plant Physiol **176**: 1095–1105

Schmelzle T, Beck T, Martin DE, Hall MN (2004) Activation of the RAS/Cyclic AMP Pathway Suppresses a TOR Deficiency in Yeast. Mol Cell Biol **24**: 338–351

Shemi A, Ben-Dor S, Vardi A (2015) Elucidating the composition and conservation of the autophagy pathway in photosynthetic eukaryotes. Autophagy **11**: 701–715

Shimobayashi M, Hall MN (2014) Making new contacts: The mTOR network in metabolism and signalling crosstalk. Nat Rev Mol Cell Biol **15**: 155–162

Tulin F, Zhang Z, Wang ZY (2021) Activation of TOR signaling by diverse nitrogen signals in plants. Dev Cell **56**: 1213–1214

Upadhyaya S, Agrawal S, Gorakshakar A, Rao BJ (2018) Modulation of TOR kinase activity in *Chlamydomonas reinhardtii*: Effect of N-starvation and changing carbon pool. bioRxiv. doi: 10.1101/312942

Vézina C, Kudelski A (1975) Rapamycin (AY-22,989), a new antifungal antibiotic. I. taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot (Tokyo) **28**: 721–726

Walther DB and TC (2009) TORC2 Plasma Membrane Localization Is Essential for Cell Viability and Restricted to a Distinct Domain. Mol Biol Cell **20**: 1565–1575

Wedaman karen P, Reinke A, Anderson S, Yates J, McCaffery JM, Powers T (2003) TOr Kinases Are in Distinct Membrane-associated Protein Complexes in Saccharomyces cerevisiae. Mol Biol Cell **14**: 1204–1220

Werth EG, McConnell EW, Couso Lianez I, Perrine Z, Crespo JL, Umen JG, Hicks LM (2019) Investigating the effect of target of rapamycin kinase inhibition on the *Chlamydomonas reinhardtii* phosphoproteome: from known homologs to new targets. New Phytol **221**: 247–260

Wullschleger S (2006) TOR signaling in growth and metabolism. Cell. doi: 10.1016/j.cell.2006.01.016

Xiong Y, Sheen J (2014) The Role of Target of Rapamycin Signaling Networks in Plant Growth and Metabolism. PLANT Physiol **164**: 499–512

Xiong Y, Sheen J (2012) Rapamycin and glucose-target of rapamycin (TOR) protein signaling in plants. J Biol Chem **287**: 2836–2842

Xu Q, Liang S, Kudla J, Luan S (1998) Molecular characterization of a plant FKBP12 that does not mediate action of FK506 and rapamycin. Plant J **15**: 511–519

Yuan HX, Xiong Y, Guan KL (2013) Nutrient Sensing, Metabolism, and Cell Growth Control. Mol Cell **49**: 379–387

Zeng Y, Li B, Lin Y, Jiang L (2019) The interplay between endomembranes and autophagy in plants. Curr Opin Plant Biol **52**: 14–22

Zhang SH, Lawton MA, Hunter T, Lamb CJ (1994) atpk1, a novel ribosomal protein kinase gene from Arabidopsis. I. Isolation, characterization, and expression. J Biol Chem **269**: 17586–17592

Zhang Z, Zhu JY, Roh J, Marchive C, Kim SK, Meyer C, Sun Y, Wang W, Wang ZY (2016) TOR Signaling Promotes Accumulation of BZR1 to Balance Growth with Carbon Availability in Arabidopsis. Curr Biol **26**: 1854–1860

Zhao Y, Wang XQ (2020) The hot issue: TOR signalling network in plants. Funct Plant Biol **48**: 1–7

Zhu L, Li S, Hu T, Nugroho YK, Yin Z, Hu D, Chu R, Mo F, Liu C, Hiltunen E (2019) Effects of nitrogen source heterogeneity on nutrient removal and biodiesel production of mono- and mix-cultured microalgae. Energy Convers Manag. doi: 10.1016/j.enconman.2019.112144 Biophys Acta - Mol Cell Biol Lipids **1861**: 1269–1281

GENERAL CONSIDERATIONS

Under non-stress conditions, microalgae are genetically programmed to synthesize and store starch to support growth and cell division, as they are evolved to be highly specialized for carbon capture and carbohydrate synthesis. On the other hand, when microalgae are subjected to stress conditions, they tend to accumulate higher amounts of storage lipids at the expense of growth.

Nitrogen starvation led to a severe inhibition of cell growth in *C. reinhardtii* CC503, *S. obliquus* BR003, *C. vulgaris* BR017, *Chlamydomonas* sp. BR020 and *Monoraphidium* sp. BR023 (Chapter 1). In addition, metabolomic and physiological analysis of these strains grown in N-starvation, N-replete and N-saturated conditions indicated that N starvation lead to typical stress-related responses such as reduction of chlorophyll and protein levels. Hence, they also markedly differ in accumulation in membrane lipid composition FA saturation and increase in TAGs.

It seems reasonable to suggest that some microalgae are programmed to accumulate lipids while others accumulate starch during the stationary phase under nutritional stress like N deplete, reinforcing the importance of evolutionary and ecological constraints in the final phenotype of a strain. Thus, it reveals a complementary finding which attributes the storage of reserve compounds not only to species type, but also to environment conditions.

The impact of rapamycin addition clearly shows that TOR inhibition causes a severely reduced growth, leading to a reduction by 50% on the cell growth (Chapter 2). The present data therefore reveals that TOR is involved in the regulation of cell growth in different strains. Next to the direct cell growth related results our data reveals that the obtained growth-phenotype was accompanied by strong accumulation of C-storage compounds, such as starch and TAGs). Changes in amino acid contents demonstrated the diverse expression pattern in relationship to the strains that will aid in specifying the role and mechanism of individual compounds in the response to the rapamycin treatment. Taken together, our findings reveal strong and different changes in carbon and nitrogen portioning in response to TOR inhibition for each strain. Further analyses using *C. reinhardtii*, together with other algae, will provide important information on the regulatory mechanism of reserve compounds accumulation and TOR signaling pathway. Furthermore, such data will be useful for the construction of algae being targeted to specific goals.

Compared to animals and yeasts, the current knowledge of TOR functions and signaling in microalgae is still limited (Chapter 3). The recent development of rapamycin-sensitive strains

and novel TOR inhibitors combined with various “omic” analyses have revealed that algal TOR is involved in many cellular processes in microalgae, as which the accumulation of energy storage molecules, autophagy, rRNA transcription in the chloroplast. However, there is still a lack of knowledgement about the molecular mechanisms by which TOR promotes photosynthetic cell growth. Efforts should focus on the identification of components of this signaling cascade that may act upstream or downstream of TOR, being able to provide valuable information about nutritional or stress inputs to this kinase. This information will help us not only to understand TOR functions in plant and algae lineages but also to develop various tools for the sustainable development of biomass production using microalgae and algal biotechnology to benefits in industrial sectors like agriculture or biofuel production.

SUPPLEMENTARY DATA

SI Table 1. P-values for significant main effects and interactions between the factors (strain, concentration and time) of five strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, *S. obliquus* BR003, *C. vulgaris* BR017 and *Monoraphidium* sp. BR023) cultivated under tree different nitrogen (N) concentration (0mM, 3.5mM and 7mM)

	AFDW	**μ**	**Chl *a***	**Chl *b***	**Chl *a*/*b***	**Protein**	**Starch**	**Lipids**
Main effects								
Strain	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Concentration	0.000**	0.000**	0.000**	0.001**	0.000**	0.000**	0.000**	0.000**
Time	0.000**	0.000**	0.000**	0.001**	0.011**	0.000**	0.000**	0.000**
Interaction effects								
Strain x Concentration	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Strain x Time	0.000**	0.000**	0.000**	0.013**	0.533NS	0.000**	0.000**	0.000**
Concentration x Time	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Strain x Dosage x Time	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
R^2	0.9899	0.9918	0.8808	0.7342	0.8298	0.9162	0.9496	0.9596

** indicate that effect and interaction are significant at *p*-value < 0.05;
NS = not significant;

SI Table 2. Relative abundance of primary metabolites of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Amino acid	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
Alanine	0.33	**Ca**	0.59	**Ab**	0.46	**Bb**	0.29	**Ba**	1.08	**Aa**	1.22	**Aa**	0.28	**Ba**	0.88	**Aa**	0.98	**Aa**	0.16	**Cb**	0.32	**Bc**	0.58	**Ab**
Arginine	0.39	**Cb**	1.66	**Aa**	1.03	**Bab**	0.71	**Bab**	1.49	**Aab**	1.35	**Aa**	0.88	**Aa**	1.34	**Aab**	0.94	**Aa**	0.69	**Aab**	0.88	**Ab**	0.50	**Ab**
Asparagine	1.08	**Aa**	0.80	**Aa**	0.94	**Aa**	0.75	**Aa**	1.18	**Aa**	0.96	**Aa**	0.77	**Aa**	1.58	**Aa**	1.13	**Aa**	0.66	**Aa**	1.76	**Aa**	1.70	**Aa**
Aspartate	0.53	**Bb**	0.75	**Aa**	0.65	**ABb**	0.34	**Bb**	0.71	**Aab**	0.68	**Ab**	0.29	**Cbc**	0.57	**Bbc**	1.13	**Aa**	0.24	**Cc**	0.47	**Bc**	0.66	**Ab**
b-Alanine	0.48	**Ba**	0.96	**Aa**	0.72	**ABb**	0.48	**Aa**	0.65	**Aa**	0.71	**Ab**	0.39	**Ba**	0.82	**Aa**	1.00	**Aab**	0.33	**Ca**	0.88	**Ba**	1.59	**Aa**
Citrulline	1.46	**Aa**	1.50	**Aa**	1.48	**Aab**	0.83	**Ab**	0.99	**Aa**	0.86	**Ac**	0.86	**Ab**	1.01	**Aa**	0.89	**Abc**	0.84	**Bb**	0.33	**Cb**	1.58	**Aa**
Glutamate	0.65	**Bb**	1.49	**Ab**	1.07	**Ab**	0.94	**Ba**	1.72	**Aa**	1.61	**Aa**	0.85	**Ba**	1.55	**Aa**	1.75	**Aa**	0.77	**Bb**	1.21	**Ab**	1.23	**Ab**
Glutamine_2	0.14	**Cab**	1.21	**Ab**	0.68	**Bb**	0.16	**Bab**	3.08	**Aa**	2.40	**Aa**	0.09	**Bb**	0.26	**Ac**	0.55	**Ab**	0.31	**Ba**	0.90	**Ab**	0.81	**Ab**
Glycine	1.30	**Ba**	1.57	**ABa**	1.44	**Aa**	0.88	**Bab**	1.01	**ABab**	1.37	**Aab**	0.96	**Bab**	1.22	**ABab**	1.18	**Aab**	0.47	**Bb**	0.63	**ABb**	1.01	**Ab**
Isoleucine	0.72	**Ab**	0.44	**Cc**	0.59	**Bbc**	1.12	**Aa**	0.54	**Bc**	0.48	**Bc**	0.91	**Aab**	0.79	**ABb**	0.71	**Bb**	0.22	**Cc**	1.14	**Ba**	2.02	**Aa**
Leucine	1.36	**Aa**	0.91	**Cb**	1.14	**Bb**	1.35	**Aa**	0.90	**Bb**	0.85	**Bc**	0.94	**Bb**	1.52	**Aa**	1.00	**Bbc**	0.30	**Cc**	1.03	**Bb**	1.84	**Aa**
Lysine	1.32	**Aa**	1.21	**Aa**	1.26	**Ab**	0.67	**Bb**	0.90	**ABab**	1.12	**Ab**	0.48	**Bb**	1.07	**Aa**	1.51	**Ab**	0.40	**Bb**	0.63	**Bb**	2.96	**Aa**
Methionine	1.09	**Ad**	1.09	**Abc**	1.09	**Ac**	1.72	**Ac**	0.97	**Cc**	1.33	**Bb**	2.12	**Ab**	1.17	**Cb**	1.69	**Ba**	2.93	**Aa**	2.20	**Ba**	1.48	**Cab**
Ornithine	0.77	**Ba**	1.68	**Aa**	1.23	**ABb**	0.63	**Bab**	0.92	**ABb**	1.15	**Ab**	0.45	**Bb**	0.99	**Ab**	1.14	**Ab**	0.40	**Bb**	0.40	**Bc**	1.80	**Aa**
Phenylalanine	0.92	**Ab**	0.57	**Bb**	0.75	**ABb**	1.34	**Aa**	0.80	**Bb**	0.66	**Bb**	0.83	**Ab**	1.28	**Ba**	0.83	**Bb**	0.29	**Ac**	1.13	**Ba**	2.04	**Ca**
Proline	0.76	**Aa**	0.84	**Aa**	0.80	**Aab**	0.83	**Aa**	0.49	**Bb**	0.64	**Ab**	0.88	**Aa**	0.82	**Aa**	0.84	**Aa**	0.89	**Aa**	0.95	**Aa**	0.97	**Aa**
Serine	0.38	**Aa**	0.28	**Bb**	0.33	**ABb**	0.25	**Bb**	0.54	**Aa**	0.46	**Aa**	0.18	**Ac**	0.17	**Ac**	0.18	**Ac**	0.06	**Cd**	0.13	**Bd**	0.47	**Aa**
Threonine	0.50	**Ab**	0.51	**Ac**	0.51	**Ac**	1.15	**Aa**	0.81	**Bb**	0.78	**Bb**	1.14	**Aa**	0.67	**Bbc**	0.67	**Bbc**	0.57	**Cb**	1.26	**Ba**	1.86	**Aa**
Tryptophan	0.97	**Ac**	0.57	**Bc**	0.77	**ABb**	6.00	**Aa**	0.74	**Bbc**	0.72	**Bb**	4.95	**Aa**	0.96	**Bb**	0.89	**Bb**	2.39	**Bb**	5.75	**Aa**	4.39	**Aa**
Tyrosine	1.07	**Aa**	0.74	**Bb**	0.91	**ABb**	1.01	**Aa**	0.47	**Bc**	0.44	**Bc**	0.89	**Aa**	1.12	**Aa**	0.84	**Ab**	0.48	**Cb**	1.25	**Ba**	2.08	**Aa**
Valine	0.89	**Aa**	0.67	**Bb**	0.79	**ABb**	0.94	**Aa**	0.74	**Bb**	0.67	**Bb**	0.79	**Ba**	1.08	**Aa**	0.83	**Bb**	0.33	**Cb**	0.81	**Bb**	1.13	**Aa**
5-Oxoproline	0.35	**Ca**	0.94	**Aab**	0.65	**Bc**	0.49	**Ba**	1.07	**Aa**	0.99	**Aab**	0.45	**Ba**	0.86	**Aab**	1.03	**Aa**	0.41	**Ba**	0.66	**Ab**	0.69	**Abc**

SI Table 2. *(continuation)* Relative abundance of primary metabolites of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Organic acid	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Benzoate	3.92 **Ab**	3.44 **Bb**	3.68 **Bb**	5.27 **Ab**	3.67 **Bb**	3.27 **Bb**	6.68 **Aa**	4.43 **Ba**	4.38 **Ba**	6.27 **Aa**	5.06 **Ba**	5.80 **Ba**
Citramalate	2.09 **Ac**	2.13 **Ab**	2.11 **Aa**	4.51 **Aa**	2.91 **Ba**	2.59 **Ba**	3.25 **Ab**	1.57 **Cc**	2.11 **Ba**	2.28 **Ac**	1.02 **Bd**	0.95 **Bb**
Citrate	0.13 **Aa**	0.08 **Bab**	0.11 **ABa**	0.07 **Ab**	0.08 **Ab**	0.07 **Ab**	0.12 **Aa**	0.11 **Aa**	0.08 **Aab**	0.13 **Aa**	0.06 **Bb**	0.07 **Bb**
Dehydroascorbate	1.30 **Ab**	0.99 **Aab**	1.15 **Abc**	0.92 **Ac**	0.87 **Ab**	0.95 **Ac**	1.06 **Bbc**	1.18 **ABab**	1.46 **Ab**	2.41 **Aa**	1.29 **Ba**	2.00 **Aa**
Fumarate	3.22 **Aa**	2.60 **Aa**	2.91 **Aa**	3.82 **Aa**	2.77 **Aa**	2.71 **Aa**	3.84 **Aa**	3.22 **Aa**	3.28 **Aa**	3.60 **Aa**	4.14 **Aa**	3.26 **Aa**
Gluconate	4.20 **Aa**	2.20 **Aa**	3.20 **Aa**	1.37 **Ab**	0.82 **Bb**	0.64 **Bb**	1.14 **Ab**	0.46 **Bbc**	0.32 **Bbc**	1.51 **Ab**	0.32 **Bc**	0.29 **Bc**
Glycerate	0.62 **Aa**	0.57 **Aa**	0.59 **Aa**	0.34 **Ab**	0.25 **Ab**	0.28 **Ab**	0.31 **Ab**	0.27 **Ab**	0.29 **Ab**	0.42 **Ab**	0.38 **Ab**	0.37 **Ab**
Ketoglutarate	1.34 **Aa**	1.26 **Ab**	1.30 **Aab**	1.15 **Aa**	1.20 **Ab**	1.04 **Ab**	1.14 **Aa**	1.41 **Ab**	1.37 **Aa**	1.24 **Ba**	2.07 **Aa**	1.44 **Ba**
Lactate	0.71 **Ab**	0.56 **Ab**	0.63 **Ab**	0.93 **Aa**	1.13 **Aa**	1.02 **Aa**	0.49 **Ab**	0.50 **Ab**	0.58 **Ab**	0.63 **Ab**	0.67 **Ab**	0.63 **Ab**
Malate	0.56 **Ab**	0.61 **Ab**	0.59 **Ab**	0.82 **Aa**	0.63 **Bb**	0.51 **Bb**	0.54 **Cb**	0.77 **Bab**	0.99 **Aa**	0.57 **Bb**	0.99 **Aa**	1.16 **Aa**
Nicotinate	1.31 **Aa**	1.21 **Aab**	1.26 **Ab**	0.90 **Bb**	1.22 **Aab**	1.13 **Ab**	0.93 **Cb**	1.37 **Ba**	1.70 **Aa**	0.81 **Cb**	1.06 **Bb**	1.73 **Aa**
Pyruvate	0.84 **Ad**	0.86 **Ac**	0.85 **Ac**	1.99 **Ab**	1.95 **Ab**	1.22 **Bc**	3.14 **Aa**	3.85 **Aa**	3.56 **Ab**	1.37 **Cc**	3.76 **Ba**	6.62 **Aa**
Succinate	0.76 **Ab**	0.81 **Ac**	0.79 **Ab**	0.75 **Bb**	0.91 **ABbc**	1.11 **Aa**	1.20 **Aa**	1.21 **Aab**	1.01 **Aab**	1.48 **Aa**	1.31 **Aa**	1.01 **Bab**
Sugar	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Cellobiose	2.14 **Ac**	1.39 **Bb**	1.77 **ABa**	5.21 **Ab**	0.53 **Bc**	0.37 **Cb**	7.16 **Aab**	2.05 **Ba**	0.26 **Cb**	7.64 **Aa**	2.09 **Ba**	0.35 **Cb**
Fructose	1.67 **Aa**	1.14 **Ba**	1.41 **ABa**	0.85 **Ab**	1.12 **Aa**	0.93 **Ab**	0.87 **Ab**	0.62 **ABb**	0.57 **Bc**	0.95 **Ab**	0.56 **Bb**	0.55 **Bc**
Fucose	2.67 **Aa**	1.32 **Ba**	2.00 **Ba**	0.81 **Ab**	0.31 **Bb**	0.30 **Bb**	0.73 **Ab**	0.38 **Bb**	0.41 **Bb**	0.91 **Ab**	0.37 **Bb**	0.32 **Bb**
Glucose_1	2.08 **Aa**	1.32 **Ba**	1.70 **ABa**	0.88 **Ab**	0.90 **Ab**	1.02 **Ab**	0.86 **Ab**	0.53 **Bc**	0.41 **Bc**	0.88 **Ab**	0.29 **Bd**	0.32 **Bc**
Maltose	1.62 **Ac**	1.04 **Bb**	1.33 **ABa**	3.93 **Ab**	0.40 **Bc**	0.28 **Cb**	5.39 **Aab**	1.54 **Ba**	0.20 **Cb**	5.76 **Aa**	1.57 **Ba**	0.26 **Cb**
Sucrose	0.01 **Ab**	0.01 **Ab**	0.01 **Ab**	0.04 **Aab**	0.26 **Aab**	0.04 **Aab**	0.03 **Aab**	0.01 **Aab**	0.01 **Aab**	0.09 **Aa**	0.01 **Aa**	0.08 **Aa**
Trehalose	3.10 **Ac**	2.01 **Bb**	2.56 **ABa**	7.84 **Ab**	0.72 **Bc**	0.51 **Cb**	10.87 **Aab**	2.96 **Ba**	0.38 **Cb**	11.52 **Aa**	2.97 **Ba**	0.49 **Cb**
Xylose	2.73 **Ab**	2.52 **Ac**	2.62 **Abc**	5.06 **Aa**	2.58 **Bc**	2.31 **Bc**	6.21 **Aa**	3.73 **Bb**	3.46 **Bab**	4.95 **ABa**	6.29 **Aa**	4.04 **Ba**
Glycerol	1.70 **Ab**	1.41 **Bb**	1.56 **ABa**	1.80 **Ab**	1.05 **Bc**	1.05 **Bb**	1.94 **Aab**	1.28 **Bb**	1.07 **Cb**	2.21 **Aa**	1.95 **Aa**	1.00 **Bb**
Glycerol 3-P	1.39 **Ba**	1.83 **Aa**	1.61 **ABc**	1.34 **Ba**	1.70 **Aa**	1.76 **Abc**	1.34 **Ba**	2.14 **Aa**	2.07 **Aab**	1.19 **Ca**	1.81 **Ba**	2.30 **Aa**

SI Table 2. *(continuation)* Relative abundance of primary metabolites of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Others	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
Putrescine	8.47 **Aa**	10.81 **Aa**	9.64 **Aa**	6.29 **Ba**	10.41 **Aa**	11.83 **Aa**	5.70 **Ba**	9.88 **Aa**	12.73 **Aa**	4.99 **Ba**	5.64 **Bb**	14.50 **Aa**
Spermidine	2.95 **Aa**	3.18 **Aab**	3.06 **Ac**	1.79 **Ba**	5.68 **Aab**	5.24 **Abc**	1.41 **Ba**	6.41 **Aa**	11.09 **Aab**	1.30 **Ba**	2.95 **Bb**	11.75 **Aa**
Adenine	2.79 **Aa**	2.13 **Aa**	2.46 **Aa**	1.19 **Ab**	1.09 **Ab**	1.38 **Ab**	1.19 **Ab**	1.13 **Ab**	1.15 **Ab**	1.40 **Ab**	1.24 **Ab**	1.53 **Ab**
Uracil	4.22 **Aa**	2.69 **Ba**	3.46 **Ba**	1.25 **Ab**	1.10 **Bb**	1.12 **Bb**	1.15 **Abc**	0.90 **Bbc**	0.83 **Bbc**	1.22 **Ac**	0.50 **Bc**	0.69 **Bc**
2-Hydroxypyridine	2.85 **Ab**	2.43 **Ab**	2.64 **Ab**	3.44 **Ab**	2.55 **Ab**	2.19 **Ab**	3.15 **Aab**	2.79 **Aab**	2.91 **Aab**	3.43 **Aa**	3.69 **Aa**	3.58 **Aa**
4-Aminobutanoate	2.60 **Aa**	1.60 **Ba**	2.10 **ABa**	0.41 **Ab**	0.40 **Bb**	0.51 **ABb**	0.33 **Ac**	0.24 **Bc**	0.27 **ABc**	0.41 **Ac**	0.16 **Bc**	0.16 **ABc**
Galactinol	3.32 **Aa**	1.83 **Aa**	2.58 **Aa**	1.98 **Aab**	0.98 **Bb**	1.20 **ABb**	1.92 **Aab**	0.95 **Bb**	0.68 **Bbc**	1.65 **Ab**	0.36 **Bc**	0.41 **Bc**
Myo-Inositol	4.19 **Aa**	2.94 **Aa**	3.57 **Aa**	1.73 **Ab**	1.22 **Ab**	1.22 **Ab**	1.75 **Ab**	0.93 **Bbc**	0.73 **Bc**	1.91 **Ab**	0.74 **Bc**	1.03 **Bbc**
Orthophosphate	1.70 **Ac**	1.54 **Ac**	1.62 **Ac**	2.01 **Ac**	1.70 **Ac**	1.64 **Ac**	2.11 **Ab**	2.02 **Ab**	2.02 **Ab**	2.38 **Aa**	2.52 **Aa**	2.21 **Aa**

SI Table 3. Relative abundance of primary metabolites of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
Alanine	1.14 **Aa**	1.15 **Ab**	1.13 **Ab**	0.63 **Bb**	1.23 **Aab**	1.31 **Aab**	0.50 **Bc**	1.40 **Aa**	1.47 **Aa**	0.39 **Cd**	0.89 **Bc**	1.30 **Aab**
Arginine	1.38 **Aa**	1.46 **Ab**	1.28 **Ab**	0.18 **Bb**	2.67 **Aa**	2.07 **Aab**	0.12 **Bbc**	3.33 **Aa**	2.47 **Aa**	0.11 **Cc**	0.26 **Bc**	2.78 **Aa**
Asparagine	1.61 **Aa**	1.63 **Ab**	1.67 **Ac**	0.34 **Bb**	2.22 **Aa**	2.07 **Ac**	0.36 **Bb**	2.96 **Aa**	3.03 **Ab**	0.28 **Cb**	0.65 **Bc**	6.57 **Aa**
Aspartate	1.18 **Aa**	1.17 **Ab**	1.14 **Ab**	0.46 **Cb**	2.08 **Aa**	1.73 **Ba**	0.33 **Cc**	2.37 **Aa**	1.97 **Ba**	0.33 **Bc**	0.32 **Bc**	1.93 **Aa**
b-Alanine	1.08 **Aa**	1.08 **Aa**	1.10 **Aa**	0.77 **Ab**	0.80 **Ab**	0.61 **Bb**	0.60 **Bc**	0.80 **Ab**	0.79 **Ab**	0.98 **Aab**	0.39 **Bc**	1.21 **Aa**
Citrulline	1.41 **Aa**	1.41 **Aab**	1.31 **Aa**	0.96 **Ba**	1.97 **Aa**	1.53 **ABa**	1.01 **Ba**	2.16 **Aa**	1.64 **ABa**	0.93 **Ba**	1.05 **Bb**	1.92 **Aa**
Glutamate	0.81 **Ab**	0.81 **Abc**	0.98 **Ab**	0.91 **Aab**	0.79 **Bc**	0.86 **ABab**	0.96 **Aa**	0.91 **Ab**	0.94 **Aa**	0.83 **Bb**	1.04 **Aa**	0.86 **Bab**
Glutamine_2	6.41 **Aa**	6.41 **Aa**	6.61 **Ab**	0.44 **Bb**	8.33 **Aa**	10.13 **Aa**	0.48 **Bb**	3.50 **Ab**	4.14 **Ac**	0.30 **Cc**	0.40 **Bc**	9.68 **Aa**
Glycine	0.80 **Aa**	0.80 **Aab**	0.88 **Ac**	0.47 **Bb**	0.87 **Aa**	0.85 **Ac**	0.57 **Bb**	1.07 **Aa**	1.29 **Ab**	0.43 **Cb**	0.64 **Bb**	3.17 **Aa**
Isoleucine	0.81 **Ab**	0.81 **Ac**	0.85 **Ac**	0.98 **Ba**	1.13 **Ab**	1.07 **ABb**	1.08 **Aa**	1.16 **Ab**	1.11 **Ab**	1.00 **Ca**	1.93 **Ba**	2.35 **Aa**
Leucine	0.53 **Ac**	0.63 **Ac**	0.51 **Ab**	1.13 **Aa**	0.58 **Bbc**	0.58 **Bb**	0.89 **Ab**	0.67 **Bb**	0.59 **Bb**	0.85 **Cb**	1.72 **Ba**	2.23 **Aa**
Lysine	0.55 **Aa**	0.75 **Ac**	0.61 **Ac**	0.54 **Ba**	1.33 **Ab**	0.72 **Bc**	0.59 **Ca**	1.94 **Aa**	1.10 **Bb**	0.49 **Ba**	0.59 **Bc**	2.42 **Aa**

SI Table 3. *(continuation)* Relative abundance of primary metabolites of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Methionine	0.57 **Ac**	0.68 **Ac**	0.61 **Ac**	0.68 **Ab**	0.75 **Ab**	0.73 **Ab**	0.93 **Aab**	0.68 **Aab**	0.83 **Aab**	0.87 **Aa**	0.79 **Aa**	1.08 **Aa**
Ornithine	1.60 **Aa**	1.71 **Ab**	1.66 **Aa**	0.62 **Cbc**	2.48 **Aa**	1.55 **Ba**	0.46 **Cc**	3.10 **Aa**	1.86 **Ba**	0.65 **Bb**	0.38 **Cc**	1.70 **Aa**
Phenylalanine	1.07 **Abc**	1.13 **Ab**	1.10 **Ab**	1.68 **Aa**	1.01 **Bb**	0.93 **Bbc**	0.98 **Ac**	0.99 **Ab**	0.81 **Bc**	1.20 **Bb**	2.26 **Aa**	1.98 **Aa**
Proline	1.84 **Aa**	1.95 **Aa**	1.89 **Aa**	2.87 **Aa**	2.15 **Aa**	2.61 **Aa**	2.84 **Aa**	2.36 **Aa**	2.54 **Aa**	2.22 **Aa**	2.39 **Aa**	1.93 **Aa**
Serine	1.34 **Aa**	1.41 **Ab**	1.44 **Ac**	1.18 **Bbc**	1.38 **Ab**	1.13 **Bd**	1.25 **Bab**	1.76 **Aa**	1.65 **Ab**	1.11 **Cc**	1.24 **Bb**	2.57 **Aa**
Threonine	1.03 **Aa**	1.11 **Ac**	1.10 **Ac**	0.83 **Bb**	1.42 **Ab**	1.23 **Ab**	1.00 **Ba**	1.38 **Ab**	1.23 **Ab**	0.77 **Cb**	1.71 **Ba**	2.75 **Aa**
Tryptophan	0.24 **Ac**	0.32 **Ac**	0.24 **Ac**	1.07 **Aa**	0.48 **Bb**	0.43 **Bb**	0.52 **Ab**	0.42 **ABb**	0.36 **Bb**	0.59 **Cb**	0.97 **Ba**	1.48 **Aa**
Tyrosine	0.57 **Ac**	0.66 **Ac**	0.58 **Ab**	1.35 **Aa**	0.69 **Bbc**	0.56 **Cb**	0.92 **Ab**	0.76 **Bb**	0.53 **Cb**	1.16 **Ba**	1.35 **ABa**	1.50 **Aa**
Valine	0.98 **Aa**	0.10 **Ac**	0.98 **Ac**	0.99 **Aa**	1.12 **Ac**	0.99 **Ac**	1.03 **Ba**	1.30 **Ab**	1.15 **ABb**	0.88 **Ca**	1.52 **Ba**	1.92 **Aa**
5-Oxoproline	0.96 **Aa**	0.15 **Ab**	0.98 **Ab**	0.70 **Bb**	1.34 **Aa**	1.29 **Aa**	0.55 **Bc**	1.38 **Aa**	1.34 **Aa**	0.40 **Cd**	0.78 **Bb**	1.42 **Aa**
Organic acid	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Benzoate	0.77 **Ab**	0.79 **Ab**	0.80 **Aa**	0.84 **Ab**	0.80 **Ab**	0.88 **Aa**	1.41 **Aa**	1.29 **Aa**	0.84 **Ba**	0.89 **Ab**	0.72 **Ab**	0.98 **Aa**
Citramalate	0.39 **Ac**	0.33 **Ad**	0.43 **Ab**	0.60 **Ab**	0.54 **ABc**	0.47 **Bb**	1.00 **Aa**	1.09 **Aa**	0.77 **Ba**	0.89 **Aa**	0.82 **Ab**	0.24 **Bc**
Citrate	4.21 **Aa**	4.28 **Aa**	4.29 **Aa**	1.85 **Bb**	4.80 **Aa**	4.29 **Aa**	1.06 **Bc**	3.23 **Ab**	3.16 **Ab**	0.93 **Ac**	0.37 **Cc**	0.65 **Bc**
Dehydroascorbate	0.47 **Ab**	0.37 **Ab**	0.55 **Ac**	0.58 **Ab**	0.59 **Ab**	0.44 **Bc**	0.56 **Bb**	0.89 **Aa**	0.76 **Ab**	0.86 **Ba**	1.00 **ABa**	1.08 **Aa**
Fumarate	0.86 **Aa**	0.79 **Aa**	0.91 **Aa**	0.72 **Aa**	0.84 **Aa**	0.75 **Aa**	0.91 **Aa**	0.95 **Aa**	0.94 **Aa**	1.01 **Aa**	0.90 **Aa**	0.98 **Aa**
Gluconate	3.18 **Aa**	3.23 **Aa**	3.15 **Aa**	3.85 **Aa**	1.16 **Bb**	0.99 **Bb**	3.57 **Aa**	0.46 **Bc**	0.34 **Cc**	3.15 **Aa**	1.35 **Bb**	0.14 **Cd**
Glycerate	0.95 **Ab**	1.00 **Ab**	0.85 **Ab**	0.95 **Cb**	1.41 **Aa**	1.17 **Ba**	1.12 **Aab**	1.00 **ABb**	0.92 **Bb**	1.33 **Aa**	0.95 **Bb**	1.34 **Aa**
Ketoglutarate	0.73 **Aa**	0.82 **Ac**	0.86 **Ab**	0.61 **Bb**	0.95 **Ab**	0.93 **Aa**	0.73 **Bab**	0.85 **Abc**	0.74 **ABa**	0.52 **Cc**	1.13 **Aa**	0.64 **Ba**
Lactate	1.37 **Aa**	1.47 **Ab**	1.45 **Aa**	1.43 **Aa**	1.38 **Ab**	1.47 **Aa**	1.50 **Aa**	1.25 **Bb**	1.42 **Aa**	1.53 **Aa**	1.56 **Aa**	0.95 **Bb**
Malate	0.58 **Ab**	0.66 **Ad**	0.69 **Ad**	0.58 **Bb**	0.96 **Ac**	0.96 **Ac**	0.89 **Ba**	1.46 **Aa**	1.47 **Ab**	0.79 **Ca**	1.20 **Bb**	2.10 **Aa**
Nicotinate	0.43 **Aa**	0.53 **Ab**	0.50 **Abc**	0.37 **Bb**	0.43 **Ab**	0.43 **ABc**	0.40 **Bab**	0.55 **Aa**	0.51 **Aab**	0.25 **Cc**	0.34 **Bc**	0.53 **Aa**
Pyruvate	0.65 **Aa**	0.69 **Ab**	0.60 **Ac**	0.32 **Cb**	0.53 **Bb**	0.70 **Ac**	0.30 **Bb**	0.95 **Aa**	1.00 **Ab**	0.15 **Cc**	0.41 **Bc**	2.56 **Aa**
Succinate	0.61 **Aa**	0.70 **Abc**	0.67 **Ab**	0.64 **Aa**	0.70 **Aab**	0.71 **Ab**	0.57 **Ba**	0.82 **Aa**	0.86 **Aa**	0.63 **ABa**	0.58 **Bc**	0.71 **Ab**
Sugar	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Cellobiose	0.80 **Ab**	0.83 **Aa**	0.90 **Aa**	1.22 **Aa**	0.35 **Bb**	0.29 **Bb**	1.25 **Aa**	0.19 **Bc**	0.21 **Bc**	1.09 **Aab**	0.67 **Ba**	0.16 **Cc**
Fructose	5.34 **Aa**	5.44 **Aa**	5.39 **Aa**	5.10 **Aa**	3.50 **Bb**	3.12 **Bb**	4.33 **Aa**	1.03 **Bd**	0.98 **Bc**	6.17 **Aa**	1.92 **Bc**	0.64 **Cd**
Fucose	1.76 **Ac**	1.86 **Aa**	1.83 **Aa**	1.94 **Abc**	1.18 **Bb**	0.84 **Cb**	2.46 **Ab**	1.02 **Bb**	0.90 **Bb**	3.75 **Aa**	2.36 **Ba**	0.15 **Cc**

SI Table 3. *(continuation)* Relative abundance of primary metabolites of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Sugar	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Glucose_1	1.85 **Ab**	1.90 **Aa**	1.93 **Aa**	2.17 **Aab**	1.46 **Bb**	1.30 **Bb**	1.81 **Ab**	0.95 **Bc**	0.99 **Bc**	2.52 **Aa**	1.85 **Ba**	0.86 **Cc**
Maltose	1.19 **Aa**	1.26 **Aa**	1.27 **Aa**	1.48 **Aa**	1.03 **Aa**	0.21 **Bb**	0.94 **Aa**	0.14 **Bb**	0.15 **Bb**	1.56 **Aa**	1.33 **Aa**	0.12 **Bb**
Sucrose	1.00 **Ac**	1.15 **Ab**	1.08 **Aa**	1.13 **Ab**	0.89 **Cc**	1.01 **Ba**	1.27 **Ab**	1.03 **Bb**	1.05 **Ba**	1.46 **Aa**	1.32 **Aa**	0.95 **Ba**
Trehalose	1.17 **Ab**	1.12 **Aa**	1.22 **Aa**	1.80 **Aa**	0.49 **Bb**	0.41 **Bb**	1.82 **Aa**	0.26 **Bc**	0.30 **Bc**	1.59 **Aab**	0.94 **Ba**	0.24 **Cc**
Xylose	0.67 **Bb**	0.60 **Ab**	0.73 **Ab**	0.68 **Ba**	0.75 **Aa**	0.81 **Aa**	0.69 **Ba**	0.84 **Aa**	0.82 **Aa**	0.74 **Ba**	0.80 **Aa**	0.82 **Aa**
Glycerol	0.67 **Ab**	0.72 **Ab**	0.65 **Aa**	0.70 **Ab**	0.62 **Bb**	0.64 **ABa**	0.83 **Aa**	0.68 **Bb**	0.65 **Ba**	0.88 **Aa**	0.91 **Aa**	0.60 **Ba**
Glycerol 3-P	0.49 **Aa**	0.41 **Ab**	0.50 **Ac**	0.46 **Aa**	0.57 **Aab**	0.53 **Abc**	0.55 **Aa**	0.67 **Aa**	0.64 **Ab**	0.50 **Ca**	0.66 **Ba**	0.82 **Aa**
Others	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Putrescine	0.66 **Aa**	0.67 **Ab**	0.70 **Aa**	0.26 **Bb**	1.01 **Aa**	0.83 **Aa**	0.29 **Cb**	1.12 **Aa**	0.78 **Ba**	0.18 **Cc**	0.25 **Bc**	0.38 **Ab**
Spermidine	1.28 **Aa**	1.35 **Ab**	1.38 **Abc**	0.73 **Aab**	0.81 **Ab**	1.00 **Ac**	0.71 **Bab**	2.74 **Aa**	4.25 **Aa**	0.64 **Bb**	1.00 **Bb**	2.02 **Ab**
Adenine	0.96 **Aa**	0.92 **Ab**	0.97 **Aab**	0.74 **Ba**	1.57 **Aa**	1.20 **Aa**	0.79 **Ba**	1.50 **Aa**	1.16 **Aa**	0.84 **Aa**	0.91 **Ab**	0.70 **Ab**
Uracil	0.99 **Aa**	1.00 **Aa**	0.95 **Aa**	0.85 **Aab**	1.19 **Aa**	1.10 **Aa**	1.07 **Aa**	1.10 **Aa**	0.82 **Aa**	0.65 **Ab**	0.55 **ABb**	0.40 **Bb**
2-Hydroxypyridine	0.90 **Aab**	0.90 **Aab**	0.81 **Aab**	0.73 **Ab**	0.86 **Ab**	0.83 **Ab**	0.96 **Aab**	0.96 **Aab**	0.86 **Aab**	1.05 **Aa**	1.03 **Aa**	1.08 **Aa**
4-Aminobutanoate	2.85 **Aa**	2.86 **Aa**	2.87 **Aa**	2.77 **Aa**	1.76 **Bb**	1.35 **Bb**	2.71 **Aa**	1.02 **Bc**	0.85 **Bc**	2.99 **Aa**	0.41 **Bd**	0.26 **Bd**
Galactinol	2.73 **Ac**	2.56 **Aa**	2.53 **Aa**	4.98 **Aab**	0.95 **Bb**	1.03 **Bb**	5.25 **Aa**	0.67 **Bb**	0.80 **Bb**	3.53 **Abc**	3.97 **Aa**	0.30 **Bc**
Myo-Inositol	0.98 **Ab**	1.00 **Ab**	0.92 **Aa**	1.16 **Ab**	0.78 **Bc**	0.76 **Bb**	1.06 **Ab**	0.65 **Bd**	0.56 **Bc**	1.77 **Aa**	1.19 **Ba**	0.60 **Cc**
Orthophosphate	0.71 **Ac**	0.67 **Ac**	0.81 **Ab**	0.76 **Ac**	0.72 **Ac**	0.74 **Ab**	0.88 **Ab**	0.80 **Bb**	0.84 **ABa**	0.99 **Aa**	0.92 **Aa**	0.74 **Bb**

SI Table 4. Relative abundance of primary metabolites of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Amino acid	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
Alanine	2.1 **Aa**	2.09 **Aa**	2.05 **Aa**	0.46 **Bb**	2.06 **Aa**	2.10 **Aa**	0.38 **Cb**	1.05 **Bb**	2.01 **Aa**	0.37 **Cb**	0.74 **Bb**	1.80 **Aa**
Arginine	1.6 **Aa**	1.33 **Aa**	1.94 **Aa**	0.27 **Bb**	1.45 **Aa**	1.63 **Aab**	0.21 **Bb**	0.71 **Ab**	1.05 **Ab**	0.17 **Cb**	0.33 **Bc**	0.64 **Ac**
Asparagine	2.3 **Aa**	2.20 **Aa**	2.38 **Aa**	0.35 **Bb**	1.21 **Ab**	0.89 **Ab**	0.27 **Bb**	0.73 **Ac**	0.93 **Ab**	0.29 **Cb**	0.92 **Bbc**	2.33 **Aa**
Aspartate	1.8 **Aa**	1.80 **Aa**	1.70 **Ab**	0.33 **Bb**	1.14 **Ab**	1.27 **Ac**	0.32 **Cb**	0.98 **Bb**	2.10 **Aab**	0.22 **Cc**	0.94 **Bb**	2.29 **Aa**
b-Alanine	2.6 **Aa**	2.86 **Aa**	2.40 **Aa**	1.24 **Ab**	1.04 **Abc**	1.19 **Ac**	0.82 **Bb**	1.18 **ABb**	1.45 **Abc**	0.46 **Cc**	0.75 **Bc**	1.90 **Aab**
Citrulline	0.7 **Aa**	0.56 **Aa**	0.79 **Aa**	0.71 **Aa**	0.68 **Aa**	0.76 **Aa**	0.69 **Aa**	0.45 **Aa**	0.67 **Aa**	0.66 **Aa**	2.17 **Aa**	0.69 **Aa**
Glutamate	1.7 **Aa**	1.73 **Aa**	1.58 **Aa**	1.51 **Aa**	1.68 **Aa**	1.56 **Aa**	1.66 **Aa**	1.64 **Aa**	1.70 **Aa**	1.57 **Aa**	1.65 **Aa**	1.50 **Aa**
Glutamine_2	4.1 **ABa**	2.42 **Ba**	5.79 **Aa**	0.49 **Bb**	2.47 **Aa**	2.32 **Ab**	0.49 **Bb**	1.95 **Aa**	1.93 **Ab**	0.39 **Cb**	2.56 **Ba**	4.35 **Aa**
Glycine	0.9 **Aa**	0.95 **Aa**	0.77 **Ab**	0.75 **Ba**	1.15 **Aa**	1.13 **Aa**	0.69 **Ba**	0.93 **Aa**	1.12 **Aa**	0.51 **Bb**	0.67 **Bb**	1.15 **Aa**
Isoleucine	0.5 **Ab**	0.51 **Ab**	0.43 **Ab**	0.88 **Aa**	0.64 **ABb**	0.53 **Bb**	0.85 **Aa**	0.73 **Aab**	0.65 **Ab**	0.74 **Aa**	1.09 **Aa**	1.00 **Aa**
Leucine	0.8 **Ab**	0.87 **Aa**	0.77 **Ab**	1.50 **Aa**	1.09 **ABa**	0.88 **Bb**	1.43 **Aa**	1.21 **ABa**	0.94 **Bb**	1.16 **Aab**	1.35 **Aa**	1.47 **Aa**
Lysine	1.1 **Aa**	1.04 **Aa**	1.12 **Aa**	0.56 **Bb**	1.05 **Aa**	1.14 **Aa**	0.61 **Cb**	0.90 **Bab**	1.36 **Aa**	0.49 **Cb**	0.68 **Bb**	1.37 **Aa**
Methionine	1 **Aa**	1.04 **Ab**	0.97 **Ab**	1.30 **Aa**	1.53 **Aa**	1.29 **Aab**	1.27 **ABa**	1.04 **Bb**	1.51 **Aa**	1.49 **Aa**	0.78 **Bb**	1.72 **Aa**
Ornithine	5.4 **ABa**	4.36 **Ba**	6.35 **Aa**	0.25 **Bb**	1.11 **Ab**	1.24 **Ab**	0.24 **Cb**	0.71 **Bc**	1.23 **Ab**	0.18 **Cb**	0.31 **Bd**	0.81 **Ac**
Phenylalanine	0.5 **Ab**	0.48 **Ac**	0.49 **Ac**	0.78 **Aa**	0.95 **Ab**	0.81 **Ab**	0.64 **Ca**	1.25 **Aa**	0.83 **Bb**	0.73 **Ca**	1.42 **Ba**	1.97 **Aa**
Proline	0.5 **Ab**	0.48 **Ac**	0.56 **Aa**	0.72 **Aa**	0.56 **Bbc**	0.60 **ABa**	0.75 **Ba**	2.10 **Aa**	0.57 **Ca**	0.70 **Aa**	0.66 **Ab**	0.60 **Aa**
Serine	0.4 **Aa**	0.42 **Aa**	0.42 **Ab**	0.27 **Bb**	0.36 **Aab**	0.36 **Ab**	0.25 **Bb**	0.29 **Bb**	0.56 **Aa**	0.25 **Cb**	0.44 **Ba**	0.60 **Aa**
Threonine	1 **Aa**	0.99 **Aa**	1.00 **Ab**	0.56 **Bb**	0.72 **Ab**	0.69 **Abc**	0.58 **Bb**	0.71 **Bb**	0.90 **Ab**	0.53 **Cb**	0.87 **Bab**	1.52 **Aa**
Tryptophan	0.4 **Ab**	0.39 **Ab**	0.35 **Ac**	2.26 **Aa**	2.37 **Aa**	1.83 **Aa**	2.95 **Aa**	2.23 **Aa**	0.99 **Bb**	2.34 **Aa**	2.06 **Aa**	2.01 **Aa**
Tyrosine	0.4 **Ab**	0.37 **Ab**	0.34 **Ac**	0.64 **Ba**	1.21 **Aa**	1.07 **Ab**	0.61 **Ca**	1.23 **Aa**	0.98 **Bb**	0.61 **Ca**	1.05 **Ba**	1.60 **Aa**
Valine	0.8 **Aa**	0.91 **Aa**	0.72 **Aa**	0.90 **Aa**	1.05 **Aa**	0.85 **Aa**	0.87 **Aa**	1.03 **Aa**	1.18 **Aa**	0.71 **Aa**	0.92 **Aa**	0.88 **Aa**
5-Oxoproline	1.4 **Ba**	1.36 **ABa**	1.46 **Aa**	0.87 **Bb**	1.09 **ABb**	1.08 **Ab**	1.12 **Bb**	0.98 **ABb**	1.23 **Ab**	0.93 **Bb**	1.02 **ABb**	1.12 **Ab**

SI Table 4. *(continuation)* Relative abundance of primary metabolites of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Organic acid	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
Benzoate	0.9 **Aa**	0.89 **Aa**	0.85 **Aa**	1.18 **Aa**	1.19 **Aa**	0.97 **Aa**	1.14 **Aa**	1.22 **Aa**	1.04 **Aa**	1.24 **Aa**	1.31 **Aa**	0.95 **Aa**
Citramalate	0.9 **Ab**	0.98 **Ab**	0.78 **Ac**	1.39 **Aa**	1.39 **Aa**	1.11 **Ab**	1.39 **Ba**	1.09 **Bab**	2.80 **Aa**	1.57 **Aa**	0.56 **Bc**	0.30 **Cd**
Citrate	3.6 **ABd**	3.91 **Aa**	3.25 **Ba**	5.26 **Ac**	2.69 **Cb**	3.30 **Ba**	6.59 **Ab**	2.19 **Bc**	2.45 **Bb**	8.59 **Aa**	2.67 **Bb**	1.07 **Cc**
Dehydroascorbate	1.3 **Aa**	1.06 **Ca**	1.48 **Ba**	1.52 **Aa**	0.97 **Ca**	0.96 **Ba**	1.75 **Aa**	0.90 **Ca**	1.18 **Ba**	1.36 **Aa**	0.74 **Ca**	1.02 **Ba**
Fumarate	1.2 **Aa**	1.34 **Aa**	0.97 **Aa**	1.00 **Aa**	1.24 **Aa**	0.84 **Aa**	1.29 **Aa**	1.16 **Aa**	0.94 **Aa**	1.11 **Aa**	1.21 **Aa**	0.91 **Aa**
Gluconate	1.4 **Ab**	1.09 **Aa**	1.61 **Aa**	3.44 **Aa**	0.28 **Bb**	0.49 **Bb**	1.97 **Aab**	0.24 **Bb**	0.26 **Bb**	1.28 **Ab**	0.33 **Bb**	0.30 **Bb**
Glycerate	1.2 **Aa**	1.34 **Ba**	1.16 **Ba**	1.25 **Ab**	0.49 **Bb**	0.65 **Bb**	1.28 **Ab**	0.55 **Bb**	0.69 **Bb**	1.28 **Ab**	1.37 **Bb**	0.52 **Bb**
Ketoglutarate	0.6 **Ac**	0.54 **Ac**	0.56 **Ac**	0.83 **Ab**	1.04 **Ab**	0.76 **Ab**	1.10 **Aa**	1.45 **Aa**	1.12 **Aa**	0.87 **Ab**	0.95 **Ab**	0.86 **Ab**
Lactate	0.2 **Aa**	0.25 **Aa**	0.19 **Ba**	0.30 **Aa**	0.28 **Aa**	0.20 **Ba**	0.22 **Aa**	0.29 **Aa**	0.14 **Ba**	0.28 **Aa**	0.26 **Aa**	0.23 **Ba**
Malate	2.6 **Ab**	2.93 **Aa**	2.23 **Aa**	3.98 **Aa**	2.73 **Ba**	2.32 **Ba**	4.04 **Aa**	3.53 **Aa**	2.13 **Ba**	3.41 **Aab**	3.07 **Aa**	2.73 **Aa**
Nicotinate	2.2 **Ca**	1.99 **Ba**	2.33 **Aa**	1.98 **Ca**	3.06 **Ba**	3.15 **Aa**	1.82 **Ca**	2.85 **Ba**	3.56 **Aa**	1.45 **Ca**	1.91 **Ba**	3.11 **Aa**
Pyruvate	0.7 **Aa**	0.70 **Ac**	0.61 **Ac**	0.51 **Ca**	3.68 **Aa**	2.03 **Bb**	0.80 **Ba**	4.02 **Aa**	3.73 **Aa**	0.60 **Ca**	1.67 **Bb**	5.31 **Aa**
Succinate	1.1 **Aab**	1.22 **Aa**	1.04 **Ab**	1.15 **Ba**	1.43 **Aa**	1.32 **ABa**	1.28 **Aa**	1.45 **Aa**	1.50 **Aa**	0.91 **Ab**	0.75 **Bb**	0.86 **ABb**
Sugar	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Cellobiose	0.7 **Ab**	0.66 **Ac**	0.72 **Ac**	1.71 **Aa**	1.47 **Aab**	1.26 **Ab**	1.58 **Aa**	1.98 **Aa**	0.93 **Bbc**	1.63 **Ba**	1.32 **Bb**	2.89 **Aa**
Fructose	0.7 **Ab**	0.60 **Ab**	0.82 **Ab**	1.49 **Aab**	0.69 **Aab**	0.66 **Aab**	1.00 **Ab**	0.71 **Ab**	0.42 **Ab**	0.98 **Aa**	3.58 **Aa**	1.08 **Aa**
Fucose	1.6 **Ab**	1.43 **Aa**	1.86 **Aa**	2.91 **Aa**	0.25 **Bc**	0.48 **Bb**	2.87 **Aab**	0.71 **Bb**	0.63 **Bb**	3.79 **Aa**	0.56 **Bb**	0.18 **Cc**
Glucose_1	0.7 **Ab**	0.56 **Ab**	0.77 **Ab**	2.10 **Aa**	0.92 **Bab**	0.83 **Bab**	1.04 **Ab**	0.91 **ABab**	0.53 **Bb**	0.99 **Ab**	2.25 **Aa**	1.38 **Aa**
Maltose	0.7 **Ab**	0.76 **Ab**	0.69 **Ac**	5.08 **Aa**	1.11 **Bab**	4.21 **Aab**	1.55 **Aab**	2.81 **Aa**	1.95 **Abc**	1.88 **Bab**	4.65 **ABa**	9.02 **Aa**
Sucrose	0.2 **Ab**	0.22 **Ac**	0.20 **Ac**	1.45 **Aa**	0.25 **Bc**	0.24 **Bbc**	1.64 **Aa**	0.80 **Bb**	0.29 **Cb**	1.95 **Aa**	1.62 **Aa**	0.63 **Ba**
Trehalose	1 **Ab**	0.91 **Aab**	1.02 **Aa**	2.09 **Aa**	0.62 **Bb**	0.59 **Bb**	2.14 **Aa**	0.93 **Bab**	0.45 **Cb**	2.17 **Aa**	1.05 **Ba**	1.12 **Ba**
Xylose	1.4 **Aa**	1.42 **Aa**	1.48 **Aa**	1.29 **Aa**	1.40 **Aa**	1.44 **Aa**	1.59 **Aa**	1.38 **Aa**	1.57 **Aa**	1.37 **Aa**	1.67 **Aa**	1.87 **Aa**
Glycerol	1 **Ab**	1.02 **Ab**	1.06 **Aa**	1.44 **Aa**	1.23 **Aa**	1.24 **Aa**	1.55 **Aa**	1.33 **ABa**	1.20 **Ba**	1.57 **Aa**	1.31 **Ba**	1.05 **Ca**
Glycerol 3-P	0.5 **Ab**	0.44 **Ab**	0.61 **Ab**	0.71 **Aa**	0.83 **Aa**	0.93 **Aa**	0.71 **Aa**	0.74 **Aa**	0.75 **Aa**	0.72 **Aa**	0.78 **Aa**	0.93 **Aa**

SI Table 4. *(continuation)* Relative abundance of primary metabolites of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Others	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
Putrescine	2.4 **Aa**	2.50 **Aa**	2.40 **Ab**	1.25 **Bb**	2.97 **Aa**	2.92 **Aab**	0.97 **Bbc**	2.91 **Aa**	3.24 **Aa**	0.86 **Bc**	0.92 **Bb**	2.90 **Aab**
Spermidine	0.4 **Ac**	0.38 **Ac**	0.45 **Ac**	0.89 **Ab**	0.76 **Ab**	0.53 **Ab**	0.81 **Aab**	0.81 **Aab**	0.67 **Aab**	0.93 **Aa**	1.03 **Aa**	1.01 **Aa**
Adenine	1.3 **Aa**	1.16 **Aa**	1.48 **Aa**	1.03 **Ab**	0.72 **Ab**	0.69 **Ab**	0.85 **Ab**	0.74 **Ab**	0.83 **Ab**	0.82 **Aab**	1.17 **Aab**	0.96 **Aab**
Uracil	1.5 **Aa**	1.23 **Ba**	1.69 **Aa**	1.36 **Aab**	0.90 **Bab**	1.17 **Aab**	1.14 **Abc**	0.70 **Bbc**	1.00 **Abc**	0.67 **Ac**	0.53 **Bc**	0.90 **Ac**
2-Hydroxypyridine	1.2 **Aa**	1.29 **Aa**	1.01 **Ba**	1.08 **Aa**	1.09 **Aa**	0.99 **Ba**	1.23 **Aa**	1.31 **Aa**	0.95 **Ba**	1.46 **Aa**	1.18 **Aa**	1.06 **Ba**
4-Aminobutanoate	2.6 **Aa**	2.44 **Aa**	2.81 **Aa**	1.47 **Aab**	0.34 **Bb**	0.62 **Bb**	1.36 **Aab**	0.23 **Bb**	0.42 **Bb**	0.77 **Ab**	0.58 **Bb**	0.17 **Bc**
Galactinol	0.5 **Ab**	0.42 **Ab**	0.65 **Aa**	4.94 **Aa**	0.51 **Bb**	0.99 **Ba**	4.28 **Aa**	0.71 **Bab**	0.43 **Ba**	4.92 **Aa**	1.28 **Ba**	0.51 **Ca**
Myo-Inositol	0.5 **Ab**	0.45 **Abc**	0.57 **Aa**	1.07 **Aa**	0.34 **Bc**	0.49 **Ba**	1.29 **Aa**	0.63 **Bab**	0.56 **Ba**	1.36 **Aa**	1.30 **Aa**	0.43 **Ba**
Orthophosphate	1.4 **Aa**	1.49 **Aa**	1.30 **Aa**	1.52 **Aa**	1.52 **Aa**	1.43 **Aa**	1.56 **Aa**	1.66 **Aa**	1.57 **Aa**	1.59 **Aa**	1.53 **Aa**	1.33 **Aa**

SI Table 5. Relative abundance of primary metabolites of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
Alanine	0.65 **Ca**	1.01 **Bb**	1.13 **Ac**	0.59 **Bb**	1.29 **Aa**	1.24 **Abc**	0.52 **Bc**	1.34 **Aa**	1.35 **Aab**	0.56 **Cbc**	1.01 **Bb**	1.44 **Aa**
Arginine	0.82 **Ba**	2.31 **Aa**	2.58 **Aa**	0.16 **Bb**	2.92 **Aa**	2.33 **Aa**	0.14 **Bb**	2.81 **Aa**	2.18 **Aab**	0.13 **Cb**	0.37 **Bb**	1.54 **Ab**
Asparagine	0.63 **Ba**	1.03 **Aa**	1.39 **Ac**	0.37 **Bb**	1.18 **Aa**	1.57 **Ac**	0.46 **Cab**	1.46 **Ba**	2.54 **Ab**	0.53 **Ba**	0.64 **Bb**	6.64 **Aa**
Aspartate	1.82 **Ba**	2.41 **Aa**	2.55 **Ab**	0.70 **Bb**	2.44 **Aa**	2.43 **Ab**	0.43 **Cd**	2.66 **Ba**	3.37 **Aa**	0.58 **Cc**	0.79 **Bb**	3.00 **Aa**
b-Alanine	1.57 **Aa**	1.36 **Aa**	1.35 **Aa**	1.31 **Aa**	1.06 **Aab**	0.93 **Aab**	0.56 **Bb**	0.99 **Aab**	0.85 **ABb**	0.54 **Bb**	0.70 **Bb**	1.68 **Aa**
Citrulline	1.02 **Ba**	1.28 **Aba**	1.41 **Aa**	1.11 **Ba**	1.47 **Aa**	1.15 **Bab**	1.15 **Aba**	1.32 **Aa**	1.07 **Bb**	0.95 **Ba**	0.81 **Bb**	1.32 **Aab**
Glutamate	0.72 **Bb**	0.76 **Abc**	0.80 **Ab**	0.78 **Ab**	0.81 **Abc**	0.80 **Ab**	1.05 **Aa**	0.88 **Bb**	0.87 **Bb**	1.00 **Aa**	1.06 **Aa**	1.00 **Aa**
Glutamine_2	0.22 **Ba**	1.75 **Aa**	1.99 **Aa**	0.15 **Ba**	1.85 **Aa**	1.70 **Aa**	0.22 **Ba**	0.92 **Ab**	1.05 **Ab**	0.21 **Ca**	0.50 **Bb**	2.37 **Aa**
Glycine	0.88 **Ba**	1.09 **Abb**	1.15 **Ab**	0.76 **Ba**	1.60 **Aa**	1.69 **Aa**	0.71 **Ba**	1.61 **Aa**	1.67 **Aa**	0.72 **Ca**	0.92 **Bb**	1.27 **Ab**
Isoleucine	1.14 **Aa**	0.87 **Bd**	0.96 **Bc**	1.00 **Aab**	1.08 **Ac**	1.05 **Abc**	0.89 **Bb**	1.29 **Ab**	1.18 **Ab**	0.89 **Cb**	1.56 **Ba**	2.38 **Aa**
Leucine	0.89 **Aa**	0.59 **Bd**	0.67 **Bb**	0.94 **Aa**	0.81 **Abc**	0.74 **Bb**	0.88 **Aba**	1.00 **Ab**	0.79 **Bb**	0.81 **Ca**	1.54 **Ba**	2.06 **Aa**
Lysine	0.65 **Aab**	0.64 **Ab**	0.64 **Ac**	0.58 **Bab**	1.82 **Aa**	1.51 **Ab**	0.76 **Ca**	1.92 **Aa**	1.44 **Bb**	0.52 **Cb**	0.84 **Bb**	2.41 **Aa**

SI Table 5. *(continuation)* Relative abundance of primary metabolites of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Methionine	0.38 **Bb**	0.58 **Aab**	0.66 **Ab**	0.50 **Ab**	0.44 **Ab**	0.42 **Ac**	0.77 **Aa**	0.53 **Bb**	0.60 **ABb**	0.83 **Ba**	0.77 **Ba**	1.79 **Aa**
Ornithine	0.84 **Ba**	2.68 **Aa**	2.01 **Aa**	0.25 **Bb**	2.48 **Aa**	1.81 **Aa**	0.17 **Bc**	2.29 **Aa**	1.65 **Aa**	0.24 **Bbc**	0.27 **Bb**	1.09 **Ab**
Phenylalanine	0.97 **Ab**	0.93 **Ac**	1.04 **Ab**	1.42 **Aa**	0.94 **Bc**	0.97 **Bb**	1.33 **Aa**	1.38 **Ab**	0.95 **Bb**	1.21 **Ba**	2.29 **Aa**	2.51 **Aa**
Proline	1.58 **Ba**	1.78 **Bb**	2.19 **Ab**	1.56 **Ba**	2.45 **Aa**	2.54 **Aab**	1.27 **Bb**	2.61 **Aa**	2.67 **Aa**	0.97 **Ac**	0.73 **Bc**	0.91 **Ac**
Serine	1.58 **Aa**	1.31 **Bc**	1.40 **Bd**	1.05 **Bb**	1.55 **Ab**	1.60 **Ac**	1.14 **Bb**	1.90 **Aa**	2.06 **Ab**	1.14 **Cb**	1.26 **Bc**	2.52 **Aa**
Threonine	1.38 **Aa**	1.02 **Bc**	1.06 **Bd**	0.78 **Bb**	1.32 **Ab**	1.39 **Ac**	0.81 **Bb**	1.60 **Aa**	1.73 **Ab**	0.80 **Cb**	1.05 **Bc**	2.53 **Aa**
Tryptophan	0.69 **Ac**	0.37 **Bc**	0.40 **Bc**	1.39 **Aa**	0.55 **Bb**	0.66 **Bb**	1.01 **Ab**	0.61 **Bb**	0.66 **Bb**	1.25 **Bab**	1.67 **Aa**	1.83 **Aa**
Tyrosine	1.43 **Aa**	0.98 **Bc**	1.05 **Bb**	1.76 **Aa**	0.99 **Bc**	1.15 **Bb**	1.45 **Aa**	1.28 **Ab**	1.01 **Bb**	1.58 **Aa**	1.70 **Aa**	1.87 **Aa**
Valine	1.06 **Aa**	0.92 **Ac**	1.06 **Ac**	0.86 **Bb**	1.21 **Ab**	1.09 **Ac**	1.02 **Ca**	1.54 **Aa**	1.34 **Bb**	0.72 **Cc**	1.43 **Ba**	1.91 **Aa**
5-Oxoproline	0.8 **Ba**	1.17 **Aa**	1.26 **Aa**	0.54 **Bc**	1.22 **Aa**	1.32 **Aa**	0.68 **Bab**	1.12 **Aa**	1.19 **Aa**	0.60 **Cbc**	0.94 **Bb**	1.42 **Aa**
Organic acid	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Benzoate	0.87 **Aa**	0.81 **Aa**	1.01 **Aa**	1.01 **Aa**	1.00 **Aa**	0.80 **Aa**	1.47 **Aa**	0.90 **Aa**	0.72 **Aa**	1.03 **Aa**	1.00 **Aa**	1.19 **Aa**
Citramalate	0.52 **Ac**	0.49 **Ab**	0.43 **Aa**	1.21 **Ab**	0.63 **Bb**	0.53 **Ba**	1.14 **Ab**	0.56 **Bb**	0.49 **Ba**	4.18 **Aa**	1.94 **Ba**	0.26 **Cb**
Citrate	2.53 **Ba**	4.74 **Aa**	5.31 **Aa**	1.17 **Bb**	4.41 **Aa**	4.29 **Aa**	1.02 **Bbc**	2.27 **Ab**	2.52 **Ab**	0.80 **Ac**	0.86 **Ac**	0.16 **Bc**
Dehydroascorbate	1.61 **Aa**	0.96 **Ba**	0.90 **Bb**	1.11 **Abc**	0.85 **Ba**	0.71 **Cc**	1.07 **Ac**	0.90 **Aa**	0.70 **Bc**	1.34 **Aab**	0.93 **Ba**	1.14 **Aa**
Fumarate	0.7 **Bc**	0.68 **Bc**	0.76 **Ab**	0.81 **Ab**	0.77 **ABb**	0.75 **Bb**	1.08 **Aa**	0.79 **Bb**	0.80 **Bb**	1.10 **Aa**	1.00 **Ba**	0.92 **Ca**
Gluconate	12.8 **Aa**	8.00 **ABa**	6.87 **Ba**	8.92 **Aa**	2.00 **Bb**	1.94 **Bb**	4.56 **Ab**	0.78 **Bc**	0.46 **Cc**	4.18 **Ab**	1.32 **Bb**	0.19 **Cd**
Glycerate	1.86 **Aa**	1.20 **Ba**	1.19 **Ba**	1.52 **Aa**	1.06 **Ba**	1.16 **Ba**	1.03 **Ab**	0.74 **Bb**	0.65 **Bb**	1.09 **Ab**	0.68 **Bb**	0.79 **Bb**
Ketoglutarate	0.99 **ABbc**	1.05 **Abc**	0.75 **Bbc**	0.89 **ABc**	0.81 **Ac**	0.79 **Bc**	1.14 **ABab**	1.01 **Aab**	0.96 **Bab**	1.05 **Aba**	1.37 **Aa**	1.10 **Ba**
Lactate	0.68 **Cc**	0.83 **Bc**	1.00 **Ab**	1.36 **Ab**	1.34 **Ab**	1.36 **Aa**	1.74 **Aa**	1.21 **Bb**	1.31 **Ba**	1.75 **Ba**	1.95 **Aa**	1.03 **Cb**
Malate	0.56 **Bb**	0.64 **ABb**	0.70 **Ac**	0.60 **Bb**	0.74 **Ab**	0.70 **Ac**	0.91 **Ba**	1.02 **ABa**	1.15 **Ab**	0.67 **Bb**	0.71 **Bb**	1.71 **Aa**
Nicotinate	1.01 **Aa**	0.80 **Bb**	0.77 **Bc**	0.73 **Bb**	0.93 **Aab**	0.90 **Ab**	0.71 **Bb**	1.07 **Aa**	0.98 **Ab**	0.59 **Cc**	0.85 **Bb**	1.15 **Aa**
Pyruvate	0.34 **Bb**	0.34 **ABc**	0.51 **Ac**	0.26 **Bb**	1.00 **Ab**	0.75 **Ac**	0.54 **Ba**	2.00 **Aa**	2.53 **Ab**	0.56 **Ca**	1.09 **Bb**	5.38 **Aa**
Succinate	0.97 **Aa**	0.65 **Bb**	0.75 **Bb**	0.81 **Ba**	0.92 **ABa**	1.08 **Aa**	0.85 **Ba**	0.99 **ABa**	1.15 **Aa**	0.86 **Ba**	0.93 **ABa**	1.06 **Aa**
Sugar	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Cellobiose	0.98 **Ab**	0.73 **Aa**	0.79 **Aa**	1.38 **Aa**	0.26 **Bc**	0.27 **Bb**	1.65 **Aa**	0.34 **Bbc**	0.13 **Cc**	1.61 **Aa**	0.42 **Bb**	0.09 **Cc**
Fructose	7.61 **Aa**	4.71 **Ba**	2.94 **Ca**	6.69 **Aa**	0.93 **Bc**	0.95 **Bb**	3.08 **Ab**	0.62 **Bd**	0.65 **Bc**	3.02 **Ab**	1.33 **Bb**	0.62 **Cc**
Fucose	1.23 **Ab**	1.01 **Ab**	1.04 **Aa**	1.79 **Aab**	0.99 **Bb**	1.26 **ABa**	1.57 **Aab**	1.27 **Ab**	1.15 **Aa**	2.03 **Aa**	2.16 **Aa**	0.19 **Bb**

SI Table 5. *(continuation)* Relative abundance of primary metabolites of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Sugar	**0h_0N**		**0h_3.5N**		**0h_7N**		**10h_0N**		**10h_3.5N**		**10h_7N**		**18h_0N**		**18h_3.5N**		**18h_7N**		**36h_0N**		**36h_3.5N**		**36h_7N**	
Glucose_1	2.21	**Aa**	1.20	**Ba**	0.85	**Cab**	2.43	**Aa**	0.88	**Bb**	1.02	**Ba**	2.03	**Aab**	0.81	**Bb**	0.87	**Bab**	1.69	**Ab**	1.43	**Aa**	0.78	**Bb**
Maltose	1.07	**Aa**	0.70	**Aa**	0.99	**Aa**	1.04	**Aa**	0.58	**Aa**	0.51	**Aab**	1.25	**Aa**	0.67	**ABa**	0.37	**Bbc**	2.91	**Aa**	1.03	**Aa**	0.16	**Bc**
Sucrose	1.04	**Ac**	1.08	**Ab**	1.11	**Aa**	1.22	**Ab**	1.07	**Bb**	1.04	**Ba**	1.66	**Aa**	1.10	**Bb**	1.10	**Ba**	1.77	**Aa**	1.53	**Ba**	1.11	**Ca**
Trehalose	1.42	**Ab**	1.05	**Aa**	1.15	**Aa**	2.03	**Aa**	0.37	**Bc**	0.38	**Bb**	2.41	**Aa**	0.49	**Bbc**	0.17	**Cc**	2.35	**Aa**	0.58	**Bb**	0.13	**Cc**
Xylose	0.65	**Bc**	0.79	**Ab**	0.85	**Ab**	0.66	**Bc**	0.92	**Aa**	0.91	**Aab**	0.99	**Aa**	0.86	**Bab**	0.89	**ABab**	0.78	**Cb**	0.90	**Bab**	1.02	**Aa**
Glycerol	0.82	**Ab**	0.78	**Ab**	0.78	**Aa**	0.89	**Ab**	0.76	**Abc**	0.75	**Aab**	1.16	**Aa**	0.71	**Bc**	0.71	**Bb**	1.22	**Aa**	1.09	**Ba**	0.79	**Ca**
Glycerol 3-P	0.99	**Aab**	0.90	**Ab**	0.78	**Bc**	0.92	**Ab**	0.98	**Ab**	0.96	**Ab**	0.94	**Aab**	0.93	**Ab**	0.91	**Ab**	1.04	**Ca**	1.27	**Ba**	1.45	**Aa**
Others	**0h_0N**		**0h_3.5N**		**0h_7N**		**10h_0N**		**10h_3.5N**		**10h_7N**		**18h_0N**		**18h_3.5N**		**18h_7N**		**36h_0N**		**36h_3.5N**		**36h_7N**	
Putrescine	0.34	**Ba**	0.70	**Aab**	0.85	**Aa**	0.47	**Ba**	0.79	**Aab**	0.66	**ABab**	0.33	**Ca**	1.05	**Aa**	0.64	**Bab**	0.52	**Aa**	0.58	**Ab**	0.51	**Ab**
Spermidine	1.98	**Aa**	1.30	**Ac**	1.06	**Ac**	0.77	**Bb**	4.05	**Aab**	3.44	**Ab**	0.83	**Bb**	5.14	**Aa**	5.06	**Aab**	1.81	**Cab**	2.75	**Bb**	8.18	**Aa**
Adenine	0.53	**Ab**	0.52	**Ab**	0.52	**Ab**	0.83	**Aa**	0.69	**Aa**	0.89	**Aa**	0.54	**Ab**	0.51	**Ab**	0.56	**Ab**	0.45	**Ab**	0.59	**Ab**	0.67	**Ab**
Uracil	1.85	**Aa**	1.34	**Ba**	1.36	**Ba**	1.24	**Ab**	1.32	**Aa**	1.11	**Aa**	1.18	**Abc**	0.88	**Bb**	0.63	**Cb**	0.92	**Ac**	0.73	**Ab**	0.53	**Bb**
2-Hydroxypyridine	0.7	**ABb**	0.64	**Bc**	0.76	**Abc**	0.77	**Ab**	0.72	**Abc**	0.71	**Ac**	0.98	**Aa**	0.75	**Cb**	0.83	**Bb**	0.97	**Aa**	1.05	**Aa**	1.08	**Aa**
4-Aminobutanoate	4.61	**Aa**	4.15	**Aa**	3.15	**Aa**	4.09	**Aa**	1.35	**Bb**	1.61	**Bb**	1.92	**Ab**	0.56	**Bc**	0.58	**Bc**	2.42	**Ab**	0.73	**Bc**	0.44	**Cc**
Galactinol	3.07	**Aa**	2.64	**Aa**	1.30	**Ba**	3.68	**Aa**	0.67	**Bb**	0.65	**Bb**	3.51	**Aa**	0.43	**Bb**	0.34	**Bc**	2.85	**Aa**	1.74	**Aa**	0.26	**Bc**
Myo-Inositol	1.64	**Aab**	1.14	**Ba**	1.04	**Ba**	1.34	**Ac**	0.80	**Bc**	0.78	**Bc**	1.43	**Abc**	0.97	**Bb**	1.01	**Bab**	1.81	**Aa**	1.27	**Ba**	0.90	**Cbc**
Orthophosphate	0.63	**Bc**	0.69	**Ac**	0.72	**Ab**	0.74	**Ab**	0.72	**Abc**	0.72	**Ab**	0.99	**Aa**	0.78	**Bb**	0.77	**Bb**	1.06	**Aa**	0.99	**Aa**	0.87	**Ba**

SI Table 6. Relative abundance of primary metabolites of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Amino acid	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
Alanine	0.5 **Ba**	0.93 **Ab**	0.96 **Ab**	0.40 **Bb**	1.34 **Aa**	1.39 **Aa**	0.45 **Cab**	1.19 **Ba**	1.45 **Aa**	0.39 **Cb**	0.68 **Bc**	1.46 **Aa**
Arginine	0.4 **Ba**	1.65 **Ab**	1.57 **Aa**	0.18 **Bb**	3.41 **Aa**	2.61 **Aa**	0.21 **Bab**	2.00 **Aab**	2.59 **Aa**	0.18 **Bb**	0.30 **Bc**	1.48 **Aa**
Asparagine	0.5 **Ba**	1.01 **Aa**	1.17 **Ac**	0.33 **Bab**	1.25 **Aa**	1.41 **Ac**	0.28 **Cb**	1.39 **Ba**	2.20 **Ab**	0.35 **Bab**	0.51 **Bb**	5.60 **Aa**
Aspartate	0.7 **Ba**	1.36 **Ab**	1.20 **Ac**	0.39 **Bb**	2.01 **Aa**	1.75 **Ab**	0.42 **Cb**	1.47 **Bb**	2.64 **Aa**	0.32 **Cc**	0.43 **Bc**	2.95 **Aa**
b-Alanine	1.7 **Ba**	2.00 **Aa**	1.63 **ABa**	2.09 **Ba**	2.53 **Aa**	1.96 **ABa**	1.59 **Ba**	2.50 **Aa**	2.06 **ABa**	1.30 **Bb**	1.39 **Ab**	1.60 **ABb**
Citrulline	1.1 **Aa**	1.01 **Aab**	1.05 **Aa**	1.06 **Aa**	1.66 **Aa**	1.54 **Aa**	0.67 **Bb**	1.05 **Aab**	1.57 **Aa**	0.71 **Bab**	0.76 **Bb**	1.18 **Aa**
Glutamate	0.8 **Ac**	0.78 **Ac**	0.81 **Ac**	0.91 **Ab**	0.90 **Ab**	0.88 **Ab**	0.99 **Aa**	1.02 **Aa**	0.94 **Aa**	0.97 **Aa**	1.08 **Aa**	1.05 **Aa**
Glutamine_2	0.3 **Ba**	2.15 **Ab**	2.05 **Ab**	0.23 **Ba**	4.30 **Aa**	3.81 **Aa**	0.24 **Ca**	0.60 **Bc**	1.34 **Ac**	0.23 **Ca**	0.65 **Bc**	3.10 **Aab**
Glycine	1.2 **Aa**	1.36 **Aa**	1.11 **Ac**	0.73 **Bb**	1.26 **Aab**	1.45 **Abc**	0.76 **Bb**	1.44 **Aa**	1.76 **Aab**	0.64 **Cb**	0.97 **Bb**	1.88 **Aa**
Isoleucine	1 **Aab**	0.87 **ABd**	0.77 **Bd**	0.91 **Bb**	1.07 **Ac**	1.06 **Ac**	1.08 **Ba**	1.25 **Ab**	1.34 **Ab**	0.95 **Cab**	1.46 **Ba**	2.61 **Aa**
Leucine	1.4 **Aab**	1.12 **Bb**	0.97 **Cc**	1.31 **Aab**	0.95 **Bc**	0.90 **Bc**	1.46 **Aa**	1.20 **Bb**	1.12 **Bb**	1.22 **Cb**	1.68 **Ba**	2.42 **Aa**
Lysine	0.5 **Bb**	0.76 **Ab**	0.80 **Ac**	0.66 **Bb**	1.83 **Aa**	1.47 **Ab**	0.89 **Ba**	1.65 **Aa**	1.45 **Ab**	0.95 **Ba**	1.06 **Bb**	2.13 **Aa**
Methionine	0.8 **Ab**	0.89 **Abc**	0.81 **Ab**	0.79 **Bb**	1.17 **Aa**	1.00 **ABab**	1.23 **Aa**	1.12 **Aab**	1.15 **Aa**	1.45 **Aa**	0.68 **Bc**	1.28 **Aa**
Ornithine	0.6 **Ba**	2.35 **Aab**	2.24 **Aa**	0.36 **Cb**	2.84 **Aa**	1.81 **Ba**	0.30 **Bb**	1.91 **Ab**	1.81 **Aa**	0.26 **Bb**	0.29 **Bc**	1.20 **Ab**
Phenylalanine	0.9 **Ab**	0.80 **Ac**	0.82 **Ab**	1.44 **Aa**	0.87 **Bc**	0.87 **Bb**	1.36 **Aa**	1.08 **Bb**	0.88 **Cb**	1.46 **Ba**	2.53 **Aa**	2.42 **Aa**
Proline	1.9 **Aa**	1.96 **Ac**	2.08 **Ab**	1.45 **Bb**	2.24 **Ab**	2.41 **Aa**	1.14 **Bc**	2.73 **Aa**	2.54 **Aa**	0.78 **Ad**	0.64 **Bd**	0.84 **Ac**
Serine	1.3 **Aa**	1.22 **Ab**	1.21 **Ac**	0.85 **Bb**	1.34 **Ab**	1.32 **Ac**	0.87 **Cb**	1.66 **Ba**	2.01 **Ab**	0.93 **Bb**	0.82 **Cc**	2.58 **Aa**
Threonine	1 **Aa**	1.02 **Abc**	0.99 **Ac**	0.69 **Bb**	1.16 **Aab**	1.21 **Ac**	0.85 **Cab**	1.32 **Ba**	1.62 **Ab**	0.70 **Cb**	0.92 **Bc**	2.69 **Aa**
Tryptophan	0.7 **Ab**	0.39 **Bc**	0.42 **Bc**	1.43 **Aa**	0.65 **Bb**	0.65 **Bb**	1.45 **Aa**	0.81 **Bb**	0.71 **Bb**	1.76 **Aa**	2.09 **Aa**	1.72 **Aa**
Tyrosine	0.7 **Ac**	0.58 **Bc**	0.64 **ABb**	0.94 **Ab**	0.68 **Bc**	0.67 **Bb**	1.18 **Aa**	0.87 **Bb**	0.69 **Cb**	1.19 **Ba**	1.61 **Aa**	1.64 **Aa**
Valine	0.9 **Aa**	1.00 **Ac**	0.87 **Ad**	0.90 **Ba**	1.21 **Ab**	1.08 **Ac**	1.04 **Ba**	1.60 **Aa**	1.52 **Ab**	0.96 **Ca**	1.40 **Bab**	2.01 **Aa**
5-Oxoproline	0.6 **Ba**	0.88 **Ab**	0.79 **Ab**	0.60 **Ba**	1.31 **Aa**	1.31 **Aa**	0.64 **Ba**	1.16 **Aa**	1.40 **Aa**	0.54 **Ca**	0.88 **Bb**	1.55 **Aa**

SI Table 6. *(continuation)* Relative abundance of primary metabolites of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Organic acid	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Benzoate	1 **Aa**	0.93 **ABa**	0.73 **Bb**	0.86 **Aa**	0.96 **Aa**	0.83 **Ab**	1.05 **Aa**	0.85 **ABa**	0.83 **Bb**	0.94 **Ba**	0.88 **Ba**	1.28 **Aa**
Citramalate	0.4 **Ad**	0.38 **Ad**	0.30 **Ab**	0.97 **Ac**	0.93 **ABb**	0.80 **Ba**	1.45 **Ab**	0.61 **Cc**	0.97 **Ba**	6.66 **Aa**	3.55 **Ba**	0.38 **Cb**
Citrate	0.3 **Cb**	1.06 **Bb**	1.54 **Aa**	0.53 **Ba**	1.63 **Aa**	1.51 **Aa**	0.39 **Ba**	0.45 **Bc**	0.69 **Ab**	0.51 **Aa**	0.40 **Ac**	0.26 **Bc**
Dehydroascorbate	1.6 **Aa**	1.19 **Ba**	1.13 **Ba**	1.34 **Ab**	0.86 **Bb**	0.76 **Bb**	1.68 **Ab**	0.80 **Bb**	0.73 **Bb**	2.07 **Aa**	1.20 **Ba**	1.01 **Ba**
Fumarate	0.8 **Aa**	0.78 **ABa**	0.80 **Ba**	1.01 **Aa**	0.93 **ABa**	0.89 **Ba**	1.14 **Aa**	0.84 **ABa**	0.76 **Ba**	1.23 **Aa**	1.10 **ABa**	0.85 **Ba**
Gluconate	6.6 **Aa**	5.01 **ABa**	3.81 **Ba**	3.82 **Ab**	0.79 **Bbc**	0.76 **Bb**	2.89 **Ab**	0.55 **Bc**	0.35 **Cc**	2.54 **Ab**	1.13 **Bb**	0.25 **Cc**
Glycerate	1.5 **Aab**	1.21 **Bab**	1.11 **Bab**	1.50 **Aa**	1.39 **Ba**	1.42 **Ba**	1.56 **Ab**	1.13 **Bb**	1.01 **Bb**	1.76 **Aab**	1.13 **Bab**	1.26 **Bab**
Ketoglutarate	0.9 **Ac**	0.95 **Ab**	0.82 **Aab**	0.97 **Abc**	1.01 **Ab**	0.98 **Aa**	1.12 **Aab**	1.00 **Ab**	0.79 **Bb**	1.22 **Ba**	1.58 **Aa**	0.87 **Cab**
Lactate	0.7 **Ab**	0.76 **Ac**	0.75 **Ac**	1.32 **Aa**	1.28 **Ab**	1.39 **Aa**	1.61 **Aa**	0.92 **Bc**	0.95 **Bb**	1.51 **Ba**	1.91 **Aa**	0.78 **Cbc**
Malate	0.5 **Ab**	0.53 **ABc**	0.41 **Bd**	0.76 **Ba**	1.25 **Aa**	1.11 **Ac**	0.68 **Ca**	1.23 **Ba**	1.77 **Ab**	0.71 **Ca**	0.97 **Bb**	2.74 **Aa**
Nicotinate	1.1 **Aa**	1.13 **Aa**	0.95 **Aab**	0.77 **Bb**	1.04 **Aa**	0.91 **Ab**	0.74 **Bb**	1.04 **Aa**	0.95 **Aab**	0.63 **Cc**	0.87 **Bb**	1.09 **Aa**
Pyruvate	0.6 **Ba**	1.33 **Aa**	1.06 **Ab**	0.21 **Bc**	1.12 **Aab**	0.96 **Ab**	0.41 **Cab**	0.79 **Bbc**	1.26 **Ab**	0.28 **Cbc**	0.57 **Bc**	2.35 **Aa**
Succinate	1.3 **Aa**	1.22 **ABb**	0.99 **Bc**	0.87 **Bb**	1.24 **Aab**	1.33 **Ab**	1.29 **Ba**	1.46 **ABa**	1.66 **Aa**	1.20 **Aa**	1.08 **Ab**	0.76 **Bd**
Sugar	**0h_0N**	**0h_3.5N**	**0h_7N**	**10h_0N**	**10h_3.5N**	**10h_7N**	**18h_0N**	**18h_3.5N**	**18h_7N**	**36h_0N**	**36h_3.5N**	**36h_7N**
Cellobiose	1.4 **Ab**	0.99 **Bb**	0.97 **Ba**	3.79 **Aa**	0.35 **Bc**	0.35 **Bb**	4.69 **Aa**	1.13 **Bb**	0.32 **Cb**	4.99 **Aa**	1.97 **Ba**	0.27 **Cb**
Fructose	8.1 **Aa**	6.13 **Aa**	7.02 **Aa**	5.61 **Ab**	1.01 **Bc**	1.02 **Bb**	3.87 **Abc**	0.89 **Bc**	0.51 **Cc**	3.58 **Ac**	2.12 **Bb**	0.68 **Cc**
Fucose	2.6 **Aa**	2.15 **Aa**	2.25 **Aa**	2.79 **Aa**	0.80 **Bc**	0.69 **Bb**	2.43 **Aa**	1.42 **Bb**	0.68 **Cb**	3.22 **Aa**	1.97 **Ba**	0.24 **Cc**
Glucose_1	2.5 **Aa**	1.81 **Ba**	2.20 **Aa**	2.51 **Aa**	0.70 **Bc**	0.73 **Bbc**	1.77 **Ab**	0.62 **Bc**	0.59 **Bc**	1.85 **Ab**	1.36 **Bb**	0.87 **Cb**
Maltose	1 **Ab**	0.75 **Ab**	0.73 **Aa**	2.86 **Aa**	0.27 **Bc**	0.26 **Bb**	3.54 **Aa**	0.95 **Bb**	0.39 **Cb**	3.76 **Aa**	1.78 **Ba**	0.27 **Cb**
Sucrose	1.1 **Ac**	1.02 **Ab**	1.10 **Aa**	1.45 **Ab**	0.80 **Bc**	0.83 **Bb**	1.54 **Aab**	1.13 **Bb**	0.83 **Cb**	1.75 **Aa**	1.61 **Aa**	0.79 **Bb**
Trehalose	2 **Ab**	1.43 **Bb**	1.40 **Ba**	5.57 **Aa**	0.48 **Bc**	0.49 **Bb**	6.93 **Aa**	1.59 **Bb**	0.31 **Cc**	7.33 **Aa**	2.90 **Ba**	0.38 **Cbc**
Xylose	0.7 **Bb**	0.75 **Ab**	0.74 **Ab**	0.78 **Ba**	1.00 **Aa**	1.03 **Aa**	0.88 **Ba**	0.90 **Aa**	0.98 **Aa**	0.79 **Ba**	0.95 **Aa**	1.01 **Aa**
Glycerol	0.8 **Ac**	0.73 **Bc**	0.69 **Bb**	0.99 **Ab**	0.78 **Bbc**	0.76 **Bab**	1.01 **Ab**	0.82 **Bb**	0.75 **Bab**	1.16 **Aa**	1.15 **Aa**	0.78 **Ba**
Glycerol 3-P	1 **Ab**	1.11 **Ab**	1.07 **Abc**	1.24 **Aa**	1.21 **Aab**	1.17 **Aab**	1.18 **Aa**	1.06 **ABb**	0.96 **Bc**	1.28 **Aa**	1.37 **Aa**	1.31 **Aa**

SI Table 6. *(continuation)* Relative abundance of primary metabolites of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Others	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
Putrescine	1 **Ba**	1.60 **Aa**	1.73 **Aa**	0.79 **Bab**	1.61 **Aa**	1.27 **Aa**	0.59 **Cbc**	2.13 **Aa**	1.23 **Ba**	0.45 **Bc**	0.66 **Ab**	0.76 **Ab**
Spermidine	0.8 **Aab**	0.59 **Ba**	0.54 **Bb**	0.50 **Ac**	0.39 **Bb**	0.39 **Bc**	0.61 **Abc**	0.59 **Aa**	0.50 **Abc**	0.80 **Ba**	0.52 **Ca**	1.14 **Aa**
Adenine	1.7 **Aa**	1.82 **Aa**	1.92 **Aa**	1.40 **Aab**	1.47 **Aa**	1.64 **Aab**	1.11 **Bb**	1.72 **Aa**	1.41 **ABb**	1.01 **Ab**	0.99 **ABb**	0.76 **Bc**
Uracil	2 **Aa**	2.12 **Aa**	1.52 **Aa**	1.20 **Bb**	1.67 **Aab**	1.44 **ABa**	1.15 **Abc**	1.26 **Ab**	0.95 **Ab**	0.85 **Ac**	0.91 **Ac**	0.50 **Bc**
2-Hydroxypyridine	0.8 **Ab**	0.81 **Ab**	0.80 **Ab**	0.91 **Ab**	0.79 **Ab**	0.84 **Ab**	0.85 **Ab**	0.81 **Ab**	0.80 **Ab**	0.95 **Aa**	1.03 **Aa**	1.02 **Aa**
4-Aminobutanoate	5.2 **Aa**	4.99 **Aa**	4.21 **Aa**	3.17 **Ab**	2.14 **Ab**	2.17 **Ab**	2.29 **Ab**	1.16 **Bc**	1.23 **Bc**	2.10 **Ab**	0.73 **Bc**	0.42 **Cd**
Galactinol	2.2 **Aa**	1.17 **Aa**	1.11 **Aa**	2.10 **Aa**	0.56 **Bb**	0.40 **Bb**	2.22 **Aa**	0.44 **Bb**	0.22 **Bb**	2.17 **Aa**	1.79 **Aa**	0.21 **Bb**
Myo-Inositol	1.4 **Ab**	1.12 **Bb**	1.00 **Ba**	1.22 **Ab**	0.49 **Bd**	0.49 **Bd**	1.32 **Ab**	0.77 **Bc**	0.71 **Bc**	1.72 **Aa**	1.35 **Ba**	0.84 **Cb**
Orthophosphate	0.7 **Ad**	0.69 **Bd**	0.67 **Bd**	0.92 **Ac**	0.79 **Bc**	0.79 **Bc**	1.00 **Ab**	0.88 **Bb**	0.85 **Bb**	1.11 **Aa**	1.01 **Ba**	0.92 **Ba**

SI Table 7. Relative abundance of lipid of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
DAG 34:1	NA	Aa	NA	Aa	NA	Ab	NA	Ba	NA	Ba	5754.5	Aa	NA	Aa	NA	Aa	NA	Ab	NA	Ba	NA	Ba	3924.2	Aa
DAG 34:2	5969.5	Ac	5341.6	Ab	5726.0	Aa	40084.2	Ab	3369.1	Bb	6408.5	Ba	65620.6	Ab	3227.3	Bb	2377.2	Ba	194660.9	Aa	78232.3	Ba	1262.3	Ca
DAG 34:3	11217.4	Ac	10144.0	Ab	11748.8	Aa	74840.5	Ab	7887.2	Bb	15624.6	Ba	94895.5	Ab	6936.7	Bb	4716.4	Ba	274342.9	Aa	134029.0	Ba	6350.7	Ca
DAG 34:4	NA	Ac	NA	Ab	NA	Aa	17957.2	Ab	NA	Bb	NA	Ba	25516.7	Ab	NA	Bb	NA	Ba	108613.1	Aa	26506.5	Ba	NA	Ca
DAG 34:5	2866.2	Ab	3438.6	Ab	3049.0	Aa	15586.4	Ab	4192.1	Ab	6164.6	Aa	18182.4	Ab	NA	Bb	NA	Ba	99860.0	Aa	20032.6	Ba	NA	Ca
DAG 34:6	3389.3	Ab	2720.4	Aa	2775.1	Aa	6574.5	Ab	NA	Aa	NA	Aa	10098.9	Ab	NA	Ba	NA	Ba	50049.6	Aa	9594.0	Ba	NA	Ca
DAG 36:1	NA	Ab	NA	Aa	NA	Aa	1217.4	Aa	NA	Ba	NA	Ba	1081.5	Aa	NA	Ba	NA	Ba	NA	Ab	NA	Aa	NA	Aa
DAG 36:2	11342.5	Ac	9253.9	Ab	8551.8	Aa	15514.7	Abc	4570.6	Bb	8214.5	Bab	17344.4	Ab	6161.9	Bb	3479.9	Bc	30835.9	Aa	14420.9	Ba	3749.6	Cbc
DAG 36:3	17462.7	Aa	12117.3	Aa	12606.6	Aa	21859.3	Aa	5307.4	Bab	9333.8	Bab	24230.3	Aa	4376.0	Bb	2595.5	Bbc	NA	Ab	NA	Ab	NA	Ac
DAG 36:4	NA	Aa	NA	Ab	NA	Aa	NA	Aa	NA	Ab	NA	Aa	NA	Aa	NA	Ab	NA	Aa	NA	Ba	34471.4	Aa	NA	Ba
DAG 36:5	12254.5	Ac	7669.2	ABb	4294.4	Bab	0.0	Bd	3806.6	ABb	10410.6	Aa	32791.9	Ab	NA	Bb	NA	Bb	83655.6	Aa	24496.9	Ba	NA	Cb
DAG 36:6	16092.8	Ab	11827.9	Aab	9848.2	Aa	20996.9	Ab	6878.9	Bb	11336.7	Ba	23006.2	Ab	4223.1	Bb	NA	Bb	49670.7	Aa	15520.8	Ba	NA	Cb
DGDG 32:0	410904.9	Aa	420934.2	Aa	457653.9	Aa	363484.9	Aab	198320.0	Bb	255743.9	Bb	291307.4	Abc	186589.1	Bb	150301.6	Bc	223263.2	Bc	208252.2	Bb	498723.8	Aa
DGDG 32:1	90080.4	Ba	116429.1	Aa	115422.6	ABb	85881.1	Aa	57205.3	Bb	76113.7	ABc	72084.1	Aa	56426.8	Ab	48159.9	Ac	39068.0	Bb	57572.1	Bb	149080.2	Aa
DGDG 32:2	45994.0	Aa	58821.9	Aa	55764.3	Ab	36994.4	Bab	39062.8	Bb	57703.4	Ab	27314.7	Ab	28483.7	Ab	25451.1	Ac	25478.5	Bb	25179.6	Bb	94728.9	Aa
DGDG 32:3	41176.4	Aa	56268.7	Aa	50714.9	Ac	NA	Cb	62636.3	Ba	87672.6	Ab	35015.1	Aa	51304.2	Aab	48290.7	Ac	38784.1	Ba	35054.9	Bb	161819.0	Aa
DGDG 32:3.1	96727.6	Aa	132756.8	Aa	121450.2	Ac	123523.7	Ba	144742.5	Ba	194724.5	Ab	129339.3	Aa	119302.0	Aa	92846.0	Ac	112201.6	Ba	134865.2	Ba	292475.8	Aa
DGDG 34:0	929370.0	Ba	1140019.3	Aa	1188645.1	Aa	410439.2	Bb	490672.0	Ab	629023.2	Ab	295194.7	Bc	408580.7	Ac	406970.6	Ac	196453.6	Bd	249758.5	Ad	278547.3	Ad
DGDG 34:1	6615217.3	Aa	6129760.5	Aa	5669417.1	Aa	4356504.5	Ab	3252506.7	Bb	4169022.5	ABb	3359294.6	ABbc	3461781.9	Ab	2309245.4	Bc	2202529.2	Ac	2802149.0	Ab	2826361.8	Ac
DGDG 34:2	6673997.0	Aa	6618319.1	Aa	6012700.1	Aa	6397609.7	Aa	4568798.0	Bb	5909850.8	ABa	5292291.9	Aa	4495130.0	Ab	2766719.4	Bb	3256301.1	Ab	4215253.6	Ab	3592936.4	Ab
DGDG 34:3	5179112.1	Aab	5420345.5	Aa	5032823.4	Aab	6080469.9	Aa	5004207.1	Aa	6561815.9	Aa	5319548.7	Aab	4733889.6	ABa	3277090.1	Bc	3969506.0	Ab	4390603.8	Aa	4258311.7	Abc
DGDG 34:4	2391349.4	Aa	3005105.4	Aa	2750166.1	Ab	2430566.2	Ba	2793756.6	Ba	3782564.1	Aa	1944773.3	ABab	2649080.5	Aa	1830626.6	Bc	1450428.9	Bb	2430702.4	Aa	2484776.1	Abc
DGDG 34:5	2574618.6	Aa	3421810.5	Aa	3014167.0	Ab	3080843.0	Ba	3542052.6	Ba	4660695.2	Aa	2894091.3	ABa	3550009.3	Aa	2420821.0	Bb	2233074.9	Aa	3054227.9	Aa	3174171.8	Ab
DGDG 34:6	1518722.5	Bb	2382038.0	Ab	2134252.3	ABb	2550214.0	Ba	2822265.1	Bab	3678317.6	Aa	2564551.2	Ba	3276426.6	Aa	2162472.7	Bb	2427810.4	Ba	2732741.5	ABab	3337867.2	Aa
DGDG 36:2	51955.7	Ba	91694.1	Aa	89667.9	Aa	40107.3	Bab	52853.1	Bb	68873.2	Ab	32434.5	Abc	44899.8	Ab	43539.6	Ac	19127.9	Ac	26092.9	Ac	26076.9	Ad
DGDG 36:3	36429.7	Ba	55111.8	Aa	48857.8	Aa	49469.4	Ba	47941.4	Aa	51564.3	Aa	NA	Bb	34484.4	Ab	33155.5	Ab	NA	Bc	NA	Ac	NA	Ac
DGDG 36:4	51086.1	Ba	81103.6	Aab	72994.8	ABc	53759.2	Ca	94082.1	Ba	130060.5	Ab	52302.8	Ba	77215.4	Aab	58264.5	ABc	22664.2	Cb	61619.8	Bb	190797.4	Aa
DGDG 36:5	51291.0	Aa	78575.0	Aab	70088.3	Ab	44001.1	Ca	96698.3	Ba	136956.4	Aa	37440.5	Bab	78232.2	Aab	71125.9	Ab	13293.9	Cb	62159.1	Bb	143432.6	Aa
DGDG 36:6	185223.6	Ba	288694.5	Aa	264021.9	ABbc	196719.6	Ba	245881.4	Bab	339592.6	Ab	166603.4	Aab	215937.5	Aab	183298.5	Ac	87261.7	Cb	182863.2	Bb	706126.6	Aa

SI Table 7. *(continuation)* Relative abundance of lipid of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
LysoPC 16:0	34134.5	**Ba**	73521.0	**Aa**	65160.9	**Aa**	44718.7	**Aa**	24909.7	**Bb**	31492.3	**ABb**	47867.6	**Aa**	38098.5	**Ab**	16095.8	**Bbc**	46368.5	**Aa**	21546.2	**Bb**	13299.0	**Bc**
LysoPC 18:3	NA	**Ba**	3570.9	**Aa**	NA	**Ba**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**
MGDG 34:1	738486.9	**Ba**	1101458.7	**Aa**	993272.0	**ABa**	289327.1	**Ab**	340707.4	**Ab**	502121.9	**Ab**	660644.6	**Aa**	317465.4	**Bb**	506227.8	**ABb**	138899.6	**Bb**	436070.4	**Ab**	261008.3	**ABb**
MGDG 34:2	463860.7	**Ba**	729176.6	**Aa**	661861.5	**ABab**	93577.2	**Cb**	471081.5	**Bab**	843318.0	**Aa**	72387.8	**Bb**	359170.9	**Ab**	458779.2	**Ab**	55873.5	**Ab**	NA	**Ac**	169189.4	**Ac**
MGDG 34:2.1	298356.9	**Ab**	414289.2	**Aa**	388497.2	**Aa**	319528.8	**Ab**	227152.0	**Ab**	322615.7	**Aab**	571845.8	**Aa**	169018.2	**Bb**	187050.8	**Bbc**	118956.9	**Bc**	394991.1	**Aa**	153429.8	**Bc**
MGDG 34:3	21576.9	**Aa**	31925.7	**Aa**	30526.2	**Aab**	16582.3	**Cab**	30208.7	**Ba**	41097.9	**Aa**	16389.0	**Bab**	31317.7	**Aa**	27492.2	**Ab**	7896.2	**Bb**	9818.1	**ABb**	19658.5	**Ab**
MGDG 34:4	381285.4	**Ab**	647946.2	**Ab**	636677.9	**Ab**	1165790.0	**Ba**	1465736.7	**Ba**	2200362.7	**Aa**	587712.3	**Ab**	1087164.1	**Aab**	992620.3	**Ab**	494453.8	**Bb**	1072745.9	**Aab**	884598.8	**ABb**
MGDG 34:5	354958.5	**Ac**	601952.6	**Ab**	579713.3	**Ac**	1977985.1	**Ba**	1941321.2	**Ba**	2879283.3	**Aa**	1547048.8	**Aab**	1626423.1	**Aa**	1452512.9	**Ab**	978593.3	**Bbc**	1814158.0	**Aa**	1384336.4	**ABb**
MGDG 34:6	NA	**Ab**	NA	**Ab**	NA	**Aa**	NA	**Bb**	1490384.3	**Aa**	NA	**Ba**	1542464.0	**Aa**	1604227.1	**Aa**	NA	**Ba**	NA	**Ab**	NA	**Ab**	NA	**Aa**
MGDG 36:6	15708.2	**Aa**	23072.6	**Aab**	21627.6	**Ac**	9917.6	**Ca**	34206.4	**Ba**	52789.1	**Ab**	10016.1	**Aa**	18036.3	**Abc**	22674.0	**Ac**	2368.5	**Ba**	8547.3	**Bc**	74554.5	**Aa**
PC 32:0	5192.8	**Aa**	NA	**Ba**	NA	**Ba**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**
PC 32:1.1	10602.9	**Aa**	10190.6	**Aa**	11579.9	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
PC 34:1	NA	**Ca**	182994.7	**Aa**	164956.4	**Ba**	NA	**Ba**	NA	**Bb**	177793.5	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Ab**
PC 34:3	15172.0	**Aa**	16150.4	**Aa**	14904.9	**Aa**	12876.1	**Aa**	12743.8	**Aa**	17989.4	**Aa**	10949.3	**Aa**	13631.1	**Aa**	11016.3	**Aa**	7511.6	**Aa**	11685.9	**Aa**	NA	**Bb**
PC 34:4	NA	**Ac**	NA	**Aa**	NA	**Aa**	3381.1	**Ab**	NA	**Ba**	NA	**Ba**	4241.1	**Aa**	NA	**Ba**	NA	**Ba**	NA	**Ac**	NA	**Aa**	NA	**Aa**
PC 36:1	125583.6	**Bc**	356415.7	**Ab**	321727.1	**Aa**	288319.6	**Bb**	455164.9	**Ab**	370886.2	**ABa**	395660.0	**Aab**	423902.0	**Ab**	294220.9	**Aa**	449527.1	**Ba**	786434.8	**Aa**	NA	**Cb**
PC 36:2	1917986.3	**Bb**	8089571.4	**Ac**	7813703.6	**Ac**	8000228.2	**Ba**	12925730.1	**Ab**	11375169.6	**ABbc**	10836674.5	**Ba**	15635365.5	**Ab**	12933043.0	**ABb**	9572776.3	**Ba**	20485387.1	**Aa**	21885026.2	**Aa**
PC 36:3	NA	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ca**	49559.8	**Aa**	43259.5	**Ba**
PC 36:3.1	84204.8	**Aa**	272756.7	**Ad**	260317.7	**Ad**	261625.0	**Ba**	708139.5	**Ac**	677372.3	**Ac**	329816.0	**Ba**	1709558.7	**Ab**	1414002.0	**Ab**	240014.5	**Ba**	2910605.2	**Aa**	2779678.1	**Aa**
PC 36:5	4469.3	**Aa**	3244.6	**Aa**	4145.2	**Aa**	2322.8	**Ba**	NA	**Bb**	5903.8	**Aa**	3007.9	**Aa**	3566.7	**Aa**	4102.2	**Aa**	2114.9	**ABa**	3436.2	**Aa**	NA	**Bb**
PC 36:6	NA	**Ab**	NA	**Ab**	NA	**Ab**	2717.5	**Aa**	1683.7	**Aa**	2845.8	**Aa**	NA	**Bb**	2114.1	**Aa**	1672.3	**Aa**	2766.9	**Aa**	1225.1	**Bab**	2813.7	**Aa**
PC 38:2	26750.9	**Bb**	89341.0	**Ab**	83336.4	**Ac**	80182.9	**Ba**	174927.5	**Aa**	147377.7	**Ab**	94991.1	**Ba**	140826.2	**Aa**	114456.9	**ABbc**	77613.4	**Ca**	144717.9	**Ba**	205696.3	**Aa**
PC 38:3.1	2022.2	**Aa**	NA	**Ad**	NA	**Ac**	3035.1	**Ba**	9075.4	**Ac**	8185.0	**Ab**	3620.7	**Ba**	13765.0	**Ab**	16082.9	**Aa**	3206.5	**Ca**	22943.6	**Aa**	15220.4	**Ba**

SI Table 7. *(continuation)* Relative abundance of lipid of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
PE 32:1	NA	Bc	10781.7	ABb	12395.3	Ac	15308.5	Bb	30680.3	Aa	20829.4	ABbc	25596.7	Bab	25663.8	Ba	42182.7	Aa	32961.2	Aa	23173.6	Aa	29066.9	Ab
PE 32:2	NA	Aa	NA	Ab	NA	Ab	NA	Aa	NA	Ab	NA	Ab	NA	Ba	3816.8	Aa	4496.0	Aa	NA	Aa	NA	Ab	NA	Ab
PE 34:1	23497.8	Ab	81212.4	Ab	70264.5	Ac	112975.2	Ba	212926.1	Aa	146884.0	Bb	172081.0	Aa	176248.6	Aa	228616.4	Aa	180278.1	Aa	150300.6	Aa	154840.6	Ab
PE 34:2	20213.7	Ab	63475.4	Ac	66834.1	Ac	111571.9	Bab	304284.6	Aa	215374.1	Ab	176900.5	Ba	261037.3	Bab	428702.2	Aa	163283.7	Ba	185386.2	Bb	294262.2	Ab
PE 34:3	NA	Ab	3921.3	Ab	3992.7	Ab	7312.4	Ba	13123.6	Aa	11098.5	ABa	10235.4	Ba	11829.9	ABa	14895.5	Aa	8549.9	Aa	12020.8	Aa	NA	Bb
PE 34:4	NA	Ab	NA	Ab	NA	Ac	NA	Bb	1934.2	Aa	1917.1	Ab	1323.5	Ba	2094.7	ABa	3012.5	Aa	NA	Bb	NA	Bb	2809.5	Aab
PE 34:5	NA	Aa	NA	Aa	NA	Ab	NA	Aa	NA	Aa	NA	Ab	NA	Ba	NA	Ba	5174.0	Aa	NA	Aa	NA	Aa	NA	Ab
PE 34:6	6042.8	Ba	10747.3	Ab	9060.1	Ab	3640.0	Bab	15620.5	Aa	16015.0	Aa	3373.7	Bab	6708.7	Ac	5176.8	ABc	1786.0	Bb	NA	Bd	8899.7	Ab
PE 36:1	18320.3	Bb	45069.1	Ab	33971.3	Ab	58186.6	Ba	124174.6	Aa	78825.7	Aa	75255.0	Ba	102775.8	Aa	116439.2	Aa	62541.5	Ba	79216.9	Aa	89642.6	Aa
PE 36:2	386046.4	Ab	1271316.7	Ab	1282271.1	Ab	1382791.5	Bab	4164931.7	Aa	3257907.8	Aa	1812601.5	Ba	3214509.5	Aa	4037547.3	Aa	1060849.5	Bab	1861462.1	Bb	3091429.0	Aa
PE 36:3	NA	Aa	27745.2	Ac	25728.4	Ac	35767.4	Ba	987206.6	Aa	756487.8	Aa	37635.0	Ba	690310.7	Ab	848395.4	Aa	22643.7	Ba	257718.5	ABc	393522.4	Ab
PE 36:3.1	1313.2	Bc	4985.3	Ac	3579.8	Ac	8325.2	Ba	14679.3	Aa	16582.7	Aa	8896.5	Ba	14749.8	Aa	14791.1	Aa	7454.7	Bb	11965.0	Ab	10069.5	Ab
PE 36:4.1	4634.0	Bc	11456.0	Ab	11564.7	Ac	15905.6	Cab	27417.4	Ba	35290.3	Aa	17762.5	Ba	29020.1	Aa	30371.1	Aa	10674.1	Bbc	17350.7	Ab	21385.3	Ab
PE 38:2	117737.6	Ba	427817.1	Abc	387641.5	Ab	200251.8	Ba	829380.1	Aa	754471.9	Aa	238492.8	Ba	536569.1	Ab	696781.4	Aa	116361.4	Ca	290291.6	Bc	619752.1	Aa
PE 38:3	8805.7	Aa	17521.6	Ab	17545.4	Ac	12723.3	Ba	147593.7	Aa	150589.2	Aab	10786.9	Ba	143439.9	Aa	171164.7	Aa	4945.9	Ca	52624.7	Bb	112583.0	Ab
PE 40:1	NA	Bb	23024.6	Aa	NA	Bc	10885.1	Ba	15285.8	Ab	15346.7	Aa	12273.3	Aa	12101.7	Ab	10689.7	Ab	8762.8	Ba	15797.0	Ab	16400.4	Aa
PE 40:2	90963.2	Bb	283290.5	Ab	267134.2	Ab	290126.4	Aa	336806.5	Ab	290504.0	Ab	397160.3	Aa	350755.2	Ab	350837.0	Ab	390261.4	Ba	527059.9	Aa	554993.9	Aa
PE 40:3	14079.8	Aa	16409.3	Aab	17823.9	Ab	6372.9	Cab	24449.9	Ba	39031.2	Aa	6060.6	Bab	13643.4	ABb	19129.6	Ab	3690.3	Bb	NA	Bc	13195.8	Ab
PG 32:0	45228.6	Ac	60859.5	Ab	48493.5	Aab	130954.8	Aa	38023.9	Bb	28812.7	Bb	108061.8	Aab	56822.5	Bb	49429.1	Bab	95308.2	Ab	106674.9	Aa	78375.2	Aa
PG 32:1	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	2707.8	Ba	NA	Cb	4077.7	Aa	4048.4	Aa	2497.6	Bb	NA	Ab
PG 34:2	19951.8	Ac	36611.1	Ac	34132.7	Ac	71722.7	Ab	88442.3	Ab	79326.9	Ac	123752.5	Ba	136226.3	Bab	216754.8	Aa	63698.7	Bbc	148384.0	Aa	148040.7	Ab
PG 34:3	5212.5	Ab	8076.2	Ab	7281.8	Ab	8415.3	Bb	14783.8	Aa	NA	Cc	13846.2	Ba	15363.2	Ba	20952.4	Aa	7467.8	Ab	11588.4	Aab	10855.9	Ab
PG 34:4	38810.0	Aa	88850.6	Aa	71025.1	Ac	92949.9	Aa	166102.8	Aa	184443.9	Abc	66754.4	Ba	162494.5	ABa	236733.9	Ab	53371.4	Ca	162192.5	Ba	790469.3	Aa
PG 36:1	14580.4	Bc	40143.9	Ac	30648.7	Ac	53669.9	Bb	86609.9	Ab	60114.3	Ab	74297.2	Ba	119738.4	Aa	132078.4	Aa	68820.3	Bb	95006.6	Ab	70838.8	Ab
PG 36:2	103971.8	Ab	293320.2	Ac	238703.1	Ac	319428.1	Cab	905185.7	Aab	613740.9	Bb	411599.5	Ba	1054457.2	Aa	1304598.2	Aa	299998.0	Bab	661330.5	Ab	853917.0	Ab
SQDG 32:0	17876129.2	Aa	16360206.3	Aa	16283770.1	Aa	12005000.4	Ab	8752447.8	Bb	10878940.1	ABb	10420566.1	Abc	8000311.4	Bb	6225713.6	Bc	8917879.0	Ac	8421257.9	Ab	9437115.2	Ab
SQDG 32:1	336794.5	Aa	373446.5	Aa	368173.5	Aa	186931.2	Ab	145187.7	Ab	181998.4	Ab	134345.7	Abc	99068.8	Ab	88525.0	Ac	73116.8	Bc	78371.9	Bb	236649.1	Ab
SQDG 32:2	115652.2	Aa	121243.1	ABa	122420.1	Ba	66301.1	Ab	50605.8	ABb	57416.0	Bb	54799.2	Ac	36738.8	ABc	27965.4	Bc	32354.1	Ad	26183.1	ABd	NA	Bd
SQDG 34:0	1002318.3	Aa	1045286.0	Aa	1012088.6	Aa	515413.7	Bb	803684.1	Ab	882470.1	Aab	384550.7	Bbc	691076.9	Ab	699561.0	Ab	248745.8	Ac	401042.9	Ac	367290.7	Ac

SI Table 7. *(continuation)* Relative abundance of lipid of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
SQDG 34:1	NA	Aa	NA	Aa	NA	Ac	NA	Ba	NA	Ba	2156475.4	Aa	NA	Ba	NA	Ba	1370235.0	Ab	NA	Aa	NA	Aa	NA	Ac
SQDG 34:2	1500374.7	Ba	2025423.1	Aab	1975344.0	Ab	1312220.1	Cab	2397189.8	Ba	3056343.0	Aa	993850.9	Bb	1762206.3	Ab	1503682.0	Ab	445650.9	Cc	1707744.4	Ab	923408.2	Bc
SQDG 34:3	4293796.4	Ba	5505243.9	Aa	5217432.8	ABa	2993091.9	Bb	3264257.7	ABb	4128431.0	Ab	2217176.7	Ab	2542011.0	Ab	2127983.2	Ac	1071683.4	Cc	2203186.2	Bb	4724686.7	Aab
SQDG 34:4	63699.7	Ba	86655.5	Aa	82805.9	ABa	43636.4	Bab	52610.5	Bb	78013.5	Aab	30319.0	Bbc	52805.6	Ab	57690.4	Ab	9292.0	Cc	37227.8	Bb	78096.6	Aab
SQDG 34:5	90562.4	Ba	117277.5	Aa	113486.7	Aa	54143.5	Bb	58334.9	ABb	76770.6	Ab	33210.0	Abc	50192.2	Abc	52594.9	Ac	9327.2	Cc	32507.0	Bc	85887.2	Ab
SQDG 34:6	53475.5	Ba	70636.9	Aa	68698.5	Ab	32305.8	Bb	36295.4	ABb	48006.6	Ac	20051.1	Bb	32783.0	ABb	41123.8	Ac	4911.9	Cc	18410.2	Bc	94924.8	Aa
TAG 42:0	21527.8	Aa	18023.5	Aa	19507.2	Aa	12657.7	Ab	9634.6	Ab	13259.8	Ab	11057.7	Ab	11025.6	Ab	6819.2	Ac	22861.1	Aa	8691.7	Bb	7410.2	Bc
TAG 42:1	26214.0	Aa	19767.1	Aa	21092.3	Aa	17016.6	Ab	11688.0	Ab	16923.9	Aa	16365.6	Ab	12401.6	ABab	7363.5	Bb	30602.6	Aa	12032.3	Bb	8247.4	Bb
TAG 42:2	5155.8	Ab	3814.2	ABa	3295.7	Ba	3053.0	Ac	2339.7	Aab	2128.4	Aab	2371.3	Ac	2310.2	Aab	1440.0	Ab	7730.4	Aa	2086.6	Bb	1509.4	Bb
TAG 44:0	98719.1	Ab	79384.6	Aa	83000.0	Aa	64272.9	Ac	37881.7	Bb	52437.9	ABb	66563.8	Ac	40872.1	Bb	26200.8	Bc	153598.9	Aa	53957.7	Bb	30389.1	Cbc
TAG 44:1	54456.5	Ab	45069.9	Aa	45035.7	Aa	48546.2	Ab	27961.5	Aa	37210.2	Aab	62654.5	Ab	32922.3	Ba	19358.4	Bb	184194.7	Aa	36506.9	Ba	28559.4	Bab
TAG 44:2	37473.6	Aa	30860.1	Aa	29135.5	Aa	20652.2	Ab	14477.0	Ab	22001.1	Aa	19873.6	Ab	15136.2	Ab	9301.2	Ab	45260.1	Aa	14770.2	Bb	10156.4	Bb
TAG 46:0	510899.5	Aa	441152.2	Aa	420053.1	Aa	329683.9	Ab	207505.1	Bb	286579.9	ABb	302935.7	Ab	228151.0	ABb	142463.5	Bc	548158.4	Aa	214141.6	Bb	166522.9	Bc
TAG 46:1	265494.8	Ab	234820.1	Aa	228319.0	Aa	254666.4	Ab	101768.3	Ba	138404.3	ABab	361368.3	Ab	136865.6	Ba	63278.2	Bb	1218569.3	Aa	182009.9	Ba	65351.5	Bb
TAG 46:2	45274.7	Ab	39806.6	Aab	37473.3	Aa	44173.4	Ab	NA	Ab	26367.0	Aa	65046.9	Ab	21927.5	Bab	NA	Ba	450825.7	Aa	65626.8	Ba	14920.9	Ca
TAG 46:2.1	46247.7	Aa	45987.1	Aa	43731.2	Aab	35835.7	ABa	34058.2	Ba	50486.9	Aa	NA	Bb	36321.1	Aa	26485.6	Ac	NA	Bb	28130.5	Aa	27834.9	Abc
TAG 46:3	13870.7	Ab	12166.6	Aa	11474.7	Aa	11359.7	Ab	5275.1	Aa	7762.5	Aa	13388.5	Ab	5413.3	Aa	NA	Aa	152701.2	Aa	15118.2	Ba	2876.8	Ba
TAG 46:3.1	7839.6	Ab	5516.9	Aa	5848.8	Aa	6141.9	Ab	3034.3	Aa	4500.6	Aa	4544.8	Ab	2704.9	Aa	2004.6	Aa	36249.2	Aa	7034.2	Ba	1617.3	Ca
TAG 46:4	NA	Ab	NA	Aa	NA	Aa	1314.5	Ab	NA	Aa	NA	Aa	1519.4	Ab	NA	Aa	NA	Aa	10747.1	Ab	1426.1	Ba	NA	Ba
TAG 48:0	2404175.7	Ab	2101606.1	Aa	1999714.9	Aa	2702692.8	Ab	1081809.9	Bb	1439723.7	Bab	2983159.5	Ab	1130772.7	Bb	720788.9	Bb	6919572.5	Aa	2247342.1	Ba	768472.4	Cb
TAG 48:1	1595155.3	Ab	1380595.4	Aa	1287700.2	Aa	1883833.0	Ab	786792.4	Ba	1118326.5	Ba	2291573.9	Ab	905544.6	Ba	534741.1	Ba	5516827.8	Aa	1295309.7	Ba	610374.2	Ba
TAG 48:2	376912.1	Aa	NA	Bc	NA	Bc	NA	Cb	190264.9	Bb	251672.9	Aa	350611.7	Aa	NA	Bc	NA	Bc	NA	Cb	284345.6	Aa	134351.4	Bb
TAG 48:3	NA	Ab	NA	Ab	NA	Aa	NA	Ab	NA	Ab	NA	Aa	145982.3	Aa	NA	Bb	NA	Ba	NA	Bb	112794.0	Aa	NA	Ba
TAG 48:4	NA	Aa	NA	Aa	NA	Ab	NA	Aa	NA	Aa	NA	Ab	NA	Ba	NA	Ba	1117.4	Aa	NA	Ba	NA	Ba	1111.5	Aa
TAG 48:5	NA	Ad	NA	Ab	NA	Aa	1560.6	Ac	NA	Bb	NA	Ba	3050.2	Ab	NA	Bb	NA	Ba	27188.3	Aa	2253.6	Ba	NA	Ca
TAG 48:6	NA	Ac	NA	Aa	NA	Aa	NA	Ac	NA	Aa	NA	Aa	1251.6	Ab	NA	Ba	NA	Ba	10664.0	Aa	NA	Ba	NA	Ba
TAG 48:7	NA	Ac	NA	Aa	NA	Aa	NA	Ac	NA	Aa	NA	Aa	1816.5	Ab	NA	Ba	NA	Ba	5110.7	Aa	NA	Ba	NA	Ba
TAG 50:0	4541793.1	Aa	3804987.5	ABa	3650994.6	Ba	3223058.0	Ab	2152347.7	Bb	2819326.8	ABa	2973449.9	Ab	2237162.1	ABb	1426064.5	Bb	3502091.4	Ab	1968546.7	ABb	1634235.9	Bb
TAG 50:1	8512754.8	Ac	7222758.8	Ab	6906568.4	Aa	15697932.2	Ab	4237448.5	Bb	5429747.2	Ba	20911116.5	Ab	4753778.1	Bb	2710726.2	Ba	69454664.1	Aa	16481514.0	Ba	3110969.1	Ca

SI Table 7. *(continuation)* Relative abundance of lipid of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 50:2	3520776.7 **Ac**	3017658.5 **Ab**	2873683.1 **Aa**	6420261.5 **Abc**	1879863.0 **Ab**	2542259.2 **Aa**	10283477.5 **Ab**	2025538.6 **Bb**	1213082.3 **Ba**	58109002.7 **Aa**	8671310.8 **Ba**	1403458.4 **Ca**
TAG 50:3	NA **Ac**	351799.7 **Aa**	NA **Aa**	1867764.9 **Abc**	NA **Aa**	NA **Aa**	3777098.5 **Ab**	NA **Ba**	116688.5 **Ba**	36263918.7 **Aa**	3733264.9 **Ba**	0.0 **Ca**
TAG 50:4	NA **Ab**	NA **Aa**	NA **Aa**	370062.2 **Ab**	NA **Aa**	NA **Aa**	1055632.0 **Ab**	NA **Aa**	NA **Aa**	19889610.3 **Aa**	1137964.6 **Ba**	0.0 **Ba**
TAG 50:5	9042.7 **Ab**	7616.7 **Aa**	8540.6 **Aa**	85672.8 **Ab**	3630.3 **Aa**	6865.5 **Aa**	228075.1 **Ab**	4515.9 **Aa**	2298.4 **Aa**	6764486.6 **Aa**	249178.4 **Ba**	2145.6 **Ba**
TAG 50:6.1	4857.3 **Ab**	3357.0 **Aa**	4293.1 **Aa**	22884.3 **Ab**	2415.4 **Aa**	3317.3 **Aa**	53173.8 **Ab**	2088.4 **Aa**	1039.1 **Aa**	1850130.9 **Aa**	47686.3 **Ba**	1034.3 **Ba**
TAG 50:7	NA **Ab**	NA **Aa**	NA **Aa**	2749.1 **Ab**	NA **Aa**	NA **Aa**	5920.0 **Ab**	NA **Aa**	NA **Aa**	93829.2 **Aa**	3761.9 **Ba**	0.0 **Ba**
TAG 50:8	NA **Ab**	NA **Aa**	NA **Aa**	1454.4 **Ab**	NA **Aa**	NA **Aa**	2898.6 **Ab**	NA **Aa**	NA **Aa**	34643.7 **Aa**	1958.4 **Ba**	NA **Ba**
TAG 50:9	NA **Ab**	NA **Aa**	NA **Aa**	NA **Ab**	NA **Aa**	NA **Aa**	NA **Ab**	NA **Aa**	NA **Aa**	12366.6 **Aa**	NA **Ba**	NA **Ba**
TAG 52:0	4095332.8 **Aa**	3384494.5 **Ba**	3175951.1 **Ba**	2590011.3 **Ab**	2080909.7 **Bb**	2640001.0 **Bb**	2208371.8 **Ac**	2060938.3 **Bc**	1305978.6 **Bc**	1976069.2 **Ac**	1623267.6 **Bc**	1509985.8 **Bc**
TAG 52:1	11772850.3 **Ab**	9842391.0 **Aa**	9483122.8 **Aa**	10815901.2 **Ab**	5726456.9 **Bb**	7716457.1 **ABab**	12186790.4 **Ab**	6501865.4 **Bab**	3854790.2 **Bc**	22488178.3 **Aa**	6599602.5 **Bab**	4423322.9 **Bbc**
TAG 52:2	17801553.2 **Ab**	14622574.9 **Aa**	14047293.8 **Aa**	18685626.0 **Ab**	9328425.9 **Ba**	12029177.2 **Bab**	20751788.9 **Ab**	10091213.3 **Ba**	6115149.3 **Bb**	60816760.6 **Aa**	15193486.5 **Ba**	6987378.5 **Cb**
TAG 52:3	4128804.0 **Ac**	3462381.6 **Ab**	3417929.1 **Aa**	7833197.7 **Abc**	2176314.4 **Bb**	2977256.8 **Ba**	11808270.2 **Ab**	2265163.3 **Bb**	1443334.3 **Ba**	59156415.7 **Aa**	8776396.1 **Bb**	1612815.1 **Ca**
TAG 52:4	2010270.5 **Ab**	1583432.9 **Aa**	1547266.8 **Aa**	4880189.4 **Ab**	1008846.1 **Aa**	1403909.8 **Aa**	7487030.1 **Ab**	1060886.8 **Ba**	656976.9 **Ba**	49710176.8 **Aa**	5593150.9 **Ba**	754155.2 **Ba**
TAG 52:5.1	66045.1 **Ab**	49026.8 **Aa**	49604.4 **Aa**	412595.8 **Ab**	21762.0 **Aa**	38394.4 **Aa**	963241.9 **Ab**	20454.6 **Aa**	10291.9 **Aa**	14168121.8 **Aa**	1098312.0 **Ba**	NA **Ba**
TAG 52:6	41159.9 **Ab**	NA **Aa**	35394.3 **Aa**	220386.7 **Ab**	15170.2 **Aa**	27720.1 **Aa**	452060.6 **Ab**	13517.5 **Aa**	6422.5 **Aa**	9704978.9 **Aa**	333569.9 **Ba**	7687.9 **Ba**
TAG 52:6.1	29019.8 **Ab**	22339.7 **Aa**	22810.6 **Aa**	202387.0 **Ab**	10089.1 **Aa**	17454.6 **Aa**	476961.8 **Ab**	9385.4 **Aa**	4353.0 **Aa**	8775173.2 **Aa**	517012.5 **Ba**	6073.1 **Ba**
TAG 52:7	6916.8 **Ab**	6927.6 **Aa**	7925.8 **Aa**	37700.4 **Ab**	3907.4 **Aa**	6308.9 **Aa**	78997.7 **Ab**	3716.6 **Aa**	2224.2 **Aa**	2557698.9 **Aa**	60914.5 **Ba**	2357.0 **Ba**
TAG 52:8	4042.1 **Ab**	1862.1 **Aa**	2344.0 **Aa**	10271.9 **Ab**	1729.1 **Aa**	2839.8 **Aa**	21484.5 **Ab**	1548.2 **Aa**	959.0 **Aa**	625327.5 **Aa**	13822.4 **Ba**	NA **Ba**
TAG 52:9	3032.6 **Ab**	3146.7 **Aa**	2918.3 **Aa**	2436.4 **Ab**	2189.9 **Aa**	2851.3 **Aa**	4004.7 **Ab**	1965.3 **Aa**	NA **Aa**	107962.3 **Aa**	2179.7 **Ba**	NA **Ba**
TAG 54:0	1213854.7 **Aa**	1027911.1 **ABa**	958074.3 **Ba**	771977.0 **Ab**	649907.5 **ABb**	785931.7 **Bb**	625291.8 **Ac**	637178.4 **ABc**	404825.7 **Bc**	482574.1 **Ac**	478347.5 **ABc**	459562.6 **Bc**
TAG 54:1	5146660.1 **Aa**	4328964.1 **Ba**	4013610.9 **Ba**	3495873.1 **Ab**	2648183.4 **Bb**	3430016.7 **Bb**	3128460.7 **Ab**	2825266.5 **Bb**	1795592.2 **Bb**	3588668.7 **Ab**	2313759.5 **Bb**	2110739.0 **Bb**
TAG 54:2	9199894.3 **Aa**	7731148.7 **ABa**	7198463.9 **Ba**	6438124.7 **Ab**	4764322.3 **Ab**	6267172.9 **Aa**	5556935.3 **Ab**	5051845.1 **ABb**	3314953.4 **Bb**	7247613.7 **Aab**	4535635.1 **Bb**	3778297.1 **Bb**
TAG 54:3	8719225.3 **Aa**	6714229.0 **Ba**	6847032.4 **Ba**	5838901.3 **Ab**	4208515.4 **Ab**	5584338.7 **Aa**	4766702.0 **Ab**	4546618.3 **Ab**	2932317.9 **Ab**	6662350.6 **Ab**	4092327.1 **Bb**	3356483.3 **Bb**
TAG 54:4	5024212.1 **Aa**	3963792.3 **Aa**	3946562.2 **Aa**	3711224.0 **Ab**	2504368.6 **Bb**	3371619.3 **ABa**	3295928.3 **Ab**	2710466.0 **ABb**	1722608.0 **Bb**	6056640.6 **Aa**	2727899.1 **Bb**	1981165.3 **Bb**
TAG 54:5	3280300.1 **Ab**	2643358.5 **Aa**	2551488.2 **Aa**	2989076.6 **Ab**	1773748.9 **Ba**	2391987.0 **ABa**	2798567.2 **Ab**	1857561.0 **Ba**	1150212.0 **Bb**	5567632.6 **Aa**	2151043.3 **Ba**	1348062.1 **Bb**
TAG 54:6	1830880.1 **Ab**	1471584.9 **Aa**	1386018.2 **Aa**	1372439.2 **Ab**	952140.3 **Aa**	1226466.5 **Aab**	1493260.2 **Ab**	981622.9 **Ba**	633091.5 **Bc**	3413108.1 **Aa**	1148507.3 **Ba**	754272.5 **Bbc**
TAG 54:7	124389.1 **Ab**	97759.2 **Aa**	92978.8 **Aa**	148469.4 **Ab**	58149.7 **Aa**	86189.2 **Aa**	178650.1 **Ab**	58449.9 **Aa**	35732.5 **Aa**	1168465.5 **Aa**	123830.8 **Ba**	42342.5 **Ba**
TAG 54:8	24417.0 **Ab**	16607.5 **Aa**	16432.3 **Aa**	29365.7 **Ab**	9789.0 **Aa**	15191.3 **Aa**	38180.5 **Ab**	8889.9 **Aa**	4477.4 **Aa**	290609.4 **Aa**	23179.0 **Ba**	4871.3 **Ba**
TAG 54:9	10429.8 **Ab**	7912.8 **Aa**	7080.5 **Aa**	7486.0 **Ab**	3313.2 **Aab**	4957.2 **Aab**	8949.7 **Ab**	2625.2 **Bb**	1087.8 **Bb**	42701.3 **Aa**	NA **Bb**	1366.5 **Bb**

SI Table 7. *(continuation)* Relative abundance of lipid of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
TAG 56:0	79335.9	Aa	64823.9	Ba	63485.3	Ba	55720.9	Ab	40578.5	Bb	54018.4	Bb	49198.0	Ab	40682.6	Bb	28470.1	Bb	53918.3	Ab	35788.6	Bb	29947.6	Bb
TAG 56:1	183759.3	Ab	152856.2	Aa	153726.8	Aa	154074.9	Ab	91703.1	Bb	119732.1	ABa	174590.4	Ab	96577.9	Bb	60932.4	Bb	356144.2	Aa	108612.6	Bab	68709.5	Bb
TAG 56:2	441394.7	Aa	347296.1	Aa	337559.8	Aa	311699.7	Ab	208704.2	Ab	285373.0	Aa	310143.7	Ab	238193.0	ABab	145952.1	Bb	490870.8	Aa	214503.7	Bb	158043.5	Bb
TAG 56:3	367566.4	Aa	283849.5	Ba	274355.3	Ba	254987.4	Ab	184982.4	Bb	252880.6	Bb	231041.1	Ab	188993.2	Bb	121032.6	Bb	302322.4	Ab	184619.3	Bb	142505.3	Bb
TAG 56:4	275806.7	Aa	213483.6	Aa	209720.6	Aa	191763.0	Abc	138575.9	Aa	189871.2	Aa	170794.4	Ac	143834.0	ABa	90559.9	Bb	263291.6	Aab	140776.8	Ba	110737.0	Bb
TAG 56:5	93558.6	Ab	69136.8	Aa	70308.1	Aa	80627.1	Ab	44639.3	Ba	67253.4	ABa	78676.0	Ab	48588.0	ABa	29543.5	Bb	215822.9	Aa	73038.4	Ba	34782.6	Cab
TAG 56:6	21888.9	Ab	13896.6	Aa	14548.2	Aa	17868.1	Ab	8666.1	Aa	15539.2	Aa	22312.0	Ab	9428.9	ABa	5839.0	Ba	106329.2	Aa	23804.3	Ba	7662.1	Ca
TAG 56:7	6053.8	Ab	4705.4	Aa	4600.3	Aa	4999.8	Ab	1875.4	Aab	4070.5	Aab	5531.4	Ab	NA	Bb	NA	Bb	29051.6	Aa	5479.5	Ba	1595.6	Cab
TAG 58:0	28585.9	Ab	23204.7	Aa	22342.0	Aa	21329.7	Ab	15518.7	Ab	20549.0	Aa	22927.2	Ab	15665.1	Bb	10053.6	Bb	39656.0	Aa	16311.2	Bab	11360.3	Bb
TAG 58:1	57701.1	Aa	43109.3	Aab	44283.5	Aa	69260.9	Abc	27215.4	Bb	39064.8	Ba	96363.6	Ab	31088.1	Bab	20597.3	Ba	268043.3	Aa	59838.8	Ba	22472.8	Ca
TAG 58:2	139704.1	Ab	106695.9	Aa	99784.9	Aa	110550.3	Ab	71502.3	Aa	96635.0	Aab	130333.5	Ab	75774.6	Ba	48559.0	Bc	268771.3	Aa	81196.3	Ba	52096.0	Bbc
TAG 58:3	146377.3	Aa	119085.8	ABa	105389.8	Ba	101966.8	Ab	77568.6	Ab	100926.9	Aa	87056.1	Ab	80401.9	ABb	51738.5	Bb	157877.2	Aa	66746.1	Bb	58761.3	Bb
TAG 58:4	160756.6	Aa	125389.6	Ba	115907.8	Ba	108522.4	Ab	87108.2	Bb	115609.9	Bb	92928.2	Abc	92435.3	Bbc	60413.6	Bbc	87690.6	Ac	72323.7	Bc	64813.4	Bc
TAG 58:5	NA	Ac	NA	Aa	NA	Aa	13801.5	Ab	NA	Ba	NA	Ba	NA	Ac	NA	Aa	NA	Aa	64050.2	Aa	NA	Ba	NA	Ba
TAG 58:6	NA	Ac	NA	Aa	NA	Ab	NA	Bc	NA	Ba	12997.3	Aa	8891.3	Ab	NA	Ba	NA	Bb	28422.0	Aa	NA	Ba	NA	Bb
TAG 60:0	10387.3	Aa	8100.6	Ba	10975.0	Aa	6296.7	Ab	4949.8	Ab	7134.9	Ab	5564.3	Ab	5268.9	Ab	3422.7	Ac	6163.8	Ab	4172.2	Ab	3926.0	Ac
TAG 60:1	19860.7	Ac	15426.6	Ab	15673.2	Aa	37740.8	Ac	9869.2	Ab	15338.5	Aa	74089.8	Ab	11144.5	Bb	6549.3	Ba	336539.0	Aa	50025.7	Ba	7940.2	Ca
TAG 60:2	48570.9	Ab	36976.6	Aa	36066.1	Aa	49381.8	Ab	25832.9	Aa	35032.6	Aa	78156.0	Ab	27203.4	Ba	17354.9	Ba	340553.8	Aa	48478.9	Ba	19284.7	Ba
TAG 60:3	43677.7	Ab	36004.4	Aa	33489.5	Aa	37105.0	Ab	25173.9	Aa	30940.2	Aa	43990.2	Ab	25441.4	ABa	16372.3	Ba	201032.9	Aa	29668.3	Ba	18169.8	Ba
TAG 60:4	49805.3	Aa	40221.3	ABa	35703.5	Ba	35247.4	Ab	27875.8	Aab	34528.0	Aa	29332.2	Ab	29355.3	Aab	18533.9	Ab	NA	Bc	23260.4	Ab	20155.7	Ab
TAG 60:5	5594.1	Aa	4501.1	Aa	4348.7	Aa	4586.6	Aa	2766.5	Bb	3618.3	ABab	5145.4	Aa	3163.3	Bab	1904.2	Bb	NA	Bb	2874.2	Aab	1925.3	Ab
TAG 60:5.1	NA	Ab	NA	Aa	NA	Aa	3089.4	Ab	NA	Aa	NA	Aa	4971.7	Ab	NA	Aa	NA	Aa	56917.5	Aa	3280.6	Ba	NA	Ba
TAG 60:6	14026.5	Ac	20060.9	Ab	17584.9	Ab	48079.3	Aab	19328.0	Bb	26819.1	ABb	67333.6	Aa	60609.6	Aa	22978.9	Bb	26445.6	Bbc	33568.0	Bab	85480.4	Aa
FA 16:0	23890500.7	Aa	17714052.0	Ba	17161412.5	Ba	20934553.4	Aa	8192391.0	Bb	10973252.7	Bb	18461051.8	Aab	10322784.3	Bb	4699738.6	Cc	14572431.8	Ab	11769561.6	Aab	5573305.7	Bbc
FA 16:1	1991151.2	Aa	NA	Bc	NA	Bb	2020068.0	Aa	595380.4	Bb	837219.2	Ba	1687284.0	Aa	691492.3	Bab	295318.8	Bab	1491348.2	Aa	1248882.1	Aa	163365.6	Bb
FA 16:2	2879934.0	Aa	2510230.9	Aa	2336779.1	Aa	2444348.6	Aab	1929013.0	Aab	2454426.9	Aa	2043788.6	Aab	1044704.3	Bc	443907.6	Bb	1819515.5	Ab	1319192.2	Abc	304508.7	Bb
FA 16:3	1511169.0	Aa	1220286.3	Aab	1183045.4	Ab	2084414.9	Aa	1762256.6	Aa	2159545.6	Aa	2157636.2	Aa	1548467.1	Aab	780843.4	Bbc	2041842.8	Aa	966091.6	Bb	388644.5	Bc
FA 16:4	52597.4	Aa	39762.9	Aa	41753.5	Aa	49523.1	Aa	17500.3	Bb	25928.5	Bb	42352.1	Aa	16581.3	Bb	7195.7	Bc	53157.8	Aa	14715.3	Bb	4475.2	Bc
FA 16:4.1	3882263.6	Bab	6687494.9	Aab	6534915.3	Ab	4573746.1	Ba	6215936.4	Bab	9126578.7	Aa	4102283.7	Bab	6747232.2	Aa	4947691.2	ABb	1796769.7	Cb	4192151.6	Bb	9168353.8	Aa

SI Table 7. *(continuation)* Relative abundance of lipid of *C. reinhardtii* CC503 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
FA 18:0	14788595.0**Aa**	11563701.0**Ba**	12536164.5**Ba**	8630916.2 **Ab**	5740262.3 **Bb**	7768917.2 **Bb**	7335283.9 **Ac**	6261083.9 **Bc**	3159366.5 **Bc**	5858367.0 **Ad**	3036257.3 **Bd**	2773854.0 **Bd**
FA 18:1	84501152.5**Aa**	64370972.8**Ba**	61062507.9**Ba**	41869863.6**Ab**	25752883.6**Bb**	35893372.5**ABb**	31730444.8**Abc**	29305750.3**Ab**	12741830.5**Bc**	26566481.4**Ac**	19669789.2**ABb**	11478436.1**Bc**
FA 18:2	39679594.8**Aa**	34361947.0**Aa**	33342348.4**Aa**	34299582.4**Aab**	28967485.1**Aab**	39711733.9**Aa**	30736064.3**Aab**	24918587.2**Aab**	12242241.3**Bb**	23534650.1**Ab**	19309349.4**ABa**	9524973.8 **Bb**
FA 18:3	70480000.3**Aa**	66576558.3**Aa**	62832350.5**Aa**	43117087.3**Bb**	45913497.4**ABb**	60856534.3**Aa**	37195546.2**ABbc**	40525626.2**Ab**	21664465.1**Bb**	25131477.5**Ac**	21583346.6**Ac**	35513660.0**Ab**
FA 20:0	326725.1 **Aa**	286173.2 **Aa**	298301.2 **Aa**	347130.2 **Aa**	187937.3 **Bab**	207828.2 **Ba**	370747.0 **Aa**	157791.6 **Bb**	67231.1 **Bb**	349856.8 **Aa**	170743.2 **Bb**	38270.7 **Cb**
FA 20:1	1346428.3 **Aa**	1017039.4 **Ba**	997263.3 **Ba**	862495.8 **ABb**	654280.7 **Bb**	971576.1 **Aa**	720116.7 **Abc**	679080.8 **Ab**	328422.7 **Bb**	450623.8 **ABc**	621725.2 **Ab**	225712.9 **Bb**
FA 20:3	84036.9 **Aa**	81271.4 **Aa**	75895.5 **Aa**	58644.8 **Ab**	44977.0 **Ab**	62239.2 **Aa**	56797.5 **Abc**	46513.6 **Ab**	21009.4 **Bb**	35554.2 **Bc**	35229.2 **Bb**	57440.8 **Aa**
FA 22:0	86512.6 **Ab**	98510.6 **Aa**	120701.4 **Aa**	119930.9 **Aab**	65506.5 **Ba**	86412.4 **ABa**	132283.6 **Aa**	65889.8 **Ba**	28173.2 **Bb**	125592.4 **Aab**	73989.8 **Ba**	28397.9 **Cb**
FA 22:1	80941.0 **Aa**	63293.2 **Ba**	59911.0 **Ba**	38971.4 **Ab**	30444.7 **Ab**	41781.8 **Ab**	31704.2 **Ab**	32991.2 **Ab**	19726.2 **Ac**	15455.9 **Ac**	25149.5 **Ab**	17702.3 **Ac**
FA 24:1	7897.9 **Aa**	6498.3 **Aa**	5824.6 **Aab**	7399.5 **Aa**	5827.1 **Aab**	7899.7 **Aa**	7090.5 **Aa**	6473.1 **Aa**	3627.1 **Bbc**	3512.2 **Ab**	3652.1 **Ab**	2563.7 **Ac**
FA 26:0	349147.0 **Aa**	311776.6 **Ba**	312163.4 **Ba**	174487.6 **Abc**	128317.3 **Bbc**	145749.7 **Bbc**	218112.5 **Ab**	182589.3 **Bb**	96424.9 **Bb**	155931.1 **Ac**	107598.0 **Bc**	94556.4 **Bc**
FA 28:0	58690.8 **Ca**	120425.7 **Ba**	183539.4 **Aa**	31862.9 **Bab**	31451.0 **Bb**	72948.6 **Ab**	18829.4 **Ab**	34368.3 **Ab**	21425.6 **Ac**	34167.3 **Aab**	13490.6 **Ab**	20827.7 **Ac**
FA 30:0	10053.5 **Bb**	22550.7 **Ba**	45406.7 **Aa**	15223.9 **ABab**	10404.1 **Ba**	27554.4 **Ab**	4502.5 **Ab**	14538.8 **Aa**	12259.9 **Ab**	27643.7 **Aa**	5855.8 **Ba**	11675.0 **Bb**
FA 30:1	3758.2 **Aa**	4357.1 **Aa**	4165.3 **Aa**	1551.6 **Bb**	1815.2 **Bb**	3807.3 **Aa**	1898.7 **Ab**	1615.7 **ABb**	774.2 **Bb**	1084.3 **Ab**	918.3 **Ab**	925.1 **Ab**

SI Table 8. Relative abundance of lipid of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
DAG 34:1	59601.8	**Ab**	54124.4	**Ab**	65866.0	**Abc**	131629.6	**Aa**	143414.3	**Aa**	129867.2	**Aa**	129671.6	**Aa**	74057.7	**Bb**	85187.1	**Bb**	NA	**Cc**	169760.3	**Aa**	40916.6	**Bc**
DAG 34:2	61042.8	**Bb**	68380.4	**Bb**	116098.6	**Aa**	69180.2	**Ca**	157876.3	**Aa**	119359.6	**Ba**	75967.7	**Aa**	45076.7	**Ab**	47679.7	**Ab**	119830.4	**Aa**	64013.3	**Bb**	27721.0	**Cb**
DAG 34:3	119331.4	**Bb**	126204.5	**Bbc**	262585.7	**Aa**	191756.7	**Ba**	284928.0	**Aa**	NA	**Cc**	173343.6	**Aab**	73297.5	**Bc**	90293.5	**Bb**	204174.0	**Aa**	151841.4	**Ab**	26873.6	**Bbc**
DAG 34:4	NA	**Ac**	NA	**Ad**	NA	**Ab**	200270.5	**Bb**	450016.7	**Aa**	NA	**Cb**	220004.0	**Aab**	98618.7	**Bc**	119845.0	**Ba**	296935.2	**Aa**	213956.8	**Ab**	NA	**Bb**
DAG 34:5	288985.0	**Ba**	229177.7	**Bb**	547127.2	**Aa**	325534.9	**Aa**	NA	**Bc**	NA	**Bb**	NA	**Bb**	256551.5	**Ab**	NA	**Bb**	NA	**Bb**	398176.4	**Aa**	51903.3	**Bb**
DAG 34:6	148342.7	**Ab**	98392.9	**Ab**	341324.8	**Ab**	629119.2	**Ba**	1667651.6	**Aa**	1365924.3	**Aa**	725481.4	**Aa**	273194.8	**Bb**	417573.8	**ABb**	961456.5	**Aa**	418098.7	**Bb**	80007.6	**Bb**
DAG 36:1	8349.7	**Aa**	8146.9	**Aa**	8951.6	**Aa**	10130.8	**Aa**	7875.4	**ABa**	5262.8	**Bb**	8457.8	**Aa**	4666.2	**Bb**	4574.9	**Bbc**	11079.7	**Aa**	6056.1	**Bab**	2157.3	**Cc**
DAG 36:2	148239.3	**Ac**	132537.7	**Ab**	169195.0	**Aab**	278634.3	**Ab**	309806.2	**Aa**	253875.6	**Aa**	350636.3	**Ab**	188273.7	**Bb**	214445.9	**Bab**	501722.2	**Aa**	360584.7	**Ba**	137558.7	**Cb**
DAG 36:3	227471.3	**Ab**	176645.7	**Ab**	331661.0	**Aab**	419506.0	**Aa**	512928.9	**Aa**	429255.1	**Aa**	456886.6	**Aa**	229924.7	**Bb**	249313.4	**Bbc**	544281.9	**Aa**	582779.0	**Aa**	93633.3	**Bc**
DAG 36:4	NA	**Ad**	NA	**Ac**	NA	**Ab**	245512.5	**Ac**	NA	**Bc**	NA	**Bb**	370213.9	**Ab**	151276.0	**Bb**	162794.2	**Ba**	564057.0	**Aa**	385905.6	**Ba**	78799.1	**Cab**
DAG 36:5	261006.2	**Bb**	343757.3	**Ba**	451503.7	**Aa**	NA	**Ac**	NA	**Ab**	NA	**Ac**	304178.9	**Aab**	NA	**Cb**	190270.9	**Bb**	374295.1	**Aa**	288807.5	**Aa**	60016.9	**Bc**
DAG 36:6	90639.7	**Bb**	93301.7	**Bb**	262529.3	**Ab**	185294.4	**Bab**	619244.9	**Aa**	491127.4	**Aa**	215047.4	**Aab**	140191.7	**Ab**	180031.1	**Abc**	235240.2	**Aa**	176288.6	**ABb**	47867.9	**Bc**
DGDG 32:0	85291.6	**Ba**	83815.6	**Ba**	108305.0	**Aa**	60549.8	**ABb**	69735.6	**Ab**	54016.1	**Bb**	45502.2	**Ac**	37752.9	**Ac**	35270.6	**Ac**	59165.3	**Ab**	39538.5	**Bc**	29587.3	**Bc**
DGDG 32:1	39212.3	**Ba**	37055.9	**Ba**	45781.0	**Aa**	15478.4	**Bb**	24260.1	**Ab**	19661.0	**Bb**	7959.4	**Bc**	12938.1	**Ac**	11714.8	**ABc**	7822.2	**ABc**	4819.0	**Bd**	10269.5	**Ac**
DGDG 32:2	147545.9	**Ba**	142138.1	**Ba**	190815.4	**Aa**	61784.0	**Bb**	88355.6	**Ab**	70849.0	**ABb**	41223.2	**Ac**	36445.2	**Ac**	31890.0	**Ac**	34447.4	**Ac**	36617.5	**Ac**	45133.0	**Ac**
DGDG 32:3	NA	**Bc**	NA	**Bb**	383920.6	**Aa**	125665.2	**Ba**	158160.2	**Aa**	NA	**Cc**	NA	**Ac**	NA	**Ab**	NA	**Ac**	43061.0	**Bb**	NA	**Cb**	156842.8	**Ab**
DGDG 32:3.1	53016.2	**Aab**	46020.3	**Ac**	64040.6	**Ab**	70735.7	**Ca**	129507.9	**Aa**	102445.8	**Ba**	49345.5	**Bb**	69836.4	**Ab**	67023.7	**ABb**	56254.6	**Aab**	24620.5	**Bd**	73214.3	**Ab**
DGDG 34:0	135483.0	**ABa**	130883.9	**Ba**	148655.7	**Aa**	50510.5	**Bb**	72243.1	**Ab**	57534.0	**Bb**	24958.9	**Bc**	50751.2	**Ac**	44149.6	**Abc**	18771.8	**ABc**	10603.1	**Bd**	31007.6	**Ac**
DGDG 34:1	2776765.9	**Aa**	2939726.2	**Aa**	2903640.6	**Aa**	1514724.0	**Cb**	2357203.0	**Ab**	2096243.3	**Bb**	1132252.1	**Bc**	1862299.2	**Ac**	1768520.8	**Ac**	843259.1	**Bd**	982047.2	**Bd**	1423300.4	**Ad**
DGDG 34:2	4535439.2	**Aa**	4798274.0	**Aa**	4800668.7	**Aa**	2182533.3	**Bb**	3831575.4	**Ab**	3482459.7	**Ab**	1263199.6	**Bc**	2644475.8	**Ac**	2458962.8	**Ac**	779817.7	**Bc**	834627.3	**Bd**	1589194.7	**Ad**
DGDG 34:3	2024259.0	**Aab**	2030726.3	**Ac**	2317443.5	**Ab**	2169394.9	**Ba**	3803849.0	**Aa**	3496918.1	**Aa**	1620118.6	**Bbc**	2817823.0	**Ab**	2725719.3	**Ab**	1196144.0	**Bc**	965739.7	**Bd**	2431092.9	**Ab**
DGDG 34:4	4883891.3	**Aa**	5318388.9	**Aa**	5271385.3	**Aa**	2036648.7	**Bb**	3500500.2	**Ab**	3121836.5	**Ab**	703674.8	**Bc**	2064616.7	**Ac**	1896959.3	**Ac**	312629.2	**Bc**	703897.3	**ABd**	1111728.3	**Ad**
DGDG 34:5	5583170.1	**Aa**	5881012.6	**Aa**	6086048.1	**Aa**	3911570.7	**Bb**	6037475.8	**Aa**	5482973.4	**Aa**	2963763.0	**Bc**	4176059.4	**Ab**	4228664.9	**Ab**	1775799.7	**Cd**	2682717.9	**Bc**	4020250.8	**Ab**
DGDG 34:6	5899517.5	**Aa**	6024223.3	**Aab**	6296180.4	**Aa**	4352941.5	**Bb**	6677870.7	**Aa**	6268790.5	**Aa**	3837500.1	**Bb**	4960678.1	**Abc**	5031174.5	**Ab**	2648223.2	**Cc**	3943925.0	**Bc**	5706928.0	**Aab**
DGDG 36:2	205097.5	**Ba**	179470.3	**Ca**	269019.5	**Aa**	72806.0	**Cb**	133529.0	**Ab**	109147.4	**Bb**	32444.2	**Bc**	78561.8	**Ac**	71996.6	**Ac**	11906.2	**Bc**	16360.9	**ABd**	35996.3	**Ad**
DGDG 36:3	203201.2	**Ba**	172634.2	**Ca**	277840.8	**Aa**	52455.3	**Bb**	137251.9	**Ab**	116999.8	**Ab**	15684.3	**Bc**	110395.4	**Ab**	96315.9	**Ab**	NA	**Bc**	28787.5	**Ac**	NA	**Bc**
DGDG 36:4	1556609.2	**Ba**	1537496.8	**Aa**	1835664.2	**Aa**	256228.7	**Bb**	474587.3	**Ab**	358901.7	**ABc**	52127.2	**Bc**	263881.5	**Ac**	222935.3	**Ad**	NA	**Bc**	50998.2	**Bd**	577123.9	**Ab**
DGDG 36:5	2797137.8	**Aa**	2894936.0	**Aa**	2976634.4	**Aa**	1059209.4	**Bb**	2024629.4	**Ab**	1773261.8	**Ab**	354566.0	**Bc**	1175063.3	**Ac**	1073464.3	**Ac**	62785.7	**Bc**	212088.7	**Bd**	1375943.8	**Ac**
DGDG 36:6	2818138.9	**Ba**	2703786.0	**Bb**	3168652.6	**Aa**	1877100.3	**Bb**	3270548.9	**Aa**	3027284.4	**Aa**	1085359.4	**Bc**	2256781.7	**Ac**	2151720.6	**Ab**	381472.1	**Bd**	454003.4	**Bd**	2226940.6	**Ab**

SI Table 8. *(continuation)* Relative abundance of lipid of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
LysoPC 16:0	33211.8	ABb	21228.7	Bc	50182.8	Aa	42444.1	Bb	86087.3	Aa	60627.4	Ba	57102.9	Ab	60330.4	Aab	50624.6	Aa	173949.0	Aa	57212.3	Bb	75521.1	Ba
LysoPC 16:0.1	2701.0	Bb	1969.5	Bc	5002.2	Aa	3547.2	Bb	8921.6	Aa	5573.3	Ba	4738.0	Ab	5509.5	Ab	4580.0	Aa	13348.5	Aa	5484.3	Bb	6219.0	Ba
LysoPC 16:2	16874.4	Aa	10025.6	Ac	37838.4	Ac	28087.8	Ba	98485.4	Ab	79545.9	Ab	41512.1	Ba	92540.9	Ab	93749.2	Ab	29785.1	Ca	153481.9	Ba	193075.2	Aa
LysoPC 16:3	7796.9	Aa	4367.5	Ac	18222.3	Ac	17593.7	Ba	73629.0	Ab	58420.9	ABbc	33467.0	Ba	88987.8	Ab	95030.8	Ab	22539.3	Ca	177442.8	Ba	294598.6	Aa
LysoPC 18:2	56903.9	Aa	31879.7	Ab	96608.5	Ac	111116.1	Ba	683432.6	Aa	521057.7	Ab	160645.4	Ba	569253.2	Aa	576993.9	Ab	115303.6	Ca	628113.3	Ba	1812148.7	Aa
LysoPC 18:3	23300.5	Aa	9961.4	Ab	42456.1	Ac	41055.5	Ba	240347.6	Aa	191777.5	ABbc	66490.6	Aa	240740.2	Aa	243323.1	Ab	48672.3	Ca	290542.9	Ba	989661.6	Aa
MGDG 34:1	424980.3	Ba	445793.8	ABa	494679.6	Aa	75442.0	Bb	347374.1	Ab	285212.4	Ab	47067.7	Bb	229045.8	Ac	224455.2	Abc	12807.1	Bb	44213.4	Bd	171506.8	Ac
MGDG 34:2	521578.6	Ba	553887.6	Aa	596274.1	Aa	26309.7	Bb	97455.2	Ab	77732.2	Ab	13806.6	Bb	78670.3	Ab	89670.4	Ab	4943.2	Bc	18218.5	Ac	43483.2	Ac
MGDG 34:2.1	396287.0	Aa	455476.6	Aa	462701.9	Aa	45676.2	Bb	528589.5	Aa	470569.0	Aa	40930.6	Bb	340416.0	Ab	371331.7	Aa	7269.8	Bb	52464.4	Bc	388023.9	Aa
MGDG 34:3	5767.8	Ba	6595.5	Ba	8785.5	Aa	NA	Cb	8678.8	Aa	6039.1	Bb	NA	Bb	6333.6	Aa	6523.0	Aab	NA	Bb	NA	Bb	7566.3	Aab
MGDG 34:4	1721801.6	Ba	2165609.6	Aa	1758982.7	Ba	91731.9	Bb	983245.9	Ab	939813.9	Ab	113224.3	Bb	1015216.0	Ab	1117089.7	Ab	16320.0	Bb	184596.5	Bc	1895818.8	Aa
MGDG 34:5	1581538.6	Aa	1938060.6	Aa	1643056.5	Ab	216301.3	Bb	1635914.8	Aa	1595646.5	Ab	191173.2	Bb	1538836.6	Aa	1688022.5	Ab	30842.0	Bb	353677.0	Bb	3106367.1	Aa
MGDG 36:5	233851.1	Aa	283888.3	Aa	280027.1	Ab	NA	Bb	223304.4	Aa	182293.7	Ab	0.0	Bb	242111.2	Aa	249558.3	Ab	NA	Bb	NA	Bb	1098709.1	Aa
MGDG 36:6	181343.0	Aa	228454.6	Aa	222222.7	Ab	14659.1	Ba	197248.0	Aab	185505.0	Ab	19577.3	Ba	238877.1	Aa	288154.7	Ab	1342.5	Ba	16197.5	Bb	1802619.4	Aa
PC 32:0	8286.9	Bb	8077.0	Bab	12029.2	Aa	10955.4	Aa	8929.4	Ba	7922.0	Bb	7844.4	Ab	6671.9	Ab	7068.2	Abc	12815.2	Aa	7429.1	Bab	5474.2	Cc
PC 32:1	35225.6	Bb	30595.1	Bb	45638.3	Aa	38819.3	Bb	48520.8	Aa	48264.8	ABa	31971.4	Ab	32727.1	Ab	34463.0	Ab	55121.5	Aa	36022.3	Bb	50443.5	Aa
PC 32:1.1	23583.3	Ab	21942.8	Ab	25804.7	Ab	24260.2	Bb	35672.5	Aa	29363.3	ABb	22310.0	Ab	22798.1	Ab	22097.7	Ab	38409.7	Aa	20831.7	Bb	44942.1	Aa
PC 32:2	126783.6	Ab	64723.7	Ac	190808.2	Ac	216048.1	Bb	546630.5	Aa	439760.0	Ab	230771.8	Aab	361088.4	Ab	364149.4	Ab	385548.5	Ba	318773.5	Bb	825508.1	Aa
PC 32:3	118861.1	Aa	61828.9	Ac	187712.6	Ac	133754.4	Ba	494211.2	Aa	399637.6	Ab	143474.3	Ba	320965.7	Ab	346402.0	Abc	216652.7	Ca	386769.5	Bab	1151604.9	Aa
PC 32:4	NA	Bb	NA	Bb	7430.3	Ab	7292.7	Ba	12679.5	Aa	10325.4	Abb	2603.5	Bab	9558.3	Aa	11056.3	Ab	2422.3	Cab	11790.0	Ba	35881.4	Aa
PC 34:1	NA	Ba	NA	Bc	235299.0	Ab	NA	Aa	NA	Ac	NA	Ac	NA	Ba	506002.8	Aa	491964.8	Aa	NA	Ba	196813.2	Ab	NA	Bc
PC 34:2	NA	Ab	NA	Ab	NA	Ac	711987.0	Ba	1304404.1	Aa	1045497.7	ABb	399845.7	Bab	1183609.9	Aa	1157840.5	Ab	624616.5	Ba	NA	Cb	3434174.0	Aa
PC 34:3	196772.6	Aa	141975.4	Ab	291713.4	Ac	155214.8	Ba	624654.3	Aa	490156.0	Abc	162584.2	Ba	621764.6	Aa	598106.8	Ab	313685.9	Ba	390681.1	Bab	2116066.2	Aa
PC 34:4	52486.9	Aa	36151.7	Ab	86117.7	Ac	47719.2	Ba	241521.8	Aa	208417.2	Ab	36139.0	Ba	221456.9	Aa	226401.9	Ab	42391.8	Ca	157627.4	Ba	805215.4	Aa
PC 34:5	34926.2	Aa	23343.2	Ab	60708.6	Ac	54352.2	Aa	299113.7	Aab	256350.0	Abc	44844.3	Ba	356367.0	Aa	384721.5	Ab	35684.4	Ba	209564.3	Bab	1657105.5	Aa
PC 34:6	13269.9	Aa	7761.9	Aa	20893.7	Ac	24311.0	Aa	143038.6	Aa	120160.8	Abc	28170.7	Ba	286689.7	Aa	347806.8	Ab	15858.1	Ba	157248.1	Ba	1577583.1	Aa
PC 36:1	231241.2	Bb	146987.1	Cc	310159.7	Ab	328641.8	Aa	369553.9	Ab	356324.4	Ab	245459.1	Bb	396533.5	Ab	453790.2	Aa	208469.8	Bb	467552.8	Aa	185711.2	Bc
PC 36:2	3214453.0	ABa	1817153.4	Bc	4381735.8	Ab	3957864.3	Aa	4748681.7	Ab	5136352.3	Ab	3435201.4	Ba	9144370.3	Aa	10566180.3	Aa	3148950.2	Ba	10326954.0	Aa	9490229.1	Aa
PC 36:3	155112.5	ABb	82837.2	Bd	240471.5	Ad	384682.9	Ba	821453.9	Aa	691059.2	Ab	199766.7	Bb	465521.5	Ac	445742.8	Ac	295279.2	Cab	635529.0	Bb	1638545.8	Aa

SI Table 8. *(continuation)* Relative abundance of lipid of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
PC 36:3.1	19357.3	Aa	17121.8	Ac	22549.8	Ac	16024.8	Aa	54629.8	Ac	75942.6	Ac	8922.3	Ca	398224.9	Bb	508428.1	Aa	9687.2	Ca	581014.0	Aa	396627.3	Bb
PC 36:5	83334.4	Aa	48702.6	Ab	169015.4	Ac	207906.5	Ba	1337939.7	Aa	1202097.9	Ab	189336.8	Ba	1109232.8	Aa	1439296.5	Ab	248624.0	Ba	869903.0	Bab	5941379.5	Aa
PC 36:6	33504.9	Aa	24165.4	Aa	55882.1	Ab	75838.0	Aa	591913.4	Aa	508648.5	Ab	64012.8	Aa	576509.0	Aa	649142.5	Ab	79263.4	Ba	362267.2	Ba	4222914.1	Aa
PC 38:2	77623.2	Ba	57250.8	Cc	102382.5	Ab	78667.3	Ba	98185.1	Ab	109674.4	Ab	46444.0	Cb	122676.0	Ba	141407.4	Aa	54911.2	Cb	80392.7	Bb	138141.7	Aa
PC 38:3	4982.5	Ba	2446.5	Bd	10480.7	Ac	3955.8	Ba	18691.2	Aa	16650.4	Ab	4293.9	Ba	14858.4	Ab	15292.3	Ab	3672.2	Ba	6928.5	Bc	28433.2	Aa
PC 38:3.1	1546.7	Ab	1282.8	Ac	1752.2	Ac	2686.0	Ba	3556.6	ABb	3915.3	Ab	2950.0	Ba	5737.7	Aa	6671.9	Aa	2946.1	Ca	5831.3	Aa	4219.5	Bb
PE 32:1	74749.8	Aa	70106.0	Ac	102230.9	Ac	81483.1	Ba	130693.2	Ab	157031.3	Ab	59457.5	Ca	271519.4	Ba	336670.0	Aa	84894.1	Aa	82696.2	Ac	79585.5	Ac
PE 32:2	24754.2	Aa	21866.0	Ac	34555.8	Ac	36210.6	Ba	72388.0	Ab	87406.8	Ab	25432.6	Ca	128622.3	Ba	151980.5	Aa	30513.0	Aa	NA	Bd	22556.2	Ac
PE 34:1	89906.9	Ba	75825.1	Bc	152689.4	Ac	139350.4	Ba	208984.4	Ab	219007.2	Ab	112082.9	Ba	267639.7	Aa	294845.3	Aa	109370.6	Ba	159076.1	Ab	167016.1	Ac
PE 34:2	532195.9	Aa	446878.7	Ac	786876.7	Ad	635232.1	Ba	1342024.4	Ab	1588647.7	Ac	423771.0	Ba	2750568.5	Aa	3049963.9	Aa	481225.0	Ca	1596230.6	Bb	2201545.4	Ab
PE 34:3	10126.3	Aa	6635.0	Aa	24537.4	Ab	10524.8	Aa	24079.7	Aa	21681.1	Ab	6148.1	Ba	26100.0	ABa	42482.6	Ab	8885.9	Ba	11340.6	Ba	84249.3	Aa
PE 34:4	2876.8	Aa	2744.2	Aa	4013.0	Ab	2612.5	Aa	8988.2	Aa	7868.1	Ab	938.8	Ba	8239.8	ABa	12834.9	Ab	1531.2	Ba	2769.4	Ba	33792.7	Aa
PE 34:5	1984.0	Aa	1674.5	Aa	3054.5	Ac	2609.2	Aa	9622.5	Aa	9381.2	Abc	3243.5	Ba	11524.1	ABa	17991.5	Ab	NA	Ba	2874.8	Ba	42225.7	Aa
PE 34:6	NA	Aa	NA	Aa	1834.6	Ab	1319.3	Aa	5946.3	Aa	5369.3	Ab	NA	Ba	5884.8	ABa	8825.1	Ab	NA	Ba	2177.8	Ba	25469.4	Aa
PE 36:1	27348.1	Aa	28724.2	Ab	54606.8	Ac	29924.2	Ba	146888.3	Aa	168672.1	Aa	25190.9	Ba	128343.7	Aa	141061.7	Aa	11523.6	Ca	50965.8	Bb	94194.0	Ab
PE 36:2	860470.2	Aa	879272.8	Ac	1485356.0	Ad	1218948.0	Ba	4308257.5	Aa	4559571.7	Ab	1110166.3	Ba	5106036.8	Aa	5505162.3	Aa	512941.3	Ba	2726482.1	Ab	3089702.9	Ac
PE 36:3	NA	Aa	NA	Ad	NA	Ad	NA	Ca	314649.1	Bb	388822.9	Ab	NA	Ba	482012.9	Aa	529214.0	Aa	NA	Ba	140167.7	Ac	162122.2	Ac
PE 36:3.1	12876.0	Aa	9943.7	Ab	25071.1	Ac	19044.5	Aa	40735.8	Aa	42220.3	Abc	10629.2	Ba	38005.3	Aa	51767.7	Ab	15776.8	Ba	22201.3	Bab	111649.4	Aa
PE 36:4	22002.6	ABb	14820.2	Bb	32894.3	Ac	31458.9	Bab	77146.1	Aa	83870.2	Ab	21070.7	Cb	72631.7	Ba	102954.5	Aa	40004.4	Ba	82717.3	Aa	77085.3	Ab
PE 36:4.1	23743.5	Aa	11335.1	Aa	47652.4	Ab	24526.8	Aa	59033.6	Aa	66459.6	Ab	15771.1	Ba	66307.7	ABa	107171.9	Ab	22253.2	Ba	34733.6	Ba	251497.4	Aa
PE 36:5	7979.3	Aa	6259.6	Aa	16514.4	Ab	8430.2	Aa	39974.2	Aa	43613.0	Ab	5681.6	Ba	53880.9	ABa	92547.4	Ab	4279.6	Ba	23039.5	Ba	278959.0	Aa
PE 36:6	3122.5	Aa	3067.4	Aa	5322.8	Ab	2039.1	Aa	10674.5	Aa	10805.8	Ab	1513.6	Ba	15480.1	ABa	27721.0	Bb	1583.6	Ba	5319.1	Ba	80154.6	Aa
PE 38:2	4530953.9	Ba	3464152.9	Cc	6115181.6	Aa	4459625.9	Aa	4897814.3	Ab	5077599.8	Abc	2659294.0	Bb	3771661.6	Ac	4579005.1	Ac	2358305.8	Bb	6642412.1	Aa	5767399.6	Aab
PE 38:3	NA	Aa	NA	Ac	NA	Ac	NA	Ba	30563.2	Ab	39754.7	Ab	1753.8	Ca	83648.8	Ba	105278.0	Aa	NA	Ba	38449.8	Ab	36228.7	Ab
PE 40:1	NA	Bb	NA	Bc	5568.1	Aab	NA	Bb	NA	Bc	5589.5	Aab	5585.7	Aa	6053.1	Aa	5989.0	Aa	5536.5	Aa	4803.9	Ab	4728.7	Ab
PE 40:2	49500.9	ABb	29860.3	Bd	69387.8	Ab	64466.0	Aab	65691.5	Ac	68931.8	Ab	58301.3	Bb	112773.8	Ab	125038.5	Aa	86138.7	Ca	148689.3	Aa	109170.7	Ba
PG 32:0	28221.2	Ba	34163.0	Aa	27836.2	Ba	8330.0	Bb	13737.1	Ab	11075.1	ABb	9647.2	Ab	11602.9	Abc	11381.5	Ab	9838.9	Bb	6734.3	Bc	15734.0	Ab
PG 32:1	13473.0	Aa	13711.2	Ab	15883.5	Ab	10647.2	Aa	14722.3	Ab	15298.0	Ab	9504.2	Ba	27960.8	Aa	32634.4	Aa	12475.3	Aa	5926.5	Bc	7944.5	ABc
PG 34:2	284158.4	Aa	336252.5	Ab	289089.6	Ab	147573.1	Bb	356288.7	Ab	363509.7	Ab	94973.7	Bb	531750.9	Aa	584305.7	Aa	110650.9	Cb	207301.7	Bc	505531.9	Aa

SI Table 8. *(continuation)* Relative abundance of lipid of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
PG 34:3	66549.1	Aa	74382.2	Abc	80195.0	Ac	35142.9	Ba	174419.5	Aab	152562.7	Abc	15937.8	Ba	214645.9	Aa	225743.1	Ab	12780.5	Ba	21451.8	Bc	617799.1	Aa
PG 34:4	15740.2	Aa	16738.1	Aa	19145.9	Ab	20980.4	Aa	48484.0	Aa	40535.3	Ab	7819.7	Aa	41390.2	Aa	42264.4	Ab	11067.0	Ba	7723.6	Ba	151393.9	Aa
PG 36:1	25626.2	Aa	24611.5	Ac	29874.5	Ac	31115.4	Ba	69709.4	Ab	74939.9	Ab	24035.5	Ba	105624.7	Aa	120720.8	Aa	27247.9	Ba	51244.4	Ab	55857.5	Ab
PG 36:2	332243.0	Aa	296639.9	Ac	386428.9	Ac	385687.4	Ba	923862.3	Ab	982482.9	Ab	255424.0	Ba	1618946.7	Aa	1747619.2	Aa	318276.6	Ba	882106.5	Ab	1058410.6	Ab
SQDG 32:0	2959146.5	Ba	3341828.9	Aa	3431755.1	Aa	1209701.5	Bb	1773062.0	Ab	1471412.6	ABb	944181.9	Ab	1125894.4	Ac	1097442.3	Ac	1231352.3	Ab	1028316.8	Ac	1239169.8	Abc
SQDG 32:1	72918.7	Ba	78504.1	Ba	89861.7	Aa	26566.6	Bb	42408.8	Ab	31726.7	Bbc	16045.2	Ac	23191.3	Ac	22627.4	Ac	15173.1	Bc	10607.4	Bd	38633.2	Ab
SQDG 32:2	84539.7	Ba	89844.6	Ba	97884.4	Aa	26849.9	Bb	37819.5	Ab	30766.6	ABc	NA	Bc	25121.4	Ac	24768.2	Ac	NA	Bc	NA	Bd	42260.6	Ab
SQDG 32:3	151915.7	Ba	164814.1	ABa	175322.6	Aa	61727.7	Bb	88197.4	Ab	71792.5	ABc	39259.8	Bc	65903.3	Ac	70966.1	Ac	26683.5	Bc	34528.3	Bd	95129.2	Ab
SQDG 34:0	673063.6	Ba	728616.7	Ba	821366.2	Aa	290280.5	Bb	412798.1	Ab	330768.1	ABb	168091.8	Ac	231562.5	Ac	225395.0	Ac	135260.7	Ac	85395.9	Ad	139419.8	Ac
SQDG 34:1	1346802.0	Ba	1469626.3	ABa	1577086.6	Aa	NA	Cb	776942.9	Ab	620376.8	Bb	NA	Bb	445450.4	Ac	436202.0	Ac	NA	Cb	323454.7	Bc	602366.1	Ab
SQDG 34:2	4197522.1	Ba	4792963.5	Aa	4928817.3	Aa	1088806.3	Bb	2278238.7	Ab	1860299.1	Abc	605882.2	Bbc	1390599.4	Ac	1373204.0	Ac	525690.0	Bc	934314.1	Bc	1918286.6	Ab
SQDG 34:3	2496651.0	Ba	2884779.4	Ba	3504580.0	Aa	1794870.3	Bb	3291608.0	Aa	2842997.9	Ab	1239649.2	Bb	2284594.4	Ab	2242424.7	Ac	1660711.1	Bb	1314107.2	Bc	3159481.0	Aab
SQDG 34:4	66016.7	Ba	69626.3	Ba	78006.9	Aa	18679.5	Bb	26814.3	Ab	24247.7	ABc	9653.6	Bc	21536.6	Ab	20861.4	Ac	6154.4	Bc	11156.9	Bc	35556.5	Ab
SQDG 34:5	49274.1	Ba	53500.0	ABa	59084.1	Aa	18385.5	Bb	28568.4	Ab	26568.4	Ac	9284.5	Bc	24609.5	Ab	24578.7	Ac	5418.9	Cc	12781.3	Bc	41175.1	Ab
SQDG 34:6	38198.6	Ba	41254.2	ABa	46392.6	Ab	14471.5	Bb	25883.3	Ab	23960.5	Ac	6878.0	Bc	26459.0	Ab	28057.8	Ac	4837.3	Cc	13627.7	Bc	53653.2	Aa
TAG 42:0	28981.1	Aa	33767.8	Aa	29069.7	Aab	24628.7	Aa	28453.5	Aa	29801.9	Aa	22906.3	Aa	19589.8	Ab	22635.7	Ab	NA	Cb	30515.5	Aa	15236.9	Bc
TAG 42:1	30838.8	Ab	32225.2	Aa	33186.3	Aa	34057.2	Aab	33883.0	Aa	31678.9	Aab	37030.6	Aab	21309.7	Bb	22103.8	Bbc	44045.7	Aa	40503.7	Aa	16392.7	Bc
TAG 42:2	5400.7	Ab	5607.9	Abc	5944.9	Aab	11170.4	Aa	7410.4	Bb	6392.4	Ba	10065.7	Aa	3549.3	Bc	4406.0	Bab	9876.7	Aa	10929.4	Aa	3282.4	Bb
TAG 44:0	105605.3	Aa	117231.9	Aa	108053.4	Aa	88903.0	Aab	85912.6	Ab	86863.9	Aa	98507.9	Aab	53151.4	Bc	56745.6	Bb	77029.5	Ab	92053.1	Ab	44092.0	Bb
TAG 44:1	69283.3	Ac	72659.8	Ab	71505.5	Aa	91002.5	Abc	62802.1	Bb	54832.9	Bab	103839.6	Aab	33862.4	Bc	37970.7	Bb	122178.3	Aa	102089.2	Aa	38488.5	Bb
TAG 44:2	33130.8	Ac	36361.0	Aa	35703.5	Aa	43694.6	Ab	36244.0	ABa	29379.5	Bab	48348.0	Ab	20002.3	Bb	20164.2	Bbc	62505.9	Aa	41206.7	Ba	14618.6	Cc
TAG 46:0	454616.5	Aa	502010.8	Aa	450679.5	Aa	381716.6	Aab	375101.8	Ab	395200.2	Aa	392524.5	Aab	264702.6	Bc	303729.8	Bb	326895.4	Ab	390722.8	Ab	225033.3	Bb
TAG 46:1	390291.4	Ac	361006.2	Ab	453626.0	Aa	551621.7	Ab	414327.0	Bb	361771.3	Ba	667282.7	Aab	178471.3	Bc	207140.0	Bb	746436.0	Aa	677161.4	Aa	103212.6	Bb
TAG 46:2	84433.4	Ac	83538.2	Abc	101587.5	Aa	146268.4	Ab	104628.8	Bb	82824.2	Bab	166056.3	Ab	46336.9	Bc	50760.6	Bb	238586.4	Aa	183352.5	Ba	NA	Cc
TAG 46:2.1	73619.9	Aa	86313.9	Aa	83269.9	Aa	63089.8	Aab	55268.0	Ab	64072.1	Ab	68690.9	Aa	39768.7	Bb	44172.3	Bc	36537.1	Ab	NA	Bc	34536.0	Ac
TAG 46:3	39477.8	Ac	35469.7	Abc	51906.7	Aa	85349.6	Ab	60650.0	ABb	48837.9	Ba	102881.1	Ab	21678.1	Bc	25943.4	Bab	178751.6	Aa	135773.0	Ba	7479.8	Cb
TAG 46:3.1	13352.7	Aa	14169.7	Aa	16070.5	Aa	NA	Bb	11564.5	Aa	12483.7	Aa	NA	Bb	6715.0	Ab	7910.1	Ab	NA	Cb	13549.5	Aa	4250.0	Bb
TAG 46:4	15611.9	Ac	17722.3	Ab	19000.0	Aa	24215.5	Abc	16090.9	ABbc	13571.0	Bab	28217.1	Ab	6660.6	Bc	8589.1	Bbc	41667.6	Aa	32519.6	Ba	1957.6	Cc
TAG 48:0	1878310.2	Aa	2069776.1	Aa	1861266.0	Aa	1388429.8	Ab	1443088.5	Ab	1412176.3	Ab	1279227.0	Ab	1058261.6	Ac	1105675.5	Ac	1279103.6	ABb	1525413.1	Ab	1075407.2	Bc

SI Table 8. *(continuation)* Relative abundance of lipid of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 48:1	2580390.8 **Ac**	2606663.0 **Ab**	2948438.2 **Aa**	4264684.5 **Ab**	2829548.1 **Bb**	2597513.4 **Ba**	5604381.1 **Ab**	1410873.3 **Bb**	1539942.9 **Bab**	8219934.7 **Aa**	4904361.5 **Ba**	1080090.1 **Cb**
TAG 48:2	2752401.5 **Ac**	2569657.6 **Abc**	3362814.3 **Aab**	4281700.1 **Abc**	3966173.3 **Ab**	3525570.5 **Aa**	4508514.8 **Ab**	1484882.9 **Bc**	1783469.9 **Bbc**	7569232.9 **Aa**	8300616.7 **Aa**	546047.1 **Bc**
TAG 48:3	2531105.8 **Ac**	2329525.9 **Ab**	3093841.2 **Aab**	4186256.6 **Abc**	4210514.0 **Ab**	3818544.8 **Aa**	5388960.4 **Ab**	1791940.3 **Bb**	2161676.8 **Bab**	11739948.3 **Aa**	11370502.2 **Aa**	927182.7 **Bb**
TAG 48:4	522970.2 **Ab**	433240.1 **Ac**	692972.5 **Aab**	1031947.6 **Aa**	947400.5 **Ab**	813484.5 **Aa**	983798.6 **Aa**	259623.4 **Bc**	326587.3 **Bbc**	1316917.7 **Ba**	2037126.8 **Aa**	76717.7 **Cc**
TAG 48:5	450838.5 **Ac**	389541.5 **Ac**	596819.3 **Aa**	964487.7 **Ab**	824512.7 **Ab**	671871.8 **Aa**	1054055.8 **Ab**	251513.5 **Bc**	319911.4 **Bab**	1596939.2 **Aa**	1914800.5 **Aa**	68284.8 **Bb**
TAG 48:6	125366.7 **Ac**	107000.0 **Abc**	163388.6 **Aab**	343274.0 **Ab**	293273.6 **Ab**	250616.8 **Aa**	434844.5 **Ab**	92031.0 **Bc**	111333.5 **Bab**	755168.8 **Ba**	929752.3 **Aa**	32605.1 **Cb**
TAG 48:7	39808.2 **Ab**	32517.9 **Ac**	53227.9 **Aab**	101158.9 **Aa**	77215.9 **ABb**	58823.5 **Ba**	98525.6 **Aa**	20764.4 **Bc**	22667.4 **Bbc**	88495.3 **Ba**	153628.3 **Aa**	5050.6 **Cc**
TAG 48:8	14019.1 **Ac**	11957.8 **Ac**	19997.2 **Aab**	47242.1 **Ab**	45505.1 **Ab**	35514.0 **Aa**	56088.2 **Aab**	12629.7 **Bc**	15475.9 **Bb**	67983.7 **Ba**	88196.6 **Aa**	4506.9 **Cb**
TAG 48:9	2679.9 **Ac**	2355.5 **Ac**	3459.9 **Ab**	8929.3 **Ab**	11224.5 **Ab**	9953.8 **Aa**	12627.8 **Ab**	4062.7 **Bc**	5535.9 **Bab**	19625.1 **Aa**	18901.9 **Aa**	2329.2 **Bb**
TAG 50:0	2957181.4 **Ba**	3403698.7 **Aa**	2867207.7 **ABa**	2174615.2 **Bb**	2373572.0 **Ab**	2183718.4 **ABb**	1982520.1 **Bc**	1716822.5 **Ac**	1859730.4 **ABc**	1644191.1 **Bc**	2208651.5 **Ac**	1929407.7 **ABc**
TAG 50:1	50922653.4 **Ac**	48381188.6 **Ac**	56587300.2 **Aab**	86667510.4 **Ab**	71773241.7 **ABb**	63730557.6 **Ba**	103781138.0 **Ab**	31280574.1 **Bc**	36697957.4 **Bbc**	136819922.2 **Aa**	115684097.3 **Ba**	17359312.7 **Cc**
TAG 50:2	17846104.7 **Ac**	17868760.5 **Abc**	22334493.9 **Aa**	29185745.6 **Ab**	22911472.2 **Ab**	19693564.9 **Aab**	35727394.2 **Ac**	9598139.3 **Bc**	10721269.8 **Bbc**	55514607.0 **Aa**	43742228.2 **Ba**	5642313.2 **Cc**
TAG 50:3	14179013.1 **Ac**	13184517.0 **Ac**	17244035.7 **Aab**	28354992.6 **Ab**	24251526.9 **Ab**	21511485.4 **Aa**	32944030.1 **Ab**	10575616.2 **Bc**	12089983.9 **Bbc**	44064126.0 **Aa**	42254980.8 **Aa**	3765087.3 **Bc**
TAG 50:4	13698435.9 **Ac**	12182317.0 **Abc**	16826141.9 **Aa**	31675709.5 **Ab**	23773043.5 **Ab**	21428508.8 **Aa**	40920978.4 **Ab**	9897415.7 **Bc**	11921038.0 **Bab**	64939228.2 **Aa**	54463280.5 **Aa**	3410880.7 **Bb**
TAG 50:5	8571862.9 **Ac**	7442600.8 **Abc**	10821620.5 **Aa**	23599409.4 **Ab**	12118699.7 **Bb**	10427452.2 **Ba**	23548170.2 **Ab**	4130663.1 **Bc**	5031403.8 **Bab**	33374098.7 **Aa**	32282593.7 **Aa**	1159656.0 **Bb**
TAG 50:6	11711.7 **Ac**	13740.1 **Ab**	8544.4 **Aa**	149511.5 **Aa**	39794.5 **Bab**	29355.6 **Ba**	81923.2 **Ab**	9049.6 **Bb**	11871.2 **Ba**	190605.3 **Aa**	78184.0 **Ba**	1555.5 **Ca**
TAG 50:6.1	4701649.4 **Ac**	4000928.5 **Ab**	5937711.1 **Aa**	17620592.5 **Ab**	6916727.4 **Bb**	6332846.9 **Ba**	25324279.0 **Ab**	2633146.7 **Bb**	3259896.4 **Ba**	38713409.8 **Aa**	26593335.4 **Ba**	846181.9 **Ca**
TAG 50:7	577474.7 **Ac**	497627.4 **Ab**	749649.2 **Aa**	2397949.0 **Ab**	1168087.6 **Bb**	914289.9 **Ba**	2803855.1 **Ab**	351592.3 **Bb**	419556.7 **Ba**	4162254.5 **Aa**	3891784.4 **Aa**	88985.3 **Ba**
TAG 50:8	240733.8 **Ac**	195638.3 **Ab**	349925.0 **Aa**	1135520.2 **Ab**	694905.3 **Ab**	647509.3 **Aa**	1410128.3 **Ab**	237279.1 **Bb**	309838.4 **Ba**	2536588.9 **Aa**	2280598.5 **Aa**	75624.8 **Ba**
TAG 50:9	65672.0 **Ad**	53446.4 **Ab**	88278.5 **Aa**	460487.9 **Ac**	286957.3 **Ab**	250243.7 **Aa**	786871.5 **Ab**	115095.4 **Bb**	146804.2 **Ba**	1569741.6 **Aa**	930971.8 **Ba**	55139.1 **Ca**
TAG 52:0	2651355.6 **Aa**	2941912.2 **Aa**	2628531.6 **Aa**	1945383.6 **Ab**	2054215.4 **Ab**	1939100.1 **Ab**	1799135.2 **Ac**	1618324.0 **Ac**	1675584.0 **Ac**	1497763.7 **Ac**	1929907.5 **Ac**	1755596.5 **Ac**
TAG 52:1	39043405.6 **Ab**	38831259.3 **Aa**	43303066.7 **Aa**	42366057.2 **Ab**	36292322.0 **ABa**	31221308.7 **Bb**	49020253.4 **Ab**	18208695.3 **Bb**	20178443.2 **Bc**	64463033.6 **Aa**	38504999.9 **Ba**	9044862.1 **Cd**
TAG 52:2	102616699.3 **Ab**	101086352.8 **Ac**	116689122.8 **Aab**	186301449.9 **Aa**	163407842.5 **Ab**	152667354.0 **Aa**	209305650.5 **Aa**	90185875.8 **Bc**	98089165.5 **Bb**	220523899.7 **Aa**	216898535.7 **Aa**	44035893.4 **Bc**
TAG 52:3	53974806.9 **Ac**	50564473.3 **Ac**	61860458.3 **Aab**	111563158.1 **Ab**	96809460.9 **Ab**	84264618.0 **Aa**	132139757.8 **Ab**	41288750.0 **Bc**	47736074.5 **Bb**	167089320.8 **Aa**	143896857.1 **Aa**	17264395.2 **Bc**
TAG 52:4	30446672.2 **Ac**	27742338.3 **Ac**	37194589.3 **Aab**	75496585.1 **Ab**	65063758.2 **Ab**	58167907.4 **Aa**	94176033.1 **Ab**	28258906.6 **Bc**	32867134.2 **Bbc**	139467359.3 **Aa**	119343299.1 **Aa**	11931178.7 **Bc**
TAG 52:5	17248421.8 **Ac**	15517226.9 **Ac**	21706209.2 **Aa**	57053422.1 **Ab**	34775809.7 **Bb**	30877038.8 **Ba**	68333792.9 **Ab**	14540982.1 **Bc**	17319522.0 **Bab**	90278945.4 **Aa**	65910390.2 **Ba**	3855724.9 **Cb**
TAG 52:5.1	21560.8 **Ab**	15661.8 **Ab**	29763.8 **Aa**	97895.5 **Ab**	127929.4 **Ab**	145821.5 **Aa**	170146.5 **Ab**	23435.4 **Ab**	30410.8 **Aa**	514713.6 **Aa**	432579.8 **Aa**	7405.7 **Ba**
TAG 52:6	10101566.0 **Ac**	8611201.4 **Ab**	12842056.0 **Aab**	48363416.4 **Ab**	23157777.0 **Bb**	21652250.7 **Ba**	57335003.0 **Ab**	9346124.5 **Bb**	10957972.1 **Bab**	80484503.2 **Aa**	56949691.3 **Ba**	2195586.2 **Cb**
TAG 52:6.1	4492.1 **Ad**	5247.8 **Ab**	11009.8 **Aa**	54062.6 **Ac**	17402.7 **ABb**	9774.0 **Ba**	357137.3 **Aa**	6441.1 **Bb**	4191.3 **Ba**	102953.9 **Ab**	72349.7 **Aa**	2987.1 **Ba**

SI Table 8. *(continuation)* Relative abundance of lipid of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 52:7	4458210.2 **Ac**	3785192.2 **Ab**	5767775.8 **Aa**	35164670.9 **Ab**	12027574.9 **Bb**	11373386.3 **Ba**	49496584.4 **Ab**	4689970.7 **Bb**	5877826.2 **Ba**	76013889.5 **Aa**	44314379.2 **Ba**	1303301.2 **Ca**
TAG 52:8	991766.0 **Ac**	769803.7 **Ab**	1317126.6 **Aa**	5974479.6 **Ab**	2885831.6 **Bb**	2702247.6 **Ba**	6418081.6 **Ab**	922732.0 **Bb**	1257175.9 **Ba**	10248831.9 **Aa**	8790298.4 **Aa**	295716.0 **Ba**
TAG 52:9	257139.9 **Ac**	194264.9 **Ab**	341112.0 **Aa**	2304922.0 **Ab**	942973.7 **Bb**	911980.8 **Ba**	2897660.8 **Ab**	418682.2 **Bb**	545139.0 **Ba**	5051331.1 **Aa**	3835608.3 **Ba**	195624.8 **Ca**
TAG 54:0	971323.3 **Aa**	1108111.8 **Aa**	956339.5 **Aa**	735671.4 **Ab**	786729.5 **Ab**	761392.1 **Ab**	687987.1 **Ab**	543943.4 **Ac**	588348.9 **Abc**	607233.9 **Bb**	799442.4 **Ab**	541715.8 **Bc**
TAG 54:1	12134760.8 **Aa**	12265746.6 **Aa**	13735701.4 **Aa**	10263627.7 **Ab**	8708367.7 **ABb**	7551382.6 **Bb**	9905434.3 **Ab**	4479798.9 **Bd**	4849934.8 **Bc**	11610402.4 **Aab**	6757962.1 **Bc**	2829056.4 **Cd**
TAG 54:2	45952269.9 **Ac**	45356315.5 **Aa**	52147286.1 **Aa**	62102441.1 **Ab**	55349417.9 **ABa**	47241876.1 **Ba**	73303708.3 **Ab**	29144511.6 **Bb**	31716285.6 **Bb**	87110117.6 **Aa**	50255388.6 **Ba**	11519739.7 **Cc**
TAG 54:3	75129272.5 **Ab**	70514552.7 **Ac**	90055843.4 **Ab**	187234012.4 **Aa**	157040487.4 **ABb**	138420530.2 **Ba**	217593851.4 **Aa**	84407348.6 **Bc**	93120809.6 **Bb**	216753212.4 **Aa**	198225439.1 **Aa**	33299954.0 **Bc**
TAG 54:4	51096306.5 **Ac**	46385423.1 **Ac**	60002300.1 **Ab**	131891049.4 **Ab**	106528297.7 **ABb**	99089964.3 **Ba**	158357743.4 **Aab**	49598862.4 **Bc**	58350075.4 **Bb**	189529276.6 **Aa**	146441801.7 **Ba**	16683962.0 **Cc**
TAG 54:5	32383480.3 **Ad**	29249686.9 **Ac**	39220978.6 **Abc**	100973664.8 **Ac**	86953568.0 **Ab**	76313517.1 **Aa**	134576541.1 **Ab**	39643457.2 **Bc**	46029634.4 **Bb**	180292351.5 **Aa**	139855539.3 **Ba**	13643393.4 **Cc**
TAG 54:6	10378633.2 **Ac**	9511411.6 **Ac**	12861104.0 **Ab**	28213264.5 **Ab**	26455593.7 **Ab**	25164419.9 **Aa**	33828009.6 **Ab**	10944290.4 **Bc**	13262905.7 **Bb**	51109938.2 **Aa**	48168943.6 **Aa**	4322861.4 **Bb**
TAG 54:7	2824678.7 **Ac**	2384806.9 **Ac**	3625229.9 **Ab**	9904003.1 **Ab**	9215938.7 **Ab**	8779930.4 **Aa**	13086923.6 **Ab**	3656667.0 **Bc**	4473300.8 **Bab**	22593105.8 **Aa**	21006474.8 **Aa**	1123067.2 **Bb**
TAG 54:8	459283.4 **Ac**	377081.7 **Ac**	652764.0 **Abc**	1085174.5 **Ab**	1275046.2 **Ab**	1225603.6 **Aa**	1221740.2 **Ab**	717181.5 **Bc**	875247.2 **ABab**	1843344.1 **Ba**	2859874.8 **Aa**	212275.0 **Cc**
TAG 54:9	66766.4 **Ac**	53042.1 **Ac**	99348.8 **Aab**	175523.2 **Ab**	182984.2 **Ab**	161506.0 **Aa**	198689.1 **Ab**	103829.0 **Bc**	132767.3 **ABa**	331528.8 **Aa**	327032.6 **Aa**	39423.5 **Bb**
TAG 56:0	104122.2 **Aa**	105410.3 **Aa**	111406.6 **Aa**	86576.5 **Aa**	73971.6 **Ab**	73497.7 **Ab**	103419.2 **Aa**	48330.3 **Bc**	49258.8 **Bc**	91118.9 **Aa**	97874.6 **Aa**	48835.6 **Bc**
TAG 56:1	804845.8 **Ac**	791734.4 **Aab**	929941.1 **Aa**	1424621.6 **Ab**	927411.1 **Ba**	751735.8 **Ba**	1726831.6 **Ab**	395915.4 **Bb**	443726.4 **Bab**	2809939.2 **Aa**	1199611.0 **Ba**	155431.8 **Cb**
TAG 56:2	3067739.4 **Ad**	2888950.1 **Abc**	3683852.2 **Aa**	5922339.4 **Ac**	4862057.2 **ABa**	3819861.2 **Ba**	7928783.0 **Ab**	2125438.3 **Bc**	2327599.9 **Bab**	10489290.6 **Aa**	4617846.2 **Bab**	672346.1 **Cb**
TAG 56:3	4242900.9 **Ac**	4197747.1 **Ab**	5619079.9 **Ab**	10528129.8 **Ab**	11226113.9 **Aa**	9930783.6 **Aa**	13240081.0 **Ab**	5539541.4 **Bb**	6192521.1 **Bb**	17059293.4 **Aa**	11629898.1 **Ba**	1953508.9 **Cc**
TAG 56:4	2216655.0 **Ab**	2120748.7 **Ab**	2732274.2 **Aab**	3186536.0 **Aa**	3865500.2 **Aa**	3374505.2 **Aa**	3296047.2 **Aa**	1864343.5 **Bb**	2125177.4 **Bb**	3141953.4 **Aa**	3484104.4 **Aa**	833722.0 **Bc**
TAG 56:5	1338873.0 **Ac**	1151034.5 **Ab**	1692776.1 **Ab**	2632131.0 **Ab**	3392798.7 **Aa**	2945195.3 **Aa**	3165180.5 **Aab**	1539449.3 **Bb**	1734550.6 **Bb**	3976329.9 **Aa**	3696841.2 **Aa**	516312.5 **Bc**
TAG 56:6	413621.4 **Ab**	386270.7 **Ab**	527257.0 **Ab**	683012.6 **Aa**	821198.4 **Aa**	753596.0 **Aa**	619806.0 **Aa**	356154.2 **Bb**	410458.3 **Bb**	647779.2 **Ba**	873928.1 **Aa**	138694.4 **Cc**
TAG 56:7	114200.1 **ABa**	99902.0 **Bc**	153511.5 **Ab**	152476.2 **Ba**	255500.8 **Ab**	241935.0 **Aa**	154901.1 **Aa**	116989.3 **Ac**	136807.9 **Ab**	NA **Bb**	341968.3 **Aa**	29943.1 **Bc**
TAG 58:0	43659.3 **Aa**	46872.9 **Aa**	45132.3 **Aa**	29838.6 **Ab**	25106.1 **Abc**	26207.8 **Ab**	31515.5 **Ab**	18511.8 **Bc**	19548.9 **Bbc**	31891.9 **Ab**	27748.8 **Ab**	16689.1 **Bc**
TAG 58:1	695870.2 **Ac**	622142.0 **Ab**	816958.0 **Aa**	1455733.7 **Ab**	735268.2 **Bb**	568517.5 **Bab**	1728082.1 **Ab**	319162.1 **Bb**	343449.0 **Bab**	3448253.0 **Aa**	1375641.3 **Ba**	127658.7 **Cb**
TAG 58:2	911292.7 **Ad**	857892.2 **Ab**	1094160.2 **Aa**	1979842.4 **Ac**	1371177.8 **ABab**	1144878.2 **Ba**	2755290.1 **Ab**	649711.9 **Bb**	672509.9 **Bab**	4107180.9 **Aa**	1688359.5 **Ba**	198298.7 **Cb**
TAG 58:3	540330.9 **Ac**	504234.3 **Abc**	660551.1 **Aa**	848000.6 **Ab**	719453.2 **ABab**	622020.0 **Bab**	935575.9 **Ab**	364260.5 **Bc**	397427.0 **Bbc**	1207793.7 **Aa**	787709.1 **Ba**	165454.7 **Cc**
TAG 58:4	308621.7 **Ac**	323533.6 **Abc**	349971.4 **Aa**	480514.5 **Abc**	415553.9 **Ab**	354425.1 **Aa**	622057.5 **Ab**	211442.6 **Bc**	225608.6 **Bab**	952400.6 **Aa**	620385.0 **Ba**	133150.0 **Cb**
TAG 58:5	208282.6 **Ac**	187593.5 **Ab**	234667.3 **Aa**	347481.9 **Aab**	121709.9 **Bc**	109697.6 **Bb**	312196.3 **Ab**	74189.7 **Bc**	90199.1 **Bb**	383190.2 **Aa**	288978.5 **Ba**	22218.7 **Cc**
TAG 58:6	148716.3 **Ac**	130014.0 **Ab**	167912.0 **Aa**	357775.5 **Ab**	104345.9 **Bb**	79872.6 **Ba**	459209.3 **Ab**	67783.2 **Bb**	75238.4 **Ba**	764232.7 **Aa**	325101.1 **Ba**	19604.6 **Ca**
TAG 60:0	11139.1 **Aa**	12430.3 **Aa**	10580.7 **Aa**	8497.2 **Ab**	7208.2 **ABb**	5552.1 **Bb**	6293.9 **Ac**	4267.0 **Bc**	4498.3 **ABb**	5054.3 **Ac**	6237.3 **Abc**	4946.1 **Ab**

SI Table 8. *(continuation)* Relative abundance of lipid of *Chlamydomonas* sp. BR020 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 60:1	470168.8 **Ab**	435798.8 **Aab**	540988.1 **Aa**	485153.1 **Ab**	300106.0 **Bbc**	237635.1 **Bb**	615464.9 **Ab**	166262.4 **Bc**	186910.3 **Bb**	1143005.8 **Aa**	580912.8 **Ba**	119241.6 **Cb**
TAG 60:2	1112211.3 **Ac**	975947.2 **Ab**	1362294.0 **Aa**	2307702.1 **Ab**	1277560.2 **Bab**	1005914.0 **Bab**	2964578.8 **Ab**	632728.6 **Bb**	732382.4 **Bab**	4773944.1 **Aa**	1839373.8 **Ba**	208056.7 **Cb**
TAG 60:3	594939.6 **Ac**	548561.0 **Aab**	685733.0 **Aa**	839312.8 **Abc**	558007.3 **ABab**	475771.9 **Bab**	1025672.7 **Ab**	260363.8 **Bb**	289669.7 **Bbc**	1649037.6 **Aa**	827639.8 **Ba**	89958.1 **Cc**
TAG 60:4	342568.4 **Ac**	323590.6 **Ab**	407071.9 **Aa**	519036.4 **Abc**	367534.7 **ABb**	307953.9 **Ba**	637944.5 **Ab**	199523.8 **Bb**	230447.2 **Bab**	1085268.9 **Aa**	668821.5 **Ba**	75692.3 **Cb**
TAG 60:5	117439.5 **Ab**	80901.8 **Bab**	134301.0 **Aa**	78762.9 **Ac**	79457.1 **Ab**	66993.1 **Ab**	77665.9 **Ac**	53535.1 **Ab**	58640.7 **Ab**	154612.6 **Aa**	116217.7 **Ba**	16681.6 **Cc**
TAG 60:5.1	162958.1 **ABa**	151705.3 **Ba**	198443.0 **Aa**	157427.4 **Aa**	69863.3 **Bb**	61930.6 **Bb**	153655.2 **Aa**	42645.5 **Bb**	52297.8 **Bb**	186891.3 **Aa**	182841.1 **Aa**	19777.8 **Bb**
TAG 60:6	124436.2 **Ac**	105607.9 **Ab**	147876.7 **Aa**	129245.0 **Abc**	51036.5 **Bc**	42076.7 **Bb**	174021.1 **Ab**	38307.0 **Bc**	48247.5 **Bb**	236443.3 **Aa**	180198.2 **Ba**	20377.6 **Cb**
FA 16:0	16409037.8 **Aa**	16401123.2 **Aa**	16112227.8 **Aa**	13237803.7 **Ab**	11629473.0 **ABb**	9275082.5 **Bb**	13826261.3 **Aab**	7010990.8 **Bc**	6386398.0 **Bbc**	11039875.0 **Ab**	14916713.1 **Ba**	5257071.3 **Cc**
FA 16:1	3582455.7 **Ba**	3393014.9 **Ba**	3817315.8 **Aa**	985837.2 **ABb**	1059946.6 **Ab**	788173.1 **Bb**	1092636.9 **Ab**	545838.5 **Bc**	514184.4 **Bc**	NA **Bc**	1105906.4 **Ab**	NA **Bd**
FA 16:2	21087231.1 **Aa**	20640021.0 **Aa**	22308069.4 **Aa**	12699031.8 **Ab**	12471901.4 **Ab**	10223858.2 **Ab**	9568985.6 **Ac**	6758084.9 **Bc**	6562711.2 **Bc**	5096861.1 **Bd**	13021236.3 **Ab**	3919362.9 **Bc**
FA 16:3	11434355.1 **Aa**	11177304.1 **Ab**	12506387.4 **Aa**	13177225.1 **Aa**	13116771.0 **Aab**	10969714.0 **Aab**	11257487.4 **Aa**	7895542.7 **Bc**	7894412.8 **Bb**	6513933.5 **Bb**	14340604.7 **Aa**	8552358.1 **Bb**
FA 16:4	65158.4 **Ab**	65660.2 **Aa**	68731.3 **Aa**	117478.7 **Aa**	72046.3 **Ba**	34774.4 **Cb**	134350.6 **Aa**	27601.0 **Bb**	23692.7 **Bb**	72017.5 **Ab**	69365.3 **Aa**	23214.9 **Bb**
FA 16:4.1	18252.8 **Aa**	17680.2 **Aa**	18997.8 **Aa**	14771.4 **Aa**	11442.9 **Aab**	10010.3 **Ab**	13015.2 **Aab**	5327.3 **Bb**	6618.9 **Bb**	7324.6 **Ab**	10868.8 **Ab**	5548.9 **Ab**
FA 18:0	3056622.4 **ABa**	3359515.3 **Aa**	2673021.0 **Ba**	2395989.9 **Ab**	1940209.7 **ABb**	1555460.7 **Bb**	2060584.5 **Ab**	1445528.5 **Bb**	1282383.2 **Bb**	1396697.5 **Ac**	1791255.0 **Ab**	682290.0 **Bc**
FA 18:1	39825596.5 **Aab**	41688413.5 **Aa**	38103453.3 **Aa**	36474640.0 **Aab**	34373433.2 **Aa**	23190768.0 **Bb**	42533822.9 **Aa**	19178154.0 **Bb**	18468524.2 **Bbc**	30456098.4 **Bb**	42233612.1 **Aa**	12762091.1 **Cc**
FA 18:2	59149335.7 **Aa**	58531511.5 **Aa**	63277251.7 **Aa**	36557043.6 **Ab**	38166657.8 **Ab**	31473844.3 **Ab**	31425304.9 **Ab**	20812580.3 **Bc**	20352139.0 **Bc**	21428685.7 **Bc**	38648513.5 **Ab**	17324300.0 **Bc**
FA 18:3	53250187.5 **Aa**	52138049.6 **Aa**	59133448.5 **Aa**	49729454.6 **Aa**	52628312.4 **Aa**	42278918.4 **Ab**	45596756.7 **Aa**	30804743.3 **Bb**	31019220.3 **Bb**	31344404.4 **Bb**	56854786.2 **Aa**	35231499.7 **Bb**
FA 18:4	29751.8 **ABa**	28367.4 **Ba**	33162.5 **Aa**	24130.4 **Ab**	24418.7 **Aa**	18119.4 **Bb**	18868.5 **Ac**	12583.2 **Bc**	11628.6 **Bc**	7988.3 **Bd**	18621.3 **Ab**	12465.5 **Bc**
FA 20:0	119289.1 **Aa**	124608.9 **Aa**	118244.7 **Aa**	111904.1 **Aa**	97931.5 **Aa**	71911.4 **Bb**	97268.2 **Aab**	63217.8 **Bb**	54782.5 **Bbc**	73928.8 **Bb**	100514.7 **Aa**	37745.9 **Cc**
FA 20:1	NA **Ac**	NA **Ac**	NA **Ab**	338739.9 **ABa**	415790.7 **Aa**	258193.8 **Ba**	371445.2 **Aa**	207682.7 **Bb**	188763.1 **Ba**	177101.6 **Bb**	268464.4 **Ab**	160140.3 **Ba**
FA 20:3	133819.2 **Ba**	125293.6 **Ba**	159420.7 **Aa**	77983.5 **Bb**	116963.8 **Aa**	80875.9 **Bb**	63048.6 **Ab**	57667.4 **Ab**	52782.5 **Ac**	30525.8 **Bc**	73367.9 **Ab**	56282.5 **Abc**
FA 22:0	53749.9 **Aa**	56037.8 **Ab**	65473.9 **Aa**	49479.9 **Ba**	77063.6 **Aa**	51829.8 **Bab**	47868.9 **Aa**	39290.7 **Ab**	43540.8 **Ab**	38615.2 **Aa**	46160.0 **Ab**	43401.3 **Ab**
FA 22:1	56110.9 **Ba**	77435.6 **Aa**	45396.9 **Ca**	31569.6 **Ab**	30320.9 **Ab**	24069.7 **Ab**	26775.8 **Abc**	16109.0 **Bc**	15496.6 **Bb**	17512.6 **Ac**	12740.5 **Ac**	19379.2 **Ab**
FA 24:1	6039.4 **Ba**	8074.9 **Aa**	4790.6 **Ca**	3333.2 **Ab**	3377.2 **Ab**	2835.3 **Abc**	2655.4 **Abc**	2207.0 **Ac**	2076.7 **Ac**	1912.4 **Bc**	1753.4 **Bc**	3125.7 **Ab**
FA 26:0	666283.1 **Ba**	547234.5 **Ca**	904797.3 **Aa**	195371.5 **Ab**	175675.8 **ABb**	119709.5 **Bb**	99962.7 **Ac**	96865.8 **Ab**	103085.7 **Ab**	60618.3 **Ac**	121669.8 **Ab**	130063.3 **Ab**
FA 28:0	110278.0 **Aa**	103878.1 **Aa**	141394.7 **Aa**	116787.2 **Aa**	206716.9 **Aa**	94102.4 **Aa**	45567.6 **Ab**	45683.0 **Ab**	80037.2 **Ab**	49446.5 **Ab**	51462.2 **Ab**	49283.9 **Ab**
FA 30:0	18480.7 **Aa**	20337.5 **Ab**	33624.1 **Aa**	37379.8 **Ba**	66621.6 **Aa**	27592.6 **Ba**	10761.3 **Aa**	14553.8 **Ab**	27671.4 **Aa**	30833.7 **Aa**	14760.8 **Ab**	18659.4 **Aa**
FA 30:1	3174.4 **Aa**	3057.4 **Aa**	2930.7 **Ba**	3595.9 **Aa**	3005.7 **Aa**	2350.8 **Ba**	2961.1 **Ab**	1858.6 **Ab**	1844.2 **Bb**	2427.7 **Ab**	2790.7 **Ab**	1460.8 **Bb**

SI Table 9. Relative abundance of lipid of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N_So		0h_3.5N_So		0h_7N_So		10h_0N_So		10h_3.5N_So		10h_7N_So		18h_0N_So		18h_3.5N_So		18h_7N_So		36h_0N_So		36h_3.5N_So		36h_7N_So	
DAG 34:1	34926.9	Ac	20287.4	Aa	18494.9	Aa	30069.7	Ac	25568.7	Aa	28156.6	Aa	77255.4	Ab	21234.5	Ba	16537.1	Ba	112932.3	Aa	23550.6	Ba	10914.1	Ba
DAG 34:2	85890.0	Aa	17300.3	Ba	15168.4	Ba	31155.0	Aa	14703.2	Ba	15401.5	Ba	71853.4	Aa	NA	Ba	11908.1	Ba	117671.8	Aa	24810.5	Ba	12043.2	Ba
DAG 34:3	29581.2	Ac	20094.5	Ab	18783.3	Aa	51463.7	Ac	24256.8	Bb	25305.4	Ba	121282.9	Ab	29260.5	Bab	21410.9	Ba	216984.9	Aa	52026.4	Ba	19902.7	Ca
DAG 34:4	12391.4	Ab	6392.6	ABa	4803.9	Bab	16396.2	Ab	NA	Ca	7400.1	Ba	NA	Ac	NA	Aa	NA	Ab	70367.2	Aa	NA	Ba	4454.2	Bab
DAG 34:5	8873.7	Ac	5525.6	Aab	4936.0	Aa	12684.8	Ac	NA	Bb	NA	Ba	28959.6	Ab	6958.0	Ba	5040.3	Ba	54332.1	Aa	8401.9	Ba	4145.1	Ba
DAG 34:6	5562.3	Ac	4218.8	Aa	3862.9	Aa	9863.6	Ac	4926.5	Aa	5179.2	Aa	25280.5	Ab	8012.6	Ba	5861.3	Ba	41374.4	Aa	7958.2	Ba	5049.8	Ba
DAG 36:1	2103.5	Ac	2428.0	Aa	1928.3	Aab	3192.3	Ac	2986.0	Aa	3263.7	Aa	7399.8	Ab	1823.6	Ba	1216.8	Bbc	9808.7	Aa	1149.0	Ba	NA	Bc
DAG 36:2	239023.9	Aa	39242.3	Ba	38250.9	Ba	94155.9	Aa	103759.9	Ba	100673.8	Ba	175097.0	Aa	70539.4	Ba	71486.9	Ba	260151.4	Aa	75672.3	Ba	40482.4	Ba
DAG 36:3	173021.2	Aa	36390.8	Ba	35674.5	Ba	65339.8	Aa	52530.1	Ba	54687.2	Ba	142228.0	Aa	44107.4	Ba	37519.6	Ba	197597.7	Aa	55283.4	Ba	25044.3	Ba
DAG 36:4	44886.2	Ac	29988.2	Ab	30963.6	Aa	61239.0	Ac	53638.2	Aab	52934.3	Aa	128193.5	Ab	55288.0	Bab	46101.9	B	231360.2	Aa	65862.8	Ba	59618.1	Ba
DAG 36:5	32762.1	Ac	23636.3	Aa	23112.8	Aa	33581.6	Ac	22482.4	Aa	24896.2	Aa	85410.1	Ab	33378.2	Ba	24665.0	Ba	138605.8	Aa	33531.6	Ba	37837.3	Ba
DAG 36:6	19038.3	Ac	16262.9	Ac	18290.6	Ab	32720.4	Ac	31389.1	Abc	32914.8	Ab	77018.7	Ab	55316.3	Ba	35214.4	Cb	100974.2	Aa	37674.5	Cab	71276.9	Ba
DGDG 32:0	221370.0	Ac	203496.2	Aab	212958.6	Ab	272004.5	Aab	182602.0	Bb	188433.2	Bbc	309658.8	Aa	184485.9	Bb	150513.6	Bc	241652.9	Abc	239161.7	Aa	261136.2	Aa
DGDG 32:1	68018.2	Aa	51515.6	Ba	52569.7	Ba	21929.2	Ab	25110.2	Ab	26768.2	Ab	24530.4	Ab	22190.6	Ab	18025.9	Ab	20777.7	Bb	24619.2	Bb	59769.4	Aa
DGDG 32:2	72026.6	Aa	47919.8	Ba	40885.3	Ba	30104.3	Ab	35844.9	Aab	36343.4	Aab	30742.3	Ab	26837.8	Ab	23166.6	Ab	26738.3	Bb	23325.4	Bb	43065.6	Aa
DGDG 32:3	71588.2	Aa	51263.1	ABb	41623.1	Bc	40792.2	Bb	78381.2	Aa	72264.9	Ab	47639.1	Bab	78990.2	Aa	66626.6	ABb	37738.0	Bb	58788.5	Bab	152829.6	Aa
DGDG 32:3.1	22083.2	Aa	20230.3	Ac	18809.5	Ac	25071.1	Ba	90631.7	Aa	80200.8	Ab	36641.7	Ba	78081.3	Aab	77898.8	Ab	49392.2	Ba	50319.7	Bbc	171849.1	Aa
DGDG 34:0	612443.4	ABa	532586.0	Ba	643455.6	Aa	322512.5	Ab	222566.7	Bb	221751.5	Bb	295063.0	Ab	194009.7	Bb	151005.7	Bb	245425.5	Ab	160487.9	ABb	143784.5	Bb
DGDG 34:1	2555996.3	Aa	2506799.6	Aa	2596965.8	Aa	1830896.3	Ab	1844437.4	Ab	1935525.8	Ab	1969270.6	Ab	1814174.3	Ab	1640732.1	Ab	1823395.8	Ab	1989709.1	Ab	2026765.6	Ab
DGDG 34:2	1487656.6	Ab	1417853.1	Ab	1360782.2	Ac	1849813.3	Bab	2394648.6	Aa	2533929.4	Aa	1952498.2	Aa	2126533.4	Aa	2053647.7	Ab	1850352.1	Bab	2138525.2	ABa	2322421.0	Aab
DGDG 34:3	565658.9	Ab	557725.4	Ab	456114.9	Ab	1392950.6	Ba	2593232.7	Aa	2740558.2	Aa	1596075.4	Ba	2596579.9	Aa	2440187.2	Aa	1640718.2	Ca	2234407.5	Ba	2811278.3	Aa
DGDG 34:4	1077565.4	Aa	747240.2	Ba	628887.1	Bb	786216.2	Ab	706921.4	Aa	760978.9	Aab	827760.1	Aab	684134.9	Aa	594499.2	Ab	670904.0	Bb	692440.4	Ba	986577.7	Aa
DGDG 34:5	1268418.7	Aa	1275237.1	Ab	1093556.3	Ab	1322614.1	Ba	1814394.6	Aa	1844966.8	Aa	1537567.8	Aa	1773359.1	Aa	1650950.7	Aa	1530779.1	Ba	1614071.7	Bab	2062059.4	Aa
DGDG 34:6	1035707.7	Ac	937906.9	Ab	806229.6	Ab	1594282.7	Bbc	2675137.8	Aa	2695872.9	Aa	2017300.9	Bab	2844685.3	Aa	2704310.3	Aa	2165238.7	Ba	2440549.5	Ba	3034656.3	Aa
DGDG 36:3	53189.4	Aa	43652.4	Aa	35790.1	Ab	41044.7	Aab	51037.5	Aa	56918.2	Ab	35906.4	Bab	59612.9	Aa	51265.3	ABb	24088.9	Bb	60282.3	Aa	79292.3	Aa
DGDG 36:4	66541.0	Aa	51461.7	Ba	NA	Ca	31482.1	Ab	NA	Bb	NA	Ba	33928.4	Ab	NA	Bb	NA	Ba	23965.9	Ab	NA	Bb	NA	Ba
DGDG 36:5	220253.2	Aa	168550.0	Bab	131800.6	Bb	74007.4	Bb	135218.7	Ab	135870.4	Ab	78662.4	Bb	127144.7	Ab	111670.7	ABb	56592.0	Cb	192537.8	Ba	531978.3	Aa
DGDG 36:6	352845.3	Aa	245726.1	Ba	196136.8	Bb	132139.4	Bb	315579.7	Aa	284876.8	Ab	134495.0	Bb	309922.1	Aa	260621.7	Ab	97360.6	Cb	361995.5	Ba	1343396.5	Aa

SI Table 9. *(continuation)* Relative abundance of lipid of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N_So		0h_3.5N_So		0h_7N_So		10h_0N_So		10h_3.5N_So		10h_7N_So		18h_0N_So		18h_3.5N_So		18h_7N_So		36h_0N_So		36h_3.5N_So		36h_7N_So	
LysoPC 16:0	27481.1	Aa	27079.9	Aa	28171.7	Aa	15233.5	Ab	15758.9	Ab	13347.9	Ab	18668.7	Ab	14334.9	Ab	14253.6	Ab	19429.2	Bb	11651.9	Cb	29388.9	Aa
LysoPC 16:0.1	1969.8	Aa	1994.6	Aa	1973.5	Aa	1173.9	Aa	1167.4	Ab	966.2	Ab	1545.1	Aa	835.8	Ab	955.9	Ab	1580.5	Ba	NA	Cc	2333.1	Aa
LysoPC 16:2	1860.9	Aa	2530.4	Aa	NA	Bc	NA	Bb	1459.9	Ab	NA	Bc	NA	Bb	994.2	Ab	1040.0	Ab	NA	Bb	NA	Bc	3631.4	Aa
LysoPC 16:3	2713.1	Aa	3277.9	Aa	793.5	Bb	NA	Ab	1553.9	Aab	NA	Ab	NA	Ab	1380.2	Ab	1568.1	Ab	NA	Bb	NA	Bb	5849.9	Aa
LysoPC 18:2	25288.0	Aa	37197.0	Aa	17561.8	Ab	5694.4	Aa	19574.3	Aa	14250.8	Ab	7867.7	Aa	21835.0	Aa	18102.5	Ab	8055.9	Ba	25226.0	Ba	94306.6	Aa
LysoPC 18:3	24069.9	Aa	41069.4	Aa	17898.6	Ab	7892.3	Aa	58148.9	Aa	37623.8	Ab	10325.0	Aa	49981.4	Aa	48639.3	Ab	8938.0	Ba	33878.7	Ba	239921.3	Aa
MGDG 34:1	352999.9	Aa	359752.2	Aa	306101.9	Aa	108979.2	Bb	342445.2	Aa	257858.4	Aab	202932.3	Ab	176162.3	Ab	151728.5	Ab	131062.8	Bb	163735.1	ABb	254022.7	Aab
MGDG 34:2	778679.8	Aa	392061.3	Ba	414340.3	Ba	43278.4	Bb	149737.1	Abc	122922.9	ABb	75849.2	Bb	213968.7	Ab	123257.3	ABb	54675.4	Bb	56395.5	Bc	419637.9	Aa
MGDG 34:2.1	77198.2	Aa	112538.5	Ab	80580.1	Ab	48639.7	Ba	245420.2	Aa	202706.2	Aa	64380.8	Aa	61601.5	Ab	75277.3	Ab	61209.1	Aa	65006.2	Ab	87508.3	Ab
MGDG 34:3	32558.7	Aa	13051.3	Aa	9993.3	Aa	2429.5	Aab	14839.0	Aab	11706.1	Aab	3014.6	Aab	8471.4	Aab	6634.4	Aab	2384.8	Ab	3473.6	Ab	5666.4	Ab
MGDG 34:4	1396832.1	Aa	974785.8	Ba	675483.0	Bb	146855.4	Bb	766255.7	Aab	634167.0	Ab	216362.3	Bb	472910.5	ABbc	543644.1	Ab	151107.2	Bb	338907.5	Bc	1131802.3	Aa
MGDG 34:5	3441846.7	Aa	2488098.6	Ba	1867885.7	Bb	917382.3	Bb	2079202.7	Aa	1882041.5	Ab	1492898.8	Ab	1978569.3	Aa	1842429.0	Ab	1213341.7	Bb	1657094.2	Ba	3382334.6	Aa
MGDG 34:6	1491626.2	Aa	1101496.8	ABbc	774258.4	Bb	325246.9	Bb	2043599.5	Aa	1646786.6	Aa	671486.1	Bb	1642072.9	Aab	1950432.5	Aa	328644.1	Bb	882170.5	Bc	1685035.3	Aa
MGDG 36:5	NA	Ba	134553.0	Aa	NA	Bb	NA	Ba	93711.0	Ab	NA	Bb	NA	Ba	97845.4	Ab	NA	Bb	NA	Ba	NA	Bc	242632.8	Aa
MGDG 36:6	96308.4	Aa	103211.2	Aa	75179.0	Ab	14283.9	Bb	80720.5	Aa	60871.0	ABb	18230.9	Bb	84266.5	Aa	83824.9	Ab	12341.2	Cb	81619.5	Ba	751130.3	Aa
PC 32:0	24169.3	Aa	22990.0	Aa	25567.1	Aa	13650.4	Ab	11787.9	ABb	10420.9	Bb	13070.3	Ab	8611.7	Bb	10930.9	ABb	21542.5	Aa	9546.2	Bb	11496.1	Bb
PC 32:1	60010.8	ABa	68525.5	Aa	55314.9	Ba	37076.6	Ab	42019.8	Abc	36595.1	Ac	34733.4	ABb	30699.6	Bc	43158.7	Abc	58067.3	Aa	49154.8	Ab	50377.2	Aab
PC 32:1.1	35311.8	Aa	39415.8	Aab	25384.2	Ab	10471.1	Bb	50653.8	Aa	32670.0	Ab	12295.6	Bb	33017.6	Aab	31485.8	ABb	NA	Cb	26011.9	Bb	66404.4	Aa
PC 32:2	12473.9	ABa	19457.4	Aab	NA	Bc	2225.9	Ba	31512.7	Aa	17826.8	Ab	3337.3	Aa	16862.4	Aab	16627.8	Ab	NA	Ba	9823.5	Bb	42531.8	Aa
PC 32:3	14819.9	Aa	22258.8	Aa	6841.3	Ab	2555.7	Ba	25184.5	Aa	15096.0	ABb	3787.3	Aa	18239.3	Aa	17412.7	Ab	2873.5	Ba	10764.2	Ba	48213.2	Aa
PC 34:1	NA	Ab	NA	Aa	NA	Aa	10108.8	Aa	NA	Ba	NA	Ba	NA	Ab	NA	Aa	NA	Aa	NA	Ab	NA	Aa	NA	Aa
PC 34:3	147289.9	Aa	205344.7	Aa	88983.6	Ab	40496.0	Ba	481604.4	Aa	265886.3	ABb	47747.7	Aa	335520.8	Aa	320935.0	Ab	45878.1	Ba	199954.6	Ba	1350004.1	Aa
PC 34:4	99435.3	Aa	145448.9	Aa	51705.4	Ab	23993.7	Aa	153013.6	Aa	95395.5	Ab	33907.5	Aa	121848.3	Aa	96305.7	Ab	29654.6	Ba	91256.5	Ba	646597.8	Aa
PC 34:5	31668.3	Aa	48306.4	Aa	17737.8	Ab	6612.7	Aa	52954.9	Aa	30315.1	Ab	7471.7	Aa	46184.2	Aa	39233.9	Ab	5826.1	Ba	24084.3	Ba	158514.3	Aa
PC 34:6	21403.1	Aa	35093.2	Aa	10993.3	Ab	5139.2	Aa	53954.5	Aa	30847.7	Ab	6261.1	Aa	55517.8	Aa	48573.7	Ab	4435.9	Ba	25137.4	Ba	206579.8	Aa
PC 36:1	478722.8	Ba	615454.8	Aa	435676.6	Ba	344935.1	Ab	430796.1	Ab	415715.5	Aa	293669.9	Bb	429300.5	Ab	459819.0	Aa	367784.3	Bab	571962.8	Aa	402786.3	Ba
PC 36:2	10925226.0	Ba	13830934.5	Aa	10075490.8	Bb	6403139.5	Bb	10397523.1	Ac	9033402.6	Ab	5483528.3	Bb	10766733.7	Abc	11136063.3	Aab	7360846.6	Bb	13476082.3	Aab	13032454.9	Aa
PC 36:3	NA	Bc	NA	Ba	68678.8	Aa	47034.1	Ab	NA	Ba	NA	Bb	NA	Ac	NA	Aa	NA	Ab	58692.0	Aa	NA	Ba	NA	Bb
PC 36:3.1	684868.0	ABa	822987.5	Aab	580784.8	Ba	375002.4	Bb	660460.3	Ab	529605.6	ABa	277966.3	Bb	690531.0	Aab	630967.4	Aa	352708.2	Cb	885007.6	Aa	674094.7	Ba

SI Table 9. *(continuation)* Relative abundance of lipid of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N_So	0h_3.5N_So	0h_7N_So	10h_0N_So	10h_3.5N_So	10h_7N_So	18h_0N_So	18h_3.5N_So	18h_7N_So	36h_0N_So	36h_3.5N_So	36h_7N_So
PC 36:5	193545.9 **Aa**	283073.2 **Aa**	99563.4 **Ab**	36804.1 **Aa**	382738.8 **Aa**	222950.3 **Ab**	47988.3 **Aa**	389521.0 **Aa**	287297.9 **Ab**	43420.3 **Ba**	309551.7 **Ba**	2127094.6 **Aa**
PC 36:6	135205.9 **Aa**	207824.2 **Aa**	87418.8 **Ab**	25629.7 **Aa**	1108955.0 **Aa**	494364.5 **Ab**	40058.8 **Aa**	851729.5 **Aa**	716290.9 **Ab**	27641.1 **Ba**	402114.1 **Ba**	4679244.8 **Aa**
PC 38:2	122997.7 **Ba**	160537.0 **Aa**	106886.8 **Ba**	83071.8 **Bb**	135707.5 **Aab**	119513.0 **Aa**	65682.7 **Bb**	113415.2 **Ab**	122119.0 **Aa**	81083.3 **Bb**	116418.5 **Ab**	130318.6 **Aa**
PC 38:3	3722.9 **Aa**	4374.9 **Aa**	3147.0 **Ab**	NA **Aa**	1696.5 **Aa**	NA **Ab**	NA **Aa**	2283.0 **Aa**	2266.7 **Ab**	NA **Ba**	2871.5 **Ba**	14987.5 **Aa**
PC 38:3.1	8170.6 **ABa**	8978.9 **Aa**	6886.1 **Ba**	4938.9 **Bb**	7864.3 **Aa**	6743.7 **Aa**	4753.5 **Bb**	7658.5 **Aa**	6688.2 **Aa**	4746.4 **Bb**	8078.2 **Aa**	6996.0 **Aa**
PE 32:1	181006.0 **Aa**	214188.2 **Ac**	185031.1 **Ab**	112627.8 **Ca**	548933.3 **Aa**	443943.4 **Ba**	102598.6 **Ca**	331289.4 **Bb**	451768.5 **Aa**	164670.3 **ABa**	218084.7 **Ac**	97994.3 **Bc**
PE 34:1	277134.8 **Aa**	320709.4 **Ab**	305217.9 **Ab**	182861.8 **Cb**	578010.4 **Aa**	481856.9 **Ba**	162445.6 **Cb**	320361.4 **Bb**	424053.7 **Aa**	239187.4 **Aab**	251861.9 **Ab**	242333.4 **Ab**
PE 34:2	1069825.8 **Aa**	1261406.4 **Ac**	1093879.0 **Ab**	675265.0 **Cab**	2969242.2 **Aa**	2366274.0 **Ba**	535838.9 **Bb**	2044119.4 **Ab**	2314037.4 **Aa**	757637.7 **Bab**	2002332.7 **Ab**	2230663.3 **Aa**
PE 34:3	39696.7 **Aa**	54717.8 **Ab**	43972.7 **Ac**	26614.6 **Ca**	99076.2 **Aa**	72556.9 **Bb**	29286.4 **Ba**	65669.3 **Ab**	85858.5 **Ab**	41608.9 **Ba**	43741.1 **Bb**	135116.3 **Aa**
PE 34:4	5670.6 **ABa**	NA **Bc**	6402.3 **Ab**	3144.0 **Ba**	17398.0 **Aa**	14128.4 **Aa**	4683.1 **Ba**	10616.4 **Ab**	13667.3 **Aa**	4119.4 **Ba**	6098.2 **Bbc**	15554.7 **Aa**
PE 34:5	5172.1 **Aa**	8832.5 **Ab**	4173.2 **Ab**	2171.3 **Ba**	22221.4 **Aa**	14355.0 **Aab**	3358.4 **Ba**	14595.1 **ABab**	19226.3 **Aa**	1921.3 **Ba**	5640.5 **Bb**	21077.2 **Aa**
PE 34:6	2685.7 **Aa**	4600.1 **Ab**	NA **Ab**	NA **Ba**	18450.6 **Aa**	13123.8 **Aa**	2219.6 **Ba**	13397.6 **Aab**	20018.8 **Aa**	NA **Ba**	3027.6 **Bb**	22111.6 **Aa**
PE 36:2	2741013.6 **Aa**	3364562.0 **Abc**	2826953.7 **Ac**	1492321.8 **Cb**	7218189.2 **Aa**	6034886.0 **Ba**	1146038.6 **Bb**	3999922.9 **Ab**	4300242.5 **Ab**	1205362.4 **Bb**	2677047.3 **Ac**	3475750.5 **Abc**
PE 36:3	51499.2 **Aa**	66606.7 **Ac**	48658.9 **Ac**	23820.3 **Ba**	596309.0 **Aa**	583133.1 **Aa**	14776.6 **Ba**	295874.4 **Ab**	265520.1 **Ab**	8975.8 **Ba**	128381.9 **Ac**	136276.3 **Ac**
PE 36:3.1	151719.9 **Aa**	173045.1 **Aa**	146879.0 **Aa**	87172.2 **Ab**	NA **Bb**	NA **Bb**	70871.4 **Ab**	NA **Bb**	NA **Bb**	106740.9 **Ab**	NA **Bb**	NA **Bb**
PE 36:4	44099.1 **Aa**	52410.3 **Ab**	45438.0 **Ac**	29890.4 **Ba**	116776.6 **Aa**	110751.1 **Ab**	29185.3 **Ca**	105436.6 **Ba**	181898.3 **Aa**	43097.0 **Ca**	124004.4 **Aa**	68849.1 **Bc**
PE 36:4.1	220613.3 **Aa**	271409.8 **Aa**	237388.5 **Aa**	117370.9 **Ba**	NA **Bb**	267776.8 **Aa**	98061.9 **Ba**	250491.8 **Aa**	300453.6 **Aa**	137242.3 **Ba**	267676.2 **Aa**	NA **Cb**
PE 36:5	94368.6 **Aa**	155278.2 **Ab**	60462.5 **Ab**	22577.7 **Ba**	599046.6 **Aa**	406186.2 **Aa**	40063.5 **Ba**	328229.7 **Aab**	430114.1 **Aa**	24481.0 **Ba**	149470.0 **Bb**	688981.5 **Aa**
PE 36:6	53629.0 **Aa**	83001.6 **Ab**	34302.1 **Ac**	14308.5 **Ba**	684265.1 **Aa**	437487.2 **ABbc**	24754.1 **Ba**	448757.5 **ABab**	611958.1 **Ab**	12053.2 **Ba**	102430.5 **Bb**	1185973.7 **Aa**
PE 38:2	6236041.3 **Aa**	6756510.0 **Aa**	6622333.3 **Ab**	3235740.3 **Bb**	5725711.8 **Aab**	5283593.7 **Ab**	2488441.7 **Cb**	3908675.5 **Bc**	5525349.2 **Ab**	2731272.6 **Cb**	4814100.3 **Bbc**	8560484.1 **Aa**
PE 38:3	12517.3 **Aa**	16579.4 **Ac**	10769.5 **Ad**	4188.3 **Ba**	75693.4 **Aa**	66824.2 **Aa**	3468.6 **Ba**	46788.5 **Ab**	52379.1 **Ab**	NA **Ca**	17103.5 **Bc**	28512.7 **Ac**
PE 40:1	20122.2 **Ba**	21038.5 **Aa**	18942.8 **Aa**	8855.7 **Bb**	13211.1 **Ab**	14790.3 **Ab**	8087.7 **Bb**	11003.8 **Ab**	12904.1 **Ab**	8548.1 **Bb**	10547.2 **Ab**	10293.4 **Ab**
PE 40:2	440047.5 **Ba**	495117.8 **Aa**	378480.0 **ABa**	284508.0 **Bb**	368054.1 **Ab**	315704.3 **ABb**	261798.6 **Bb**	324586.0 **Ab**	401456.5 **ABb**	384894.5 **Ba**	443136.0 **Aa**	376959.5 **ABa**
PE 40:3	7816.7 **Aa**	NA **Bb**	7448.2 **Aa**	NA **Bc**	10016.0 **Aa**	NA **Bb**	2673.0 **Ab**	NA **Bb**	NA **Bb**	NA **Ac**	NA **Ab**	NA **Ab**
PG 32:0	45951.2 **Aa**	53389.5 **Aa**	45628.6 **Aa**	20470.4 **Bb**	49862.3 **Aa**	29714.0 **Bab**	19071.7 **Ab**	26003.5 **Ab**	28325.6 **Ab**	22173.5 **Bb**	18998.9 **Bb**	43831.2 **Aab**
PG 32:1	17245.2 **Aa**	20446.5 **Ab**	16000.0 **Ab**	8435.6 **Bb**	27185.4 **Aa**	23259.6 **Aa**	6670.7 **Cb**	13896.2 **Bc**	27426.9 **Aa**	10299.9 **Ab**	5088.1 **ABd**	4396.3 **Bc**
PG 34:2	166981.0 **Ba**	231846.4 **Ab**	179397.7 **Bc**	NA **Cb**	316093.6 **Aa**	254717.1 **Bb**	NA **Cb**	217374.4 **Bb**	304330.4 **Aab**	NA **Bb**	NA **Bc**	309889.9 **Aa**
PG 34:3	17270.1 **Aa**	21741.2 **Aab**	11490.2 **Ab**	4408.5 **Ba**	44171.3 **Aa**	25954.6 **ABb**	4924.1 **Ba**	25134.3 **ABab**	29214.5 **Ab**	NA **Ba**	12786.2 **Bb**	63288.0 **Aa**
PG 34:4	31418.8 **Aa**	42721.8 **Aa**	24492.8 **Ab**	6916.1 **Ba**	143367.1 **Aa**	88440.8 **ABb**	8777.2 **Aa**	75504.4 **Aa**	86704.2 **Ab**	4918.1 **Ba**	33702.2 **Ba**	386644.7 **Aa**

SI Table 9. *(continuation)* Relative abundance of lipid of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N_So		0h_3.5N_So		0h_7N_So		10h_0N_So		10h_3.5N_So		10h_7N_So		18h_0N_So		18h_3.5N_So		18h_7N_So		36h_0N_So		36h_3.5N_So		36h_7N_So	
PG 36:1	105255.8	Aa	111475.4	Abc	110344.3	Ab	71563.5	Bb	186868.8	Aa	168158.1	Aa	55752.4	Cb	130214.9	Bb	161005.2	Aa	62500.9	Bb	97684.8	Ac	80692.6	ABb
PG 36:2	1104754.8	Aa	1176600.3	Abc	1125037.5	Ab	647076.8	Bb	1775498.1	Aa	1587492.2	Aa	494230.2	Bb	1387826.4	Ab	1555661.4	Aa	499753.2	Bb	1095928.5	Ac	1058196.6	Ab
SQDG 32:0	5160100.7	Aa	4887323.8	Aa	5062432.2	Aa	3001919.0	Ab	2415083.8	Ab	2442353.5	Ab	3050068.1	Ab	2908015.0	Ab	2503348.2	Ab	2997191.3	Ab	3030098.9	Ab	3613859.9	Ab
SQDG 32:1	231702.7	Aa	217508.3	Aa	204529.3	Aa	58546.2	Ab	86057.9	Ab	81544.0	Ab	56171.4	Ab	76615.0	Ab	68945.1	Ab	37083.4	Bb	51036.3	Bb	176008.3	Aa
SQDG 32:2	19788.2	Aa	19284.2	ABa	17957.6	Ba	NA	Ab	NA	Ab	NA	Ab	3497.6	Ab	NA	Bb	NA	Bb	2341.3	Ab	NA	Bb	NA	Bb
SQDG 32:3	13638.5	Aa	12794.5	Aa	12314.1	Aa	NA	Bb	2751.4	Ab	3048.5	Ab	NA	Ab	2188.3	Ab	2391.9	Ab	NA	Bb	2621.6	Ab	4690.9	Ab
SQDG 34:0	430540.4	Aa	389628.0	Ba	438946.9	ABa	193469.7	Ab	171490.2	Bb	187093.5	ABb	214246.9	Ab	187056.7	Bb	145262.0	ABb	177810.7	Ab	118599.2	Bb	128442.3	ABb
SQDG 34:1	2229234.4	Aa	1940372.1	Aa	2084238.1	Aa	577317.6	Bb	1607547.5	Aa	1638386.6	Abc	779744.3	Bb	1555256.3	Aa	1256590.3	Ac	NA	Cc	944983.9	Bb	1892026.3	Aab
SQDG 34:2	1941765.2	Aa	1864199.2	Aa	1719938.4	Aab	1130157.5	Bb	1915119.0	Aa	2019682.0	Aab	1131074.8	Bb	1533017.7	ABab	1612368.5	Ab	1030195.9	Bb	1319010.7	Bb	2115626.5	Aa
SQDG 34:3	3043717.3	Aa	3049968.8	Ab	2789384.4	Ac	1756361.9	Bb	4472025.5	Aa	4336485.2	Ab	1947107.8	Bb	4471805.3	Aa	4113282.9	Ab	1494145.5	Cb	2840512.7	Bb	6670922.1	Aa
SQDG 34:4	40998.0	Aa	41578.9	Aa	35442.7	Ab	15880.9	Bb	27000.5	Ab	28137.8	Abc	15418.3	Ab	21426.9	Ab	20747.4	Ac	8916.4	Cb	18566.1	Bb	47243.7	Aa
SQDG 34:5	22195.5	Aa	22270.8	Aa	17987.6	Aab	6958.9	Bb	12866.4	Ab	14291.6	Ab	6668.2	Bb	10302.8	ABbc	13659.0	Ab	3475.8	Bb	7216.7	Bc	21368.9	Aa
SQDG 34:6	18751.6	Aa	19344.2	Aa	16774.7	Ab	4892.5	Bb	13623.1	Ab	14474.4	Ab	4748.2	Cb	10975.2	Bbc	17281.4	Ab	1942.9	Cb	6700.3	Bc	28591.4	Aa
TAG 42:0	18590.4	Ab	17511.1	Aa	18341.2	Aa	15860.0	Ab	10808.4	Aa	14970.3	Aa	21131.0	Aab	13285.8	Ba	12111.3	Ba	27245.1	Aa	13422.1	Ba	12343.3	Ba
TAG 42:1	15131.7	Ac	15442.2	Aa	18717.3	Aa	17828.4	Abc	9153.7	Ba	11956.7	Bb	22910.1	Ab	9603.0	Ba	NA	Cc	30981.7	Aa	11072.9	Ba	9406.5	Bb
TAG 42:2	2134.9	Ac	NA	Bb	NA	Bb	2721.4	Ac	NA	Bb	2254.7	Aa	4916.5	Ab	NA	Cb	1660.4	Ba	7331.8	Aa	1654.1	Ba	1972.5	Ba
TAG 44:0	58188.0	Aab	54389.2	Aa	60108.0	Aa	48443.4	Ab	34502.5	Aa	43664.9	Aab	57902.7	Aab	36528.2	Ba	37287.8	Bb	77896.2	Aa	39831.7	Ba	36526.7	Bb
TAG 44:1	37802.4	Ab	39188.2	Aa	36427.4	Aa	37072.5	Ab	25835.1	Aa	30248.4	Aa	50126.0	Ab	26778.5	Ba	25691.7	Ba	87581.6	Aa	28761.6	Ba	32583.2	Ba
TAG 44:2	22451.7	Ab	20674.5	Aa	22395.6	Aa	22378.2	Ab	13707.2	Ba	15117.9	ABab	28974.2	Ab	13281.9	Ba	13273.7	Bb	39560.4	Aa	15325.4	Ba	13553.9	Bb
TAG 46:0	182369.6	Ab	157776.5	Aa	193448.2	Aa	174224.0	Ab	110065.1	Aa	136779.2	Aab	226769.0	Ab	107161.4	Ba	112726.2	Bb	365745.3	Aa	119306.2	Ba	116982.2	Bb
TAG 46:1	NA	Ac	NA	Ab	NA	Aa	NA	Ac	NA	Ab	NA	Aa	169356.3	Ab	NA	Bb	NA	Ba	297515.9	Aa	78854.0	Ba	NA	Ca
TAG 46:2	NA	Ad	NA	Ab	NA	Aa	31322.4	Ac	NA	Bb	NA	Ba	57296.4	Ab	NA	Bb	NA	Ba	112388.8	Aa	23921.1	Ba	NA	Ca
TAG 46:2.1	37420.0	Aa	37176.0	Aa	37180.5	Aa	25158.2	Bb	28126.7	ABab	37671.3	Aa	NA	Bc	26482.8	Aab	30764.8	Aa	NA	Bc	24318.4	Ab	33307.1	Aa
TAG 46:3	5216.0	Ac	5066.5	Aa	4901.3	Aa	11077.1	Ac	4243.8	Aa	4759.0	Aa	25265.6	Ab	7315.8	Ba	6306.5	Ba	54458.5	Aa	7600.0	Ba	3480.2	Ba
TAG 46:3.1	6176.2	Ab	5448.5	Aa	5556.7	Aa	6520.0	Ab	3055.4	Bb	4304.2	Ba	6173.4	Ab	2940.7	Bb	3450.7	Ba	8984.4	Aa	3239.2	Bab	3564.4	Ba
TAG 46:4	NA	Ad	NA	Aa	NA	Aa	1756.6	Ac	NA	Ba	NA	Ba	4684.5	Ab	1316.3	Ba	904.7	Ba	10933.0	Aa	NA	Ba	NA	Ba
TAG 48:0	877361.9	Ad	753938.9	Aa	876857.2	Aa	4050115.8	Ac	494802.4	Ba	585454.9	Ba	8594256.3	Ab	641261.8	Ba	436574.2	Ba	16684849.4	Aa	1592627.3	Ba	415994.7	Ba
TAG 48:1	356761.7	Ac	432913.8	Aa	306843.5	Aa	1017402.1	Ac	303735.4	Ba	360267.3	Ba	2123296.8	Ab	358557.1	Ba	286304.4	Ba	4931052.2	Aa	616857.0	Ba	242920.6	Ba
TAG 48:2	88447.0	Ad	102966.4	Aa	NA	Aa	291428.5	Ac	71096.6	Ba	85666.7	Ba	670283.9	Ab	92345.2	Ba	71425.3	Ba	1614262.1	Aa	171379.4	Ba	50249.7	Ba

SI Table 9. *(continuation)* Relative abundance of lipid of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N_So		0h_3.5N_So		0h_7N_So		10h_0N_So		10h_3.5N_So		10h_7N_So		18h_0N_So		18h_3.5N_So		18h_7N_So		36h_0N_So		36h_3.5N_So		36h_7N_So	
TAG 48:3	25114.3	Ad	25818.9	Aa	21590.0	Aa	168992.5	Ac	25707.4	Ba	27971.1	Ba	429407.2	Ab	42026.5	Ba	28873.0	Ba	1122505.4	Aa	97869.6	Ba	15710.7	Ba
TAG 48:4	4747.6	Ad	4851.0	Aa	3512.0	Aa	24919.3	Ac	2769.3	Ba	2648.9	Ba	49631.9	Ab	4824.6	Ba	3544.4	Ba	121519.4	Aa	9971.6	Ba	1453.9	Ba
TAG 48:5	764.5	Ad	NA	Aa	NA	Aa	7458.6	Ac	NA	Ba	NA	Ba	17340.2	Ab	1799.8	Ba	NA	Ba	52354.6	Aa	2529.1	Ba	NA	Ba
TAG 48:6	NA	Ad	NA	Aa	NA	Aa	3764.9	Ac	NA	Ba	NA	Ba	10138.9	Ab	1324.2	Ba	NA	Ba	27685.4	Aa	NA	Ba	NA	Ba
TAG 48:7	NA	Ab	NA	Ab	NA	Aa	NA	Ab	NA	Ab	NA	Aa	NA	Ab	NA	Ab	NA	Aa	2943.6	Aa	758.3	Ba	NA	Ca
TAG 50:0	1193989.1	Ac	979182.5	Aa	1225418.9	Aa	2245144.2	Ac	605427.2	Ba	668504.7	Ba	4501948.4	Ab	601597.8	Ba	541759.2	Ba	7988425.6	Aa	761525.3	Ba	561094.5	Ba
TAG 50:1	6899376.7	Ad	6106301.7	Ab	5518185.0	Aa	39803128.6	Ac	7157808.0	Bb	7931794.0	Ba	82500723.2	Ab	8150809.4	Bab	5059264.9	Ba	131626461.9	Aa	23644273.2	Ba	2075442.8	Ca
TAG 50:2	1143042.9	Ad	1371345.6	Aa	1371855.3	Aa	13335930.9	Ac	1466090.7	Ba	1684813.7	Ba	34095668.7	Ab	1927382.1	Ba	1232327.5	Ba	68727966.0	Aa	7574957.7	Ba	670635.2	Ba
TAG 50:3	705456.0	Ad	678205.6	Ab	516300.6	Aa	9618439.3	Ac	897184.5	Bb	980706.6	Ba	27459842.1	Ab	1698145.4	Bab	815816.6	Ba	64996866.2	Aa	8087449.7	Ba	240692.1	Ca
TAG 50:4	194278.9	Ad	166647.5	Aa	124750.7	Aa	2590850.9	Ac	256600.2	Ba	266791.0	Ba	6949156.2	Ab	510479.6	Ba	248037.6	Ba	17323860.0	Aa	1369718.9	Ba	64508.8	Ba
TAG 50:5	51390.9	Ad	174367.8	Aa	35430.5	Aa	794380.4	Ac	54252.7	Ba	56994.4	Ba	2082693.2	Ab	111801.4	Ba	60342.6	Ba	5549670.0	Aa	347249.3	Ba	15230.9	Ba
TAG 50:6.1	18633.4	Ac	19714.8	Aa	14597.3	Aa	283650.9	Ac	27840.4	Aa	27304.9	Aa	880704.9	Ab	74235.5	Ba	39151.1	Ba	2554601.8	Aa	168879.8	Ba	9044.2	Ba
TAG 50:7	3389.4	Ac	4162.5	Aa	2754.3	Aa	26120.8	Ac	3215.5	Aa	4168.0	Aa	74152.0	Ab	9266.3	Ba	NA	Ba	225738.8	Aa	15963.5	Ba	NA	Ba
TAG 50:8	1648.4	Ac	2170.7	Aa	NA	Aa	18307.7	Ac	1120.5	Aa	1572.3	Aa	47404.2	Ab	4604.9	Ba	2595.9	Ba	165427.6	Aa	7580.6	Ba	NA	Ba
TAG 50:9	NA	Ac	NA	Aa	NA	Aa	13521.7	Ac	NA	Aa	NA	Aa	42157.1	Ab	3278.4	Ba	1737.0	Ba	142985.4	Aa	5102.7	Ba	NA	Ba
TAG 52:0	895704.8	Ab	820758.1	Aa	932795.1	Aa	664455.1	Ab	505399.2	Aa	538343.1	Ab	964949.9	Ab	494699.1	Ba	489095.7	Bb	1385056.8	Aa	462425.1	Ba	526489.9	Bb
TAG 52:1	4293230.5	Ad	4036415.3	Aa	3818622.3	Aa	12325581.7	Ac	3327402.4	Ba	3942922.5	Ba	25756092.7	Ab	3040185.6	Ba	2160362.2	Ba	44525360.0	Aa	4702711.1	Ba	1377995.3	Ba
TAG 52:2	12747897.3	Ad	11680518.2	Ab	9383274.7	Aab	65518373.8	Ac	21327826.2	Bb	22348388.3	Ba	110092597.1	Ab	21123567.3	Bb	14728543.8	Bab	164416202.3	Aa	40836427.7	Ba	5572904.6	Cb
TAG 52:3	3002694.6	Ad	2806192.9	Aa	2427444.8	Aa	22508344.2	Ac	4282750.9	Ba	4684329.7	Ba	49468128.8	Ab	4694167.1	Ba	3093652.8	Ba	98109037.5	Aa	13765619.5	Ba	1224535.9	Ca
TAG 52:4	1887557.0	Ad	1702043.4	Ab	1612960.1	Aa	14568630.8	Ac	3035996.6	Bb	3387102.7	Ba	37106491.8	Ab	4330000.6	Bab	2539580.2	Ba	78681822.1	Aa	12902566.7	Ba	938555.2	Ca
TAG 52:5	264355.7	Ad	253064.7	Aa	238359.5	Aa	3133951.5	Ac	572858.9	Ba	651896.2	Ba	8071115.4	Ab	878221.2	Ba	530799.4	Ba	18703968.4	Aa	1724262.1	Ba	133133.4	Ba
TAG 52:5.1	117311.7	Aa	157594.7	Aa	102028.7	Aa	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab
TAG 52:6	154030.0	Ad	133917.4	Aa	123186.4	Aa	1222879.2	Ac	195590.6	Ba	230872.2	Ba	3279637.3	Ab	473333.8	Ba	236009.3	Ba	7654020.6	Aa	890330.9	Ba	75213.4	Ba
TAG 52:6.1	64409.5	Aa	78728.5	Aa	55611.9	Aa	43302.2	ABa	41280.0	Bbc	68215.4	Aa	NA	Bb	50348.2	Ab	49435.0	Aa	64620.5	Aa	18079.8	Bc	14222.6	Bb
TAG 52:7	53272.7	Ac	54932.1	Aa	41817.4	Aa	448894.5	Ac	71206.5	Aa	84927.3	Aa	1345254.2	Ab	180812.7	Ba	94468.5	Ba	3295222.7	Aa	295311.3	Ba	23784.5	Ba
TAG 52:8	17890.9	Ac	19802.1	Aa	14674.9	Aa	80070.1	Ac	16255.1	Aa	21235.8	Aa	217120.6	Ab	40006.6	Ba	24197.8	Ba	537312.6	Aa	47677.7	Ba	7639.9	Ba
TAG 52:9	6933.3	Ac	12234.1	Aa	5601.1	Aa	33549.3	Ac	9168.0	Aa	12102.3	Aa	87586.6	Ab	30063.7	Ba	16522.2	Ba	220749.4	Aa	26805.0	Ba	4829.5	Ba
TAG 54:0	314720.9	Aa	272266.7	Ba	325044.0	Ba	217976.7	Ab	189989.8	Bb	207364.3	Bb	264747.4	Ab	185337.6	Bb	183658.9	Bb	317681.2	Ab	195466.4	Bb	191786.3	Bb
TAG 54:1	1331662.5	Ac	1101949.0	Aa	1287124.8	Aa	1399392.5	Ac	877512.1	Aa	1027476.8	Aa	2143707.2	Ab	783355.3	Ba	716498.5	Ba	3633242.1	Aa	816013.9	Ba	672637.8	Ba

SI Table 9. *(continuation)* Relative abundance of lipid of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N_So	0h_3.5N_So	0h_7N_So	10h_0N_So	10h_3.5N_So	10h_7N_So	18h_0N_So	18h_3.5N_So	18h_7N_So	36h_0N_So	36h_3.5N_So	36h_7N_So
TAG 54:2	4311575.5 **Ad**	4185194.5 **Aa**	3639678.2 **Aa**	13252819.0**Ac**	5977913.4 **Ba**	7008794.1 **Ba**	24573238.3 **Ab**	5060081.1 **Ba**	3736043.5 **Ba**	37298171.2 **Aa**	5997778.1 **Ba**	1780143.9 **Ba**
TAG 54:3	8098768.0 **Ad**	7155154.3 **Ab**	6081840.0 **Ab**	40316247.9**Ac**	19176410.6**Bab**	21082501.9**Ba**	67104695.8 **Ab**	18355503.0**Bab**	13963100.5**Bab**	100949843.3**Aa**	24979493.6**Ba**	4686598.1 **Cb**
TAG 54:4	2820411.8 **Ad**	2499528.9 **Aa**	2466116.4 **Aa**	13022337.1**Ac**	4930161.0 **Ba**	5428366.9 **Ba**	25616809.9 **Ab**	4816480.8 **Ba**	3540665.5 **Ba**	47569065.9 **Aa**	8533198.7 **Ba**	1529027.9 **Ca**
TAG 54:5	1903439.3 **Ad**	1832505.2 **Ab**	1775322.4 **Aa**	7080661.9 **Ac**	3663401.3 **Aab**	4116068.8 **Aa**	14705066.3 **Ab**	4399747.0 **Bab**	3052447.9 **Ba**	29690897.4 **Aa**	7051365.2 **Ba**	1254702.6 **Ca**
TAG 54:6	922905.6 **Ac**	877836.0 **Aa**	925898.9 **Aa**	1919568.5 **Ac**	887700.5 **Aa**	1021827.0 **Aa**	3905861.8 **Ab**	1193114.9 **Ba**	889805.8 **Ba**	9424129.3 **Aa**	2091831.2 **Ba**	433995.0 **Ca**
TAG 54:7	316477.5 **Ac**	293224.4 **Ab**	289910.2 **Aa**	822862.8 **Ac**	405910.2 **Ab**	503888.4 **Aa**	1648243.5 **Ab**	834412.4 **Bab**	495838.9 **Ba**	4072258.3 **Aa**	1156744.1 **Ba**	183540.2 **Ca**
TAG 54:8	106488.6 **Ac**	110568.9 **Aa**	94950.3 **Aa**	218606.2 **Ac**	85393.3 **Ba**	126063.3 **ABa**	363896.9 **Ab**	177848.0 **Ba**	120121.9 **Ba**	767056.1 **Aa**	186274.4 **Ba**	40991.5 **Ca**
TAG 54:9	29354.1 **Ac**	31898.9 **Ac**	27199.7 **Ab**	74698.4 **Ac**	45339.6 **Abc**	64500.2 **Aab**	126296.7 **Ab**	121807.4 **Ba**	74022.7 **Ba**	218794.2 **Aa**	82398.7 **Bab**	29198.7 **Cab**
TAG 56:0	71176.7 **Ab**	64891.7 **Aa**	74512.0 **Aa**	71367.5 **Ab**	51561.8 **Aa**	59193.8 **Aab**	85472.2 **Ab**	58972.8 **Ba**	47949.3 **Bb**	116346.9 **Aa**	71215.1 **Ba**	49563.6 **Bab**
TAG 56:1	226059.7 **Ad**	200197.4 **Aa**	190969.0 **Aa**	402937.9 **Ac**	246363.3 **Ba**	273948.6 **ABa**	602398.1 **Ab**	211650.2 **Ba**	149705.4 **Ba**	1112784.1 **Aa**	343495.9 **Ba**	138817.3 **Ca**
TAG 56:2	426488.5 **Ad**	408472.5 **Aa**	383459.7 **Aab**	1094498.0 **Ac**	693262.5 **Ba**	748192.3 **ABa**	1725747.8 **Ab**	613874.9 **Ba**	426218.3 **Bab**	2924803.4 **Aa**	779043.9 **Ba**	313127.5 **Cb**
TAG 56:3	533808.3 **Ad**	418756.4 **Ab**	594390.8 **Aab**	2504091.8 **Ac**	1495428.3 **Ba**	1560708.6 **Ba**	4153288.8 **Ab**	1612232.5 **Ba**	983565.4 **Bab**	6525434.9 **Aa**	2016494.7 **Ba**	489535.3 **Cb**
TAG 56:4	332986.3 **Ac**	169475.1 **Ab**	570987.8 **Aa**	689500.6 **Abc**	437651.5 **Aab**	402140.5 **Aa**	1113596.3 **Ab**	443465.7 **Bab**	273966.1 **Ba**	1743979.5 **Aa**	668846.8 **Ba**	169008.9 **Ca**
TAG 56:5	334702.8 **ABb**	111620.3 **Ba**	688278.4 **Aa**	442297.5 **Ab**	294276.6 **Aa**	251629.9 **Aa**	755879.5 **Ab**	364239.8 **ABa**	198713.2 **Ba**	1421727.5 **Aa**	576175.6 **Ba**	153452.7 **Ba**
TAG 56:6	228734.2 **Aa**	59685.1 **Aa**	512537.0 **Aa**	121949.1 **Aa**	99021.3 **Aa**	63153.9 **Aa**	199244.3 **Aa**	103471.1 **Aa**	58081.5 **Aa**	402182.1 **Aa**	171947.4 **Aa**	49145.5 **Aa**
TAG 56:7	84524.7 **ABa**	23017.2 **Ba**	186153.7 **Aa**	57101.6 **Aa**	42743.6 **Aa**	31651.5 **Ab**	92041.0 **Aa**	78413.1 **Aa**	36435.4 **Aab**	196040.6 **Aa**	111289.6 **ABa**	32295.9 **Bb**
TAG 58:0	37310.6 **Ac**	33717.0 **Aab**	37747.6 **Aa**	44177.2 **Ac**	27399.3 **Bb**	30559.9 **Ba**	59882.6 **Ab**	31498.7 **Bab**	25785.8 **Ba**	77881.5 **Aa**	42364.7 **Ba**	27834.5 **Ca**
TAG 58:1	95995.6 **Ad**	89753.0 **Aa**	84231.0 **Aab**	151860.0 **Ac**	111925.7 **Aa**	130322.3 **Aa**	210623.1 **Ab**	107392.4 **Ba**	73112.2 **Bb**	347240.0 **Aa**	133838.8 **Ba**	61114.8 **Cb**
TAG 58:2	503068.8 **Ad**	454429.5 **Ab**	352899.7 **Ab**	1026936.4 **Ac**	790249.5 **Aab**	894203.3 **Aa**	1461687.4 **Ab**	682520.9 **Bab**	482479.4 **Bb**	2342361.1 **Aa**	876201.7 **Ba**	398581.5 **Cb**
TAG 58:3	244791.6 **Ad**	205026.9 **Ab**	269560.1 **Aa**	727587.2 **Ac**	478809.3 **Aab**	486575.9 **Aa**	1130831.4 **Ab**	535848.2 **Ba**	348688.5 **Ba**	1899829.4 **Aa**	672560.1 **Ba**	249055.5 **Ca**
TAG 58:4	113134.7 **Ad**	132958.9 **Ab**	85531.1 **Aa**	247668.1 **Ac**	181497.0 **Ab**	199871.1 **Aa**	393815.6 **Ab**	191680.0 **Bb**	127926.1 **Ba**	773479.3 **Aa**	325249.5 **Ba**	107893.1 **Ca**
TAG 58:5	10635.0 **Aa**	12928.3 **Aa**	NA **Bb**	NA **Ab**	NA **Ab**	NA **Ab**	NA **Ab**	NA **Ab**	NA **Ab**	NA **Bb**	NA **Bb**	8189.0 **Aa**
TAG 58:6	NA **Bc**	7944.3 **Ab**	NA **Ba**	NA **Ac**	NA **Ac**	NA **Aa**	26158.4 **Ab**	NA **Bc**	NA **Ba**	46371.9 **Aa**	17521.7 **Ba**	NA **Ca**
TAG 60:0	66187.6 **Aa**	66065.3 **Aa**	62585.0 **Aa**	75234.7 **Aa**	43076.5 **Bb**	47255.3 **Bab**	79158.2 **Aa**	53603.6 **Bab**	39814.5 **Bb**	78298.6 **Aa**	60087.4 **Bab**	48239.3 **Bab**
TAG 60:1	179796.6 **Ad**	163978.3 **Ab**	159601.0 **Ab**	270105.6 **Ac**	253618.8 **Aa**	298311.9 **Aa**	358252.1 **Ab**	229673.9 **Bab**	150156.6 **Cb**	487659.6 **Aa**	289841.8 **Ba**	161176.4 **Cb**
TAG 60:2	167869.8 **Ad**	164843.0 **Ab**	124666.5 **Ab**	323547.4 **Ac**	313936.1 **Aa**	368233.6 **Aa**	457725.6 **Ab**	284500.8 **Ba**	196207.5 **Bb**	675770.0 **Aa**	306053.5 **Ba**	153811.4 **Cb**
TAG 60:3	65069.9 **Ad**	62418.9 **Ab**	66611.1 **Ac**	214779.0 **Ac**	203445.4 **Aa**	209897.9 **Aa**	342497.8 **Ab**	247704.1 **Ba**	161948.5 **Cab**	538686.1 **Aa**	225922.2 **Ba**	100129.9 **Cbc**
TAG 60:4	37948.4 **Aa**	42197.8 **Ac**	32850.3 **Ab**	NA **Bb**	63698.5 **Ab**	73264.9 **Aa**	NA **Cb**	73829.1 **Ab**	48149.4 **Bb**	NA **Cb**	101900.3 **Aa**	35812.3 **Bb**
TAG 60:5	11414.1 **Ac**	14684.2 **Ac**	9757.9 **Ab**	29711.7 **Ab**	35580.1 **Ab**	30715.4 **Aa**	NA **Cc**	53098.2 **Aa**	28227.7 **Ba**	98641.8 **Aa**	46662.7 **Bab**	21171.6 **Cab**

SI Table 9. *(continuation)* Relative abundance of lipid of *Scenedesmus obliquus* BR003 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N_So		0h_3.5N_So		0h_7N_So		10h_0N_So		10h_3.5N_So		10h_7N_So		18h_0N_So		18h_3.5N_So		18h_7N_So		36h_0N_So		36h_3.5N_So		36h_7N_So	
TAG 60:5.1	8007.2	**Ad**	11568.3	**Ab**	8302.3	**Aa**	48177.6	**Ac**	22884.0	**Bab**	27905.0	**Ba**	92051.9	**Ab**	28527.0	**Bab**	20093.1	**Ba**	143547.0	**Aa**	41371.5	**Ba**	12461.8	**Ca**
TAG 60:6	8873.2	**Ac**	12408.7	**Ab**	7193.5	**Ab**	24014.1	**Ac**	18330.7	**Ab**	21001.9	**Aab**	55575.3	**Ab**	41349.6	**ABa**	28185.9	**Ba**	88146.4	**Aa**	40921.2	**Ba**	32528.1	**Ba**
FA 16:0	8043628.3	**Ac**	7366681.2	**Aa**	8450180.7	**Aa**	10452974.5	**Ab**	4686789.1	**Bb**	5979066.3	**Bb**	11757800.6	**Ab**	4521616.3	**Bb**	4370813.3	**Bb**	15351890.6	**Aa**	6234721.5	**Bab**	4125602.0	**Bb**
FA 16:1	1521808.8	**Ab**	1571535.4	**Aa**	1620831.0	**Aa**	1187932.7	**Ab**	489299.9	**Bb**	702301.2	**Bb**	1455739.1	**Ab**	596674.1	**Bb**	552693.1	**Bb**	2053248.2	**Aa**	686199.1	**Bb**	455319.9	**Bb**
FA 16:2	1773099.1	**Ab**	1904104.1	**Aa**	1888659.6	**Aa**	2011871.6	**Ab**	442423.6	**Bb**	780833.9	**Bb**	1883264.1	**Ab**	407494.1	**Bb**	442138.6	**Bb**	3383208.0	**Aa**	605069.4	**Bb**	355534.4	**Bb**
FA 16:3	2245471.3	**Ab**	2566960.0	**Aa**	2296031.9	**Aa**	3526015.3	**Aa**	1148704.0	**Bb**	2056802.5	**Ba**	3712577.2	**Aa**	1220362.9	**Bb**	1318615.8	**Bab**	4412122.1	**Aa**	1401089.3	**Bab**	586061.9	**Bb**
FA 16:4	62050.9	**ABa**	50223.9	**Ba**	70861.5	**Aa**	74086.5	**Aa**	6322.1	**Bb**	13914.9	**Bb**	69103.2	**Aa**	9388.0	**Bb**	8696.9	**Bb**	63309.4	**Aa**	11304.3	**Bb**	2583.0	**Bb**
FA 16:4.1	1080643.8	**Aa**	1469826.0	**Aa**	860371.3	**Ab**	118360.5	**Bb**	1551209.6	**Aa**	1352856.5	**Ab**	249794.6	**Bb**	1468252.9	**Aa**	1409739.9	**Ab**	159174.0	**Cb**	1226729.9	**Ba**	2795097.9	**Aa**
FA 18:0	2360900.4	**Cb**	2969076.6	**Ba**	3527074.8	**Aa**	2359620.2	**Ab**	1317958.9	**Bb**	NA	**Cc**	3363101.1	**Aa**	1127314.4	**Bb**	1003479.6	**Bb**	NA	**Bc**	1285824.0	**Ab**	785551.5	**Ab**
FA 18:1	25849773.4	**Ac**	25214059.7	**Aa**	25601258.6	**Aa**	33205835.6	**Abc**	20240570.3	**Ba**	24705724.5	**Bab**	37847387.8	**Ab**	19472832.4	**Ba**	19552193.3	**Bab**	54180840.7	**Aa**	22745985.7	**Ba**	16181771.8	**Bb**
FA 18:2	14529287.8	**Ab**	17407175.1	**Aa**	15648810.6	**Aa**	12623201.4	**Ab**	7300857.2	**Bb**	11442711.0	**ABab**	16661742.4	**Ab**	8072681.9	**Bb**	8739817.0	**Bb**	22398343.5	**Aa**	10230946.3	**Bb**	7917981.6	**Bb**
FA 18:3	17555771.7	**Aa**	18918432.8	**Aa**	17417222.5	**AA**	19198164.7	**Aa**	16971867.9	**Aa**	22317173.5	**Aa**	20938564.8	**Aa**	19098397.3	**Aa**	18232878.6	**Aa**	27723021.7	**Aa**	17948903.0	**Aa**	18356949.9	**Aa**
FA 20:0	NA	**Ba**	218648.8	**Aa**	NA	**Ba**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**
FA 20:1	624658.0	**Ab**	613988.3	**Aa**	634829.7	**Aa**	819417.5	**Aab**	462958.8	**Ba**	581370.2	**Ba**	888534.7	**Aa**	628097.7	**Ba**	556775.9	**Ba**	1043978.5	**Aa**	671065.3	**Ba**	630767.1	**Ba**
FA 20:3	96993.1	**Aa**	110563.2	**Aa**	96388.3	**Aa**	73311.3	**Ac**	52668.5	**Ac**	67106.0	**Ac**	78810.5	**Abc**	87225.0	**Abc**	73897.4	**Abc**	107378.2	**Aab**	92235.1	**Aab**	97062.9	**Aab**
FA 22:1	107712.1	**Ab**	102028.7	**Ac**	99297.3	**Ac**	NA	**Bc**	134481.4	**Abc**	141928.2	**Ab**	141947.9	**Ba**	181195.2	**Aa**	164435.5	**ABb**	156965.8	**Ba**	162342.7	**Bab**	250563.0	**Aa**
FA 24:1	32746.3	**Ba**	NA	**Cc**	70146.2	**Ac**	NA	**Bb**	91269.9	**Ab**	97323.4	**Ab**	NA	**Cb**	123778.9	**Aa**	108564.4	**Bb**	NA	**Cb**	87875.7	**Bb**	134882.2	**Aa**

SI Table 10. Relative abundance of lipid of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
DAG 34:1	48488.3	**Ab**	37588.8	**Ab**	51033.0	**Aa**	63592.9	**Ab**	42885.3	**Ab**	42430.9	**Aa**	106679.4	**Aa**	21346.5	**Bb**	26424.8	**Bab**	NA	**Bc**	131024.4	**Aa**	2823.3	**Bb**
DAG 34:2	57490.6	**Ab**	48688.6	**Aa**	57626.3	**Aa**	42328.2	**Ab**	49767.3	**Aa**	48518.2	**Aa**	50640.8	**Ab**	19248.1	**Bb**	23345.7	**Bb**	103802.4	**Aa**	62299.9	**Ba**	7299.6	**Cb**
DAG 34:3	140600.4	**Ab**	108155.8	**Aa**	124162.1	**Aa**	112360.0	**Ab**	91808.0	**Aa**	100301.9	**Aa**	116859.4	**Ab**	32647.4	**Bb**	40701.2	**Bb**	227945.1	**Aa**	82558.2	**Ba**	8775.9	**Cb**
DAG 34:4	125414.5	**Ab**	98216.4	**Aa**	123085.0	**Aa**	112275.5	**Ab**	87026.4	**Aa**	104215.6	**Aa**	149103.4	**Ab**	41331.4	**Bb**	56946.8	**Bb**	291466.4	**Aa**	109048.5	**Ba**	9050.8	**Cc**
DAG 34:5	207524.4	**Ab**	170640.3	**Aa**	214811.3	**Aa**	NA	**Bc**	205521.3	**Aa**	230681.5	**Aa**	228023.6	**Ab**	78911.9	**Bb**	86892.6	**Bb**	454228.1	**Aa**	169847.0	**Ba**	21434.2	**Cb**
DAG 34:6	160015.6	**Ac**	133786.3	**Aab**	171062.5	**Aab**	227833.8	**Abc**	204695.8	**Aa**	234842.4	**Aa**	279907.0	**Ab**	75806.9	**Bb**	92720.1	**Bbc**	659744.4	**Aa**	164997.7	**Bab**	24440.3	**Cc**
DAG 36:1	5587.1	**Ac**	4167.0	**Aa**	5340.6	**Aa**	7869.9	**Ac**	5878.9	**Aa**	5226.3	**Aa**	15753.6	**Ab**	5418.4	**Ba**	5248.6	**Bab**	25100.5	**Aa**	7350.4	**Ba**	NA	**Cb**
DAG 36:2	87320.7	**Ad**	86978.7	**Ab**	101835.5	**Aa**	167557.2	**Ac**	111963.2	**Bb**	101844.9	**Ba**	258105.0	**Ab**	69015.9	**Bb**	83661.2	**Ba**	501261.0	**Aa**	240425.3	**Ba**	7100.3	**Cb**
DAG 36:3	168384.2	**Ac**	125515.6	**Ab**	149946.5	**Aa**	190655.8	**Ac**	150413.0	**Ab**	131080.3	**Aa**	374023.1	**Ab**	63910.2	**Bb**	85115.0	**Bab**	797487.2	**Aa**	402678.0	**Ba**	8196.9	**Cb**
DAG 36:4	159808.7	**Ac**	127756.5	**Ab**	144784.7	**Aa**	212959.1	**Ac**	138829.1	**Bb**	129691.4	**Bab**	324082.4	**Ab**	49145.0	**Bc**	63756.7	**Bbc**	668101.2	**Aa**	279943.6	**Ba**	NA	**Cc**
DAG 36:5	176694.5	**Ab**	144385.0	**Aa**	151675.4	**Aa**	118284.7	**Ab**	138359.6	**Aa**	154439.2	**Aa**	140014.2	**Ab**	44870.3	**Bb**	54878.9	**Bb**	284226.6	**Aa**	118696.6	**Ba**	19603.0	**Cb**
DAG 36:6	121766.5	**Ab**	85963.4	**Aab**	116799.5	**Aa**	98054.2	**Ab**	126826.2	**Aa**	134329.3	**Aa**	108102.7	**Ab**	34758.5	**Bc**	47391.8	**Bb**	222456.7	**Aa**	58570.2	**Bbc**	12835.4	**Cb**
DGDG 32:0	111343.8	**Aa**	107704.4	**Aa**	100654.9	**Aa**	70707.1	**Ac**	72515.7	**Ab**	72916.6	**Ab**	77380.0	**Abc**	58108.8	**Bb**	62422.9	**Bb**	86428.8	**Ab**	62628.4	**Bb**	75193.2	**ABb**
DGDG 32:1	35796.4	**Aa**	35885.7	**Aa**	34156.1	**Aa**	7871.3	**Bb**	22515.1	**Ab**	23085.9	**Ab**	11164.5	**Bb**	17035.1	**Ac**	16865.6	**Ac**	10992.4	**Bb**	13111.7	**ABc**	17426.4	**Ac**
DGDG 32:2	129021.8	**Aa**	105823.2	**Ba**	101127.0	**Ba**	35627.1	**Bb**	64664.2	**Ab**	69958.6	**Ab**	33508.2	**Bb**	53404.6	**Ab**	54495.9	**Ab**	39769.9	**Cb**	54622.3	**Bb**	99107.5	**Aa**
DGDG 32:3	237532.5	**Aa**	197886.4	**Aa**	208371.6	**Ab**	53932.4	**Bb**	181349.0	**Aab**	188235.2	**Abc**	53916.0	**Bb**	147260.5	**Abc**	148921.2	**Ac**	71233.2	**Cb**	127212.4	**Bc**	311614.7	**Aa**
DGDG 32:3.1	28170.4	**Bb**	31933.4	**ABc**	38692.5	**Ac**	12062.7	**Bc**	60808.9	**Aa**	62786.0	**Aa**	22756.8	**Bbc**	45454.3	**Ab**	50282.7	**Ab**	55534.7	**Ba**	31314.9	**Cc**	68792.9	**Aa**
DGDG 34:0	65348.5	**Aa**	65725.2	**Ab**	67012.5	**Ab**	36941.7	**Ba**	142427.2	**Aa**	159832.6	**Aa**	37264.7	**Ba**	177626.1	**Aa**	191941.5	**Aa**	27503.0	**Ba**	57609.0	**Bb**	100632.0	**Ab**
DGDG 34:1	2071068.1	**Ba**	2184602.3	**ABa**	2500024.3	**Aa**	720343.3	**Bb**	1967730.1	**Aa**	1715656.0	**Ab**	1065203.3	**Bb**	1576366.2	**Ab**	1630860.7	**Ab**	1021402.7	**Bb**	1551715.0	**Ab**	1467276.4	**Ab**
DGDG 34:2	2736961.4	**Aa**	2816745.2	**Aa**	3081179.1	**Aa**	631568.0	**Bc**	2775960.5	**Aa**	2454446.3	**Ab**	964421.9	**Bbc**	2017574.0	**Ab**	2208796.9	**Ab**	1121828.2	**Cb**	1818367.6	**Bb**	2301074.2	**Ab**
DGDG 34:3	1231033.3	**Aab**	1300374.1	**Ac**	1495793.7	**Ab**	579772.4	**Bc**	2548513.5	**Aa**	2247591.7	**Aa**	971863.1	**Bb**	1861225.0	**Ab**	2039271.5	**Aa**	1450861.2	**Ba**	1358052.8	**Bc**	2078230.7	**Aa**
DGDG 34:4	3215081.7	**Ba**	3270020.9	**Ba**	3713968.4	**Aa**	470138.7	**Bb**	2546278.4	**Ab**	2367253.3	**Ab**	532765.5	**Bb**	1990814.9	**Ac**	2169599.1	**Ab**	531234.2	**Cb**	1693605.8	**Bc**	2234715.8	**Ab**
DGDG 34:5	3831406.0	**Ba**	3914190.2	**Ba**	4551955.6	**Aa**	1078277.1	**Bc**	4125810.3	**Aa**	3658621.3	**Ab**	1480772.2	**Bbc**	3083774.0	**Ab**	3334828.1	**Ab**	1805567.5	**Cb**	2991410.0	**Bb**	3822093.1	**Ab**
DGDG 34:6	4415164.0	**Ba**	4550077.9	**Bab**	5637679.8	**Aa**	1423329.0	**Bc**	5161564.9	**Aa**	4368288.2	**Ab**	2248417.6	**Bbc**	3846658.8	**Ab**	4264026.1	**Ab**	2927590.6	**Bb**	3908408.2	**ABb**	4793585.6	**Aab**
DGDG 36:2	41891.6	**Aa**	43015.3	**Ac**	48236.5	**Ac**	10179.2	**Cb**	74862.0	**Bb**	98940.8	**Ab**	11506.8	**Cb**	102350.3	**Ba**	129433.0	**Aa**	6819.4	**Cb**	56256.7	**Bbc**	91232.7	**Ab**
DGDG 36:3	49869.1	**Aa**	49286.4	**Ac**	55957.4	**Ab**	5861.2	**Bb**	66117.4	**Ab**	70404.4	**Aa**	6090.3	**Bb**	79129.9	**Aa**	74126.7	**Aa**	3379.3	**Bb**	78277.4	**Aa**	82031.8	**Aa**
DGDG 36:4	334604.4	**Aa**	349462.4	**Aa**	364734.3	**Ab**	23009.5	**Bb**	237430.9	**Ab**	242546.6	**Ac**	20443.1	**Bb**	245964.0	**Ab**	237777.7	**Ac**	NA	**Cb**	230065.4	**Bb**	698035.8	**Aa**
DGDG 36:5	830960.0	**Ba**	835249.9	**Ba**	950694.6	**Ab**	75047.6	**Bb**	801263.6	**Aa**	765716.0	**Ac**	79559.0	**Bb**	680172.6	**Ab**	687907.1	**Ac**	37009.1	**Cb**	460071.8	**Bc**	1070265.4	**Aa**
DGDG 36:6	1133821.7	**Ba**	1200583.0	**ABb**	1358580.0	**Aab**	187509.3	**Bb**	1594014.7	**Aa**	1514795.1	**Aa**	277205.0	**Bb**	1213471.5	**Ab**	1254635.8	**Ab**	231518.9	**Cb**	632783.2	**Bc**	1532103.6	**Aa**

SI Table 10. *(continuation)* Relative abundance of lipid of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
LysoPC 16:0	52209.8	**Cb**	166891.8	**Ba**	216845.7	**Aa**	35415.8	**Bb**	82229.1	**Ab**	80194.8	**Abc**	45305.3	**Bb**	79820.8	**Abc**	70633.7	**ABc**	103768.5	**Aa**	49161.5	**Bc**	109893.8	**Ab**
LysoPC 16:0.1	3880.9	**Cb**	15479.9	**Ba**	19949.9	**Aa**	2843.1	**Bb**	7154.9	**Ab**	7238.2	**Abc**	3231.7	**Bb**	6950.3	**Ab**	6568.0	**Ac**	8861.5	**Aa**	4533.9	**Bb**	9734.9	**Ab**
LysoPC 16:2	27572.0	**Ba**	74503.2	**Ab**	111423.9	**Ac**	14651.8	**Ba**	114061.3	**Aab**	100619.8	**Ac**	31527.7	**Ba**	155371.9	**Aa**	160925.8	**Ab**	43496.0	**Ba**	83375.7	**Bb**	475638.1	**Aa**
LysoPC 16:3	15737.7	**Ba**	51606.4	**ABc**	85183.0	**Ac**	11016.8	**Ba**	116060.6	**Aab**	109219.6	**Ac**	29866.5	**Ba**	170611.3	**Aa**	178515.6	**Ab**	37827.5	**Ba**	80563.8	**Bbc**	685116.3	**Aa**
LysoPC 18:2	46728.4	**Ba**	196897.7	**ABc**	316764.1	**Ac**	41434.1	**Ba**	513475.1	**Aab**	420511.6	**Abc**	139458.5	**Ba**	766154.3	**Aa**	584144.5	**Ab**	218529.6	**Ca**	458120.8	**Bbc**	3170119.8	**Aa**
LysoPC 18:3	26355.9	**Ba**	106090.6	**ABc**	174835.5	**Ac**	19537.6	**Ba**	304372.1	**Aab**	262732.0	**Abc**	68953.0	**Ba**	376432.8	**Aa**	368423.7	**Ab**	95376.9	**Ba**	218358.5	**Bbc**	1900874.6	**Aa**
MGDG 34:1	187851.1	**Ba**	240011.3	**ABb**	301290.8	**Ab**	40370.7	**Bb**	433191.3	**Aa**	386096.9	**Aa**	66322.9	**Bb**	258978.2	**Ab**	292792.5	**Ab**	NA	**Bb**	69167.5	**Bc**	153763.7	**Ac**
MGDG 34:2	168958.4	**Ca**	237076.3	**Ba**	279190.4	**Aa**	15255.8	**Bb**	179289.0	**Ab**	157358.7	**Ab**	22789.2	**Bb**	170644.8	**Ab**	170134.3	**Ab**	4392.0	**Ab**	30672.4	**Ac**	NA	**Ac**
MGDG 34:2.1	136092.1	**Ba**	166103.4	**ABb**	218732.2	**Abc**	18829.1	**Bb**	338167.0	**Aa**	281690.2	**Ab**	28677.3	**Bb**	144096.4	**Ab**	195236.2	**Ac**	9380.3	**Bb**	52829.0	**Bc**	407996.3	**Aa**
MGDG 34:3	2642.4	**Ba**	2854.0	**Bc**	5537.2	**Ab**	NA	**Bb**	9190.3	**Aa**	9282.6	**Aa**	NA	**Bb**	6351.2	**Ab**	7558.2	**Aab**	NA	**Bb**	NA	**Bd**	5442.0	**Ab**
MGDG 34:4	449246.6	**Ba**	742863.3	**Aa**	832887.6	**Ac**	26318.4	**Bb**	980309.3	**Aa**	873347.8	**Ac**	35196.6	**Bb**	1043067.9	**Aa**	1301158.8	**Ab**	23037.5	**Cb**	347063.4	**Bb**	2166782.2	**Aa**
MGDG 34:5	529970.1	**Ba**	812393.3	**ABb**	976802.7	**Ac**	43460.3	**Bb**	1341185.2	**Aa**	1233594.3	**Abc**	57418.0	**Bb**	1326896.9	**Aa**	1619978.8	**Ab**	39492.6	**Cb**	466027.7	**Bb**	3004791.1	**Aa**
MGDG 34:6	NA	**Aa**	NA	**Ab**	NA	**Ac**	NA	**Ba**	NA	**Bb**	1384767.3	**Ab**	NA	**Ba**	1465604.3	**Aa**	1691724.4	**Ab**	45899.2	**Ba**	NA	**Bb**	3548924.7	**Aa**
MGDG 36:5	78541.7	**Aa**	123131.6	**Aa**	120015.2	**Ab**	NA	**Ba**	155868.1	**Aa**	140863.6	**Ab**	NA	**Ba**	135780.7	**Aa**	160793.1	**Ab**	NA	**Ba**	61628.5	**Ba**	574085.5	**Aa**
MGDG 36:6	58602.7	**Aa**	90424.3	**Abc**	99001.1	**Ac**	4956.4	**Ba**	194169.6	**Aab**	182173.9	**Abc**	5444.9	**Ba**	200208.9	**Aa**	235944.4	**Ab**	2268.9	**Ba**	27880.2	**Bc**	908889.4	**Aa**
PC 32:0	4601.0	**Bb**	8038.5	**Bab**	11895.1	**Aa**	7317.2	**Ab**	6993.3	**Ab**	6947.1	**Ab**	8284.9	**Ab**	7659.7	**Ab**	8139.5	**Aab**	16057.1	**Aa**	11685.1	**Ba**	10711.4	**Bab**
PC 32:1	9597.7	**Bb**	18636.2	**Bb**	32339.3	**Ab**	9930.7	**Bb**	26545.1	**Aab**	28318.8	**Ab**	17186.4	**Bb**	33467.5	**Aa**	39471.5	**Ab**	47892.1	**Ba**	39879.1	**Ba**	65991.7	**Aa**
PC 32:1.1	14217.4	**Cb**	26230.3	**Ba**	37987.5	**Ab**	8149.7	**Bb**	30092.3	**Aa**	28333.3	**Ac**	12042.6	**Bb**	29922.4	**Aa**	33502.6	**Abc**	28735.8	**Ba**	24376.7	**Ba**	92714.4	**Aa**
PC 32:2	59964.1	**Ca**	179163.7	**Bb**	340690.8	**Ab**	32038.2	**Ba**	313488.9	**Aa**	289172.5	**Ab**	72394.0	**Ba**	333046.4	**Aa**	384769.6	**Ab**	138753.5	**Ba**	169847.3	**Bb**	981556.9	**Aa**
PC 32:3	62718.1	**Ba**	238042.1	**ABbc**	464332.6	**Ab**	26210.6	**Ba**	472052.6	**Aab**	437007.5	**Ab**	71545.5	**Ba**	528817.9	**Aa**	634749.5	**Ab**	124682.6	**Ba**	212855.2	**Bc**	1846028.6	**Aa**
PC 32:4	3338.2	**Ba**	12735.3	**ABa**	20717.2	**Ab**	NA	**Ba**	14694.5	**Aa**	13525.2	**Ab**	1653.6	**Ba**	17155.0	**Aa**	20752.4	**Ab**	2410.1	**Ba**	8365.6	**Ba**	81422.7	**Aa**
PC 34:1	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Ba**	388385.5	**Aa**	NA	**Ba**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**
PC 34:2	119100.2	**Ba**	368536.2	**Bc**	734540.0	**Ac**	NA	**Ba**	772983.4	**Ab**	698704.5	**Ac**	NA	**Ba**	1098338.7	**Aa**	1084247.9	**Ab**	NA	**Ba**	NA	**Bd**	3538294.6	**Aa**
PC 34:3	82485.8	**Ba**	291934.0	**ABb**	541443.6	**Ac**	37257.1	**Ba**	593756.0	**Aa**	557279.6	**Ac**	93755.6	**Ba**	770817.3	**Aa**	909184.2	**Ab**	195868.6	**Ba**	314650.5	**Bb**	2545890.8	**Aa**
PC 34:4	29341.2	**Aa**	85856.5	**Aa**	169904.1	**Abc**	9948.5	**Aa**	155988.2	**Aa**	146041.2	**Ac**	18912.9	**Ba**	255637.0	**Aa**	317593.7	**Ab**	27998.6	**Ba**	87923.5	**Ba**	1208301.6	**Aa**
PC 34:5	24717.5	**Aa**	97043.3	**Aa**	196801.4	**Ab**	11233.2	**Aa**	239175.1	**Aa**	226131.0	**Ab**	27001.5	**Ba**	429539.0	**Aa**	561528.4	**Ab**	36249.9	**Ba**	127380.6	**Ba**	2233983.6	**Aa**
PC 34:6	15222.6	**Aa**	54997.4	**Ab**	141693.9	**Ac**	6358.7	**Aa**	199359.8	**Aab**	188267.0	**Ac**	17584.2	**Ba**	472132.3	**Aa**	648875.8	**Ab**	23189.1	**Ba**	79421.4	**Bb**	2291920.3	**Aa**
PC 36:1	80192.1	**Bb**	146889.1	**Bc**	358845.2	**Aa**	131715.1	**Bb**	389400.1	**Ab**	474540.2	**Aa**	197373.0	**Bb**	380715.8	**Ab**	409848.9	**Aa**	347987.8	**Ba**	821213.8	**Aa**	NA	**Cb**
PC 36:2	1401149.1	**Cb**	3370475.1	**Bc**	8324610.6	**Aab**	1716879.1	**Bb**	5812624.4	**Ab**	6229927.3	**Ac**	2480633.0	**Bab**	7385764.8	**Ab**	7575685.5	**Abc**	4266669.0	**Ba**	10430358.2	**Aa**	10069783.7	**Aa**

SI Table 10. *(continuation)* Relative abundance of lipid of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
PC 36:3	25877.6	Ba	72841.1	ABbc	157965.1	Ab	23538.0	Ba	207662.1	Aa	181382.3	Ab	52590.4	Aa	NA	Ac	NA	Ac	113872.1	Ba	173510.3	Bab	928788.1	Aa
PC 36:3.1	43370.7	Bb	122330.5	Bc	372407.3	Aa	61257.0	Bb	253183.7	Ab	288757.4	Aa	123973.9	Bab	325107.4	Aab	304198.6	Aa	222635.2	Ba	376623.8	Aa	361170.7	Aa
PC 36:5	51362.8	Aa	219073.8	Aa	499061.3	Ab	31638.0	Aa	835136.6	Aa	741234.5	Ab	101524.8	Ba	1069431.2	Aa	1256235.9	Ab	198418.8	Ba	481527.8	Ba	6422661.9	Aa
PC 36:6	23582.4	Aa	99688.7	Aa	252022.7	Ab	14776.6	Aa	530592.8	Aa	485643.7	Ab	46726.1	Ba	621266.7	Aa	807406.3	Ab	80299.0	Ba	224059.2	Ba	3817578.1	Aa
PC 38:2	20625.0	Bb	37695.5	Bb	82432.4	Ab	25810.5	Bb	110299.9	Aa	113446.5	Aab	39996.6	Bab	110599.0	Aa	113917.6	Aab	68789.6	Ba	125409.5	Aa	143545.1	Aa
PC 38:3	2699.8	Ba	6644.8	ABb	8428.2	Ac	NA	Ba	14986.6	Aa	15626.3	Ab	2134.9	Ba	18197.8	Aa	19608.4	Ab	1886.6	Ba	4644.9	Bb	39221.3	Aa
PC 38:3.1	2403.0	Ba	2233.8	Bb	4244.2	Aa	2682.0	Ba	4572.1	Aa	4365.8	Aa	2836.0	Aa	3893.7	Aa	4031.5	Aa	2631.1	Aa	3789.5	Aa	3472.1	Aa
PE 32:1	7470.8	Ab	11935.9	Ab	24067.9	Ab	9015.5	Ab	47281.0	Ab	59728.7	Ab	18046.4	Bb	71044.4	ABb	97947.6	Aab	202481.8	Ba	303022.9	Aa	152956.0	Ba
PE 34:1	21360.2	Bb	38646.7	ABc	75215.2	Ac	27237.1	Bb	168633.3	Ab	197059.3	Aab	30127.8	Cb	170052.4	Bb	241643.9	Aa	134791.4	Ba	238227.1	Aa	173104.3	Bb
PE 34:2	51247.4	Bb	92243.8	ABc	213167.3	Ac	61869.4	Bb	422093.3	Ab	471074.7	Ab	85345.5	Cb	534832.4	Bab	717403.4	Aa	298027.4	Ba	664391.6	Aa	740050.1	Aa
PE 34:3	3830.3	Aa	6174.5	Ab	9326.9	Ab	3081.4	Ba	19330.0	Aab	24973.6	Ab	4466.6	Ca	32742.6	Ba	68911.9	Aa	8175.6	Ba	16206.0	Bab	86024.1	Aa
PE 34:4	NA	Aa	NA	Ab	NA	Ac	NA	Ba	7318.1	ABab	9209.6	Ac	NA	Ca	12491.9	Ba	23870.0	Ab	1020.8	Ba	4004.6	Bab	45540.4	Aa
PE 34:5	2226.3	Aa	1918.4	Aa	3661.4	Ac	NA	Aa	8064.1	Aa	10955.7	Ac	NA	Ca	18247.5	Ba	40321.2	Ab	NA	Ba	4574.3	Ba	64812.2	Aa
PE 34:6	NA	Aa	1225.5	Aa	2324.4	Ac	NA	Aa	6498.1	Aa	8610.7	Ac	NA	Ca	13478.4	Ba	30638.4	Ab	NA	Ba	3148.4	Ba	45709.7	Aa
PE 36:1	16746.7	Ba	24175.7	Bc	50959.9	Ac	12863.6	Ca	150380.8	Ba	176917.5	Aa	15592.6	Ca	138087.4	Ba	170015.4	Aa	16913.1	Ba	66975.3	Ab	82891.7	Ab
PE 36:2	236689.3	Ba	461360.0	Bc	1169401.5	Ac	235501.8	Ba	2823482.6	Aa	3081569.0	Aa	266726.3	Ca	2425396.8	Ba	2948359.0	Aa	300026.2	Ba	1522756.4	Ab	1861602.1	Ab
PE 36:3	NA	Ba	14867.4	ABc	30875.6	Ac	NA	Ba	138830.1	Aa	158785.8	Aa	NA	Ba	96973.3	Ab	107326.4	Ab	NA	Ba	33480.2	Ac	52714.6	Ac
PE 36:3.1	3067.6	Aa	4196.1	Ab	8741.0	Ac	4200.4	Ba	20317.1	Aa	22950.9	Ab	5676.9	Aa	NA	Ab	NA	Ac	9779.3	Ca	24296.8	Ba	83346.0	Aa
PE 36:4	NA	Ab	NA	Ac	NA	Ac	NA	Cb	9902.7	Bb	14129.5	Ab	2526.7	Cb	20394.1	Ba	27268.8	Aa	9060.5	Ca	19267.5	Aa	14930.5	Bb
PE 36:4.1	5803.8	Aa	8789.6	Aa	15861.0	Ac	6024.3	Aa	40419.9	Aa	47343.0	Ac	9249.8	Ba	58117.8	Aa	100382.7	Ab	17177.8	Ba	44746.6	Ba	252904.7	Aa
PE 36:5	3628.9	Aa	6417.2	Aa	15081.0	Ac	2482.5	Aa	39381.0	Aa	52803.1	Ac	5385.0	Ca	88436.1	Ba	172868.5	Ab	9241.6	Ba	34758.3	Ba	329735.6	Aa
PE 36:6	1921.6	Aa	3388.0	Ab	5011.6	Ab	NA	Aa	14609.6	Aab	19480.2	Ab	1855.0	Ca	36576.7	Ba	79132.2	Aa	2722.9	Ba	8273.6	Bab	104639.1	Aa
PE 38:2	910803.1	Ba	1182793.3	Ba	1680584.5	Ab	643183.7	Ba	1192753.2	Aa	1407862.2	Ab	608242.8	Ba	1238870.2	Aa	1477011.1	Ab	770497.7	Ca	1237644.4	Ba	2381357.6	Aa
PE 38:3	NA	Aa	NA	Ab	NA	Ab	NA	Ba	25175.7	Aa	26823.8	Aa	NA	Ba	23813.6	Aa	27079.1	Aa	NA	Aa	NA	Ab	NA	Ab
PE 40:1	NA	Aa	NA	Ac	NA	Ac	NA	Ba	6189.2	Ab	7289.8	Ab	NA	Ba	5783.3	Ab	6468.4	Ab	NA	Ba	10067.6	Aa	9718.5	Aa
PE 40:2	21713.7	Bb	25713.9	Bc	91559.3	Ac	32953.3	Bb	95233.9	Ab	106472.3	Ac	52772.1	Bb	134237.5	Ab	157045.0	Ab	95417.0	Ba	236571.8	Aa	208318.4	Aa
PE 40:3	NA	Aa	NA	Aa	NA	Ac	NA	Ba	NA	Ba	22436.0	Ab	NA	Ba	NA	Ba	40682.6	Aa	NA	Aa	NA	Aa	NA	Ac

SI Table 10. *(continuation)* Relative abundance of lipid of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
PG 32:0	NA	Ab	NA	Ab	NA	Aa	5090.1	Aa	NA	Bb	NA	Ba	NA	Ab	NA	Ab	NA	Aa	NA	Bb	9121.0	Aa	NA	Ba
PG 32:1	2867.3	Ba	3001.5	Aa	2707.7	Ca	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab	NA	Ab
PG 34:2	85535.3	Aa	NA	Bb	NA	Ba	NA	Ac	NA	Ab	NA	Aa	NA	Ac	NA	Ab	NA	Aa	58847.3	Bb	111589.9	Aa	NA	Ca
PG 34:3	50052.1	Ca	62311.5	Ba	70683.9	Aa	NA	Ac	NA	Ab	NA	Ab	NA	Ac	NA	Ab	NA	Ab	9271.4	Ab	NA	Bb	NA	Bb
PG 34:4	NA	Ba	15797.3	Aab	18742.6	Ab	2996.5	Ba	27968.3	Aa	27551.8	Ab	NA	Ba	27042.7	Aa	NA	Bc	NA	Ba	5983.5	Bb	149104.2	Aa
SQDG 32:0	2995195.4	Aa	NA	Ba	NA	Ba	NA	Ab	NA	Aa	NA	Aa	NA	Ab	NA	Aa	NA	Aa	NA	Ab	NA	Aa	NA	Aa
SQDG 32:1	65488.4	Aa	63423.2	Aa	50606.4	Ba	8281.4	Ab	NA	Bb	NA	Bb	9439.6	Ab	NA	Bb	NA	Bb	NA	Ac	NA	Ab	NA	Ab
SQDG 32:2	65437.7	Aa	61396.1	Aa	59777.1	Aa	15385.9	Bb	30801.4	Ab	26476.8	Ab	11139.8	Bb	27780.0	Ab	30452.8	Ab	9445.1	Cb	19249.6	Bc	66630.0	Aa
SQDG 32:3	118427.7	Aa	93249.7	Ba	89874.4	Ba	24077.8	Bb	63235.8	Ab	60457.7	Ab	19269.3	Bb	56982.5	Ab	64799.3	Ab	18341.8	Bb	34734.5	Ac	NA	Cc
SQDG 34:1	1172130.9	Aa	1151463.3	Aa	1137111.3	Aa	NA	Bc	NA	Bb	849712.7	Ab	NA	Bc	NA	Bb	780236.9	Ab	292974.7	Ab	NA	Bb	NA	Bc
SQDG 34:2	4241010.0	Aa	4169533.7	Aa	3235835.0	Ba	NA	Bb	2096655.0	Ab	2050479.4	Ab	456335.6	Bb	1716626.7	Ab	NA	Bc	NA	Ab	NA	Ac	NA	Ac
SQDG 34:3	2974943.8	Aa	3161032.5	Aa	2610914.2	Aa	902186.9	Bb	2628065.8	Aa	2501841.1	Aa	793993.4	Ab	NA	Bc	NA	Bb	1255529.0	Ab	1146589.3	Ab	NA	Bb
SQDG 34:4	56220.8	Aa	55471.0	ABa	50147.5	Ba	5983.1	Bb	25703.9	Ab	23630.7	Ab	NA	Bb	24315.0	Ab	25341.5	Ab	2923.6	Cb	15375.9	Bc	52720.8	Aa
SQDG 34:5	55892.7	Aa	61265.3	Aa	53200.3	Ab	8071.4	Bb	40925.6	Ab	40840.6	Ac	6114.6	Bb	38126.4	Ab	42109.0	Abc	4173.8	Cb	17387.6	Bc	83111.9	Aa
SQDG 34:6	50917.1	Aa	53842.4	Aa	50454.0	Aa	6935.3	Bb	44061.0	Aab	41319.7	Ab	5410.4	Bb	41228.0	Ab	45472.2	Ab	4342.6	Cb	15686.5	Bc	99001.3	Ab
TAG 42:0	25359.3	Aa	30605.8	Aa	27302.0	Aa	25046.3	Aa	19764.9	Abc	18638.1	Aab	26072.4	Aa	13125.2	Bc	14611.0	Bb	26206.1	Aa	25564.7	Aab	12028.6	Bb
TAG 42:1	27773.6	Aa	29675.9	Aa	31983.9	Aa	30262.8	Aa	19267.7	Bbc	16224.3	Bb	30215.6	Aa	12568.6	Bc	14405.8	Bb	28601.8	Aa	27016.5	Aab	10448.9	Bb
TAG 42:2	5217.0	Ab	6051.0	Aa	6374.8	Aa	6129.5	Ab	3673.0	Bb	3749.6	Bb	7051.0	Ab	2569.5	Bb	2494.9	Bb	10147.9	Aa	6265.2	Ba	2578.0	Cb
TAG 44:0	82549.3	Aa	85638.6	Aa	94336.1	Aa	72358.7	Aa	63094.0	Aab	54495.8	Ab	82015.9	Aa	40468.4	Bb	42543.6	Bb	86374.7	Aa	71797.3	Aa	37927.4	Bb
TAG 44:1	56861.9	Ac	63332.9	Aa	68181.9	Aa	68989.7	Abc	41357.8	Bbc	36858.4	Bb	87146.3	Ab	29380.1	Bc	30553.4	Bb	148717.0	Aa	56964.0	Bab	38568.1	Cb
TAG 44:2	39822.1	Aab	42233.5	Aa	35608.1	Aa	39077.6	Ab	21913.5	Bbc	20970.4	Bb	38215.6	Ab	14803.3	Bc	17093.3	Bb	51953.2	Aa	28362.1	Bb	13019.2	Cb
TAG 46:0	289613.3	Aa	299672.0	Aa	359624.1	Aa	277178.9	Aa	250469.7	Aab	210064.4	Ab	307167.1	Aa	172085.8	Bb	188660.2	Bb	319796.0	Aa	286801.9	Aa	176730.7	Bb
TAG 46:1	386832.3	Ad	347648.4	Aa	404544.2	Aa	641517.4	Ac	345304.2	Ba	334559.9	Bab	789915.8	Ab	202220.3	Bb	234309.5	Bb	1322922.8	Aa	441264.0	Ba	81263.0	Cc
TAG 46:2	85543.5	Ac	73935.4	Aa	91084.4	Aa	110788.6	Abc	59670.9	Ba	59227.8	Bab	140396.2	Ab	NA	Cb	44330.2	Bb	273201.6	Aa	84800.1	Ba	NA	Cc
TAG 46:2.1	54246.2	Aa	53667.5	Aa	50192.9	Aa	40731.6	Aa	NA	Bb	NA	Bb	NA	Ab	NA	Ab	NA	Ab	NA	Bb	49346.8	Aa	NA	Bb
TAG 46:3	50615.2	Ac	39843.9	Aab	42992.4	Aa	93649.7	Ab	31591.5	Bb	30882.5	Bab	119225.4	Ab	16754.7	Bb	23406.0	Bab	271294.1	Aa	65858.9	Ba	3903.3	Cb
TAG 46:3.1	11428.0	Aab	11934.2	Aa	9146.5	Aa	NA	Bc	5127.2	Ab	4130.3	Ab	8142.5	Ab	3294.4	Bb	3588.1	Bb	12448.8	Aa	9507.9	Aa	3476.5	Bb
TAG 46:4	17355.2	Ac	11750.1	Aab	14501.5	Aa	26836.3	Ab	11921.9	Bab	13734.9	Ba	33105.1	Ab	7790.1	Bb	12058.8	Ba	71884.8	Aa	18696.6	Ba	NA	Cb
TAG 48:0	1384296.8	Ba	1401341.0	Ba	1859239.9	Aa	1301987.5	Aa	1104752.5	Aab	925226.2	Ab	1442323.0	Aa	725708.2	Bb	830905.8	Bb	1727880.6	Aa	1493943.8	Aa	639559.9	Bb

SI Table 10. *(continuation)* Relative abundance of lipid of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 48:1	1661599.2 **Ad**	1458606.6 **Aab**	2159684.2 **Aa**	2712376.8 **Ac**	1354152.3 **Bb**	1130951.4 **Bb**	3952472.2 **Ab**	830493.7 **Bb**	822372.5 **Bb**	8175172.0 **Aa**	2276707.7 **Ba**	567523.9 **Cb**
TAG 48:2	1803321.5 **Ad**	1476625.5 **Ab**	1737924.3 **Aa**	2829997.1 **Ac**	1230373.8 **Bb**	1056648.3 **Bab**	3836366.2 **Ab**	600156.3 **Bb**	727954.4 **Bb**	8419145.4 **Aa**	2636889.3 **Ba**	164389.7 **Cb**
TAG 48:3	2152427.2 **Ad**	1703743.3 **Ab**	2015374.4 **Aa**	3790933.3 **Ac**	1482467.5 **Bb**	1171647.2 **Bab**	5768578.7 **Ab**	623467.7 **Bb**	719372.5 **Bab**	14981131.7 **Aa**	3916047.4 **Ba**	51531.6 **Cb**
TAG 48:4	382142.4 **Ac**	306562.8 **Ab**	345653.3 **Aa**	681178.0 **Ab**	232217.8 **Bb**	200186.9 **Bab**	880711.2 **Ab**	80279.5 **Bb**	99866.3 **Bb**	2120964.5 **Aa**	760016.7 **Ba**	7447.9 **Cb**
TAG 48:5	347274.6 **Ac**	258511.2 **Ab**	291474.3 **Aa**	620469.8 **Ab**	215022.2 **Bb**	185219.0 **Bab**	881507.4 **Ab**	74887.0 **Bb**	97239.3 **Bab**	2249436.5 **Aa**	732307.4 **Ba**	8526.5 **Cb**
TAG 48:6	161563.6 **Ad**	111580.8 **Ab**	134170.2 **Aa**	358174.5 **Ac**	95499.6 **Bb**	82602.6 **Ba**	620509.7 **Ab**	32827.2 **Bb**	42616.3 **Ba**	1827725.0 **Aa**	477815.6 **Ba**	4430.5 **Ca**
TAG 48:7	44812.5 **Ac**	30053.3 **Ab**	34922.7 **Aa**	57472.8 **Abc**	20890.2 **Bb**	20415.0 **Bab**	77824.8 **Ab**	7842.3 **Bb**	10358.7 **Bab**	182949.7 **Aa**	95597.4 **Ba**	2111.8 **Cb**
TAG 48:8	19992.8 **Ad**	13720.7 **Ab**	17096.3 **Aa**	38951.7 **Ac**	15916.9 **Bb**	14934.4 **Ba**	55875.7 **Ab**	4521.4 **Bb**	6773.7 **Ba**	139725.2 **Aa**	51644.7 **Ba**	1364.6 **Ca**
TAG 48:9	4740.6 **Ac**	3199.0 **Ab**	4052.9 **Aab**	11486.1 **Ab**	6475.0 **Bab**	6410.3 **Ba**	14637.5 **Ab**	1539.2 **Bb**	2130.8 **Bab**	38173.8 **Aa**	11631.7 **Ba**	NA **Cb**
TAG 50:0	1816389.6 **Ba**	1795634.6 **Ba**	2596840.4 **Aa**	1699897.6 **Aa**	1606830.4 **Aa**	1399604.6 **Ab**	1753172.3 **Aa**	1144612.7 **Aa**	1295231.3 **Ab**	1738835.5 **Aa**	1475605.2 **Aa**	1179819.7 **Ab**
TAG 50:1	34193672.0 **Ad**	32015956.6 **Ab**	36497778.9 **Aa**	65706794.3 **Ac**	25188616.4 **Bbc**	20071784.1 **Bb**	90306132.4 **Ab**	11623535.5 **Bc**	12709845.0 **Bbc**	135258887.8 **Aa**	59149935.3 **Ba**	2536060.7 **Cc**
TAG 50:2	11109850.2 **Ad**	9554659.2 **Ab**	11635714.5 **Aa**	17256316.6 **Ac**	7434785.5 **Bbc**	6237063.8 **Bab**	26689252.2 **Ab**	3357905.6 **Bc**	3919674.0 **Bb**	54770461.3 **Aa**	19006206.3 **Ba**	1159019.8 **Cb**
TAG 50:3	8810658.4 **Ac**	7457007.3 **Ab**	8543942.3 **Aa**	17775298.4 **Ab**	6800774.6 **Bb**	6026498.0 **Ba**	23094386.4 **Ab**	2876538.9 **Bb**	3534872.5 **Bab**	40602222.1 **Aa**	18581199.4 **Ba**	187490.7 **Cb**
TAG 50:4	8178632.9 **Ad**	6328727.8 **Ab**	7169289.6 **Aa**	19798879.9 **Ac**	5733905.1 **Bb**	4754430.9 **Bab**	30296977.2 **Ab**	1996232.1 **Bb**	2670821.3 **Bab**	64306888.7 **Aa**	20985032.6 **Ba**	90641.1 **Cb**
TAG 50:5	6031626.8 **Ad**	4722250.5 **Ab**	5245273.9 **Aa**	13321850.1 **Ac**	2757622.6 **Bb**	2285962.0 **Bab**	19028032.1 **Ab**	934827.5 **Bb**	1232481.6 **Bab**	42234021.5 **Aa**	12001353.4 **Ba**	69908.1 **Cb**
TAG 50:6	13047.5 **Ac**	10733.8 **Aab**	9192.8 **Aa**	39571.9 **Ab**	6371.8 **Bab**	2851.2 **Ba**	41871.9 **Ab**	NA **Bb**	1875.9 **Ba**	143194.6 **Aa**	24918.1 **Ba**	NA **Ca**
TAG 50:6.1	4339596.7 **Ad**	3284941.7 **Ab**	3750182.8 **Aa**	12943799.7 **Ac**	2059147.4 **Bb**	1663754.0 **Ba**	20994223.9 **Ab**	731959.4 **Bb**	982729.6 **Ba**	47504249.6 **Aa**	10112401.2 **Ba**	59928.5 **Ca**
TAG 50:7	707272.6 **Ad**	526610.4 **Ab**	569378.8 **Aa**	1557457.5 **Ac**	318338.9 **Bb**	274588.0 **Ba**	2496217.2 **Ab**	108508.0 **Bb**	153388.0 **Ba**	6916455.5 **Aa**	1931041.7 **Ba**	23774.1 **Ca**
TAG 50:8	355370.2 **Ad**	246157.6 **Ab**	283655.8 **Aa**	970499.0 **Ac**	229111.1 **Bb**	206571.0 **Ba**	1446784.9 **Ab**	73906.9 **Bb**	111421.1 **Ba**	4236259.7 **Aa**	1130192.3 **Ba**	22923.9 **Ca**
TAG 50:9	135415.7 **Ad**	94229.1 **Ab**	109636.9 **Aa**	601771.1 **Ac**	124983.4 **Bb**	109002.9 **Ba**	987136.9 **Ab**	37788.1 **Bb**	56734.4 **Ba**	3090286.9 **Aa**	603695.6 **Ba**	15821.0 **Ca**
TAG 52:0	1337600.0 **Aa**	1393024.7 **Aa**	2063626.0 **Aa**	1413515.3 **Aab**	1419640.1 **Aab**	1073061.6 **Aab**	1370202.4 **Ab**	972916.4 **Ab**	1064937.6 **Ab**	1490583.4 **Aab**	1206873.5 **Aab**	1096909.5 **Aab**
TAG 52:1	19804556.1 **Ad**	17619929.8 **Aab**	22033559.9 **Aa**	38365996.7 **Ac**	17630531.6 **Bab**	15503692.2 **Bab**	50788432.8 **Ab**	10642793.5 **Bb**	11413381.1 **Bbc**	76875298.0 **Aa**	20531882.5 **Ba**	3222720.9 **Cc**
TAG 52:2	59467416.6 **Ac**	56494822.6 **Ab**	67857532.1 **Aa**	131165925.2 **Ab**	57976750.3 **Bb**	49067294.8 **Bab**	157036067.4 **Ab**	28887124.4 **Bb**	34221198.9 **Bbc**	198250176.9 **Aa**	117986567.4 **Ba**	5762468.1 **Cc**
TAG 52:3	31674098.9 **Ad**	27234163.2 **Ab**	32005155.5 **Aa**	64753840.9 **Ac**	24198332.8 **Bb**	20906912.1 **Bab**	91941755.7 **Ab**	10003966.4 **Bb**	12520009.8 **Bab**	150723738.1 **Aa**	68558960.3 **Ba**	1562897.6 **Cb**
TAG 52:4	23382526.0 **Ad**	19111210.0 **Ab**	21743741.3 **Aa**	53787424.6 **Ac**	17704178.7 **Bb**	14623735.6 **Bab**	76119264.0 **Ab**	5448110.8 **Bb**	7028229.1 **Bab**	140798593.4 **Aa**	56625081.0 **Ba**	945762.8 **Cb**
TAG 52:5	9178774.5 **Ad**	7254184.8 **Ab**	8061667.0 **Aa**	28220510.3 **Ac**	7645852.0 **Bb**	6453968.6 **Ba**	40500026.9 **Ab**	2650364.5 **Bb**	3545811.0 **Ba**	74650946.0 **Aa**	24649343.8 **Ba**	NA **Ca**
TAG 52:5.1	11534.0 **Ac**	11545.0 **Ab**	10070.6 **Aa**	30178.0 **Abc**	9896.1 **Ab**	8004.8 **Aa**	45409.4 **Ab**	3069.1 **Bb**	NA **Ba**	206341.2 **Aa**	56796.8 **Ba**	2145.5 **Ca**
TAG 52:6	6535523.7 **Ad**	5126840.0 **Ab**	5811218.7 **Aa**	23615822.2 **Ac**	4397033.3 **Bb**	3613385.5 **Ba**	34199125.5 **Ab**	1227388.8 **Bb**	1729493.3 **Ba**	68714333.9 **Aa**	21124266.0 **Ba**	76857.6 **Ca**
TAG 52:6.1	7233.2 **Aa**	5431.0 **Aa**	5584.4 **Aa**	NA **Aa**	7294.9 **Aa**	3990.6 **Aa**	NA **Aa**	2392.7 **Aa**	NA **Aa**	62192.3 **Aa**	6778.1 **Aa**	1681.3 **Aa**

SI Table 10. *(continuation)* Relative abundance of lipid of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 52:7	3839460.7 **Ad**	2848355.1 **Ab**	3342500.9 **Aa**	20492738.2 **Ac**	2580748.4 **Bb**	2076333.5 **Ba**	32055071.5 **Ab**	697431.2 **Bb**	978012.2 **Ba**	67468313.4 **Aa**	16417138.1 **Ba**	81343.9 **Ca**
TAG 52:8	1398039.8 **Ad**	935999.8 **Ab**	1074870.8 **Aa**	3534891.1 **Ac**	718824.0 **Bb**	667552.9 **Ba**	4928888.1 **Ab**	192155.5 **Bb**	294422.1 **Ba**	13156559.7 **Aa**	3711877.1 **Ba**	71203.8 **Ca**
TAG 52:9	497450.5 **Ac**	284869.4 **Ab**	349156.9 **Aa**	1832879.9 **Ab**	350693.3 **Bb**	313655.4 **Ba**	2387628.9 **Ab**	99766.6 **Bb**	150382.5 **Ba**	6855868.4 **Aa**	1489099.1 **Ba**	45223.0 **Ca**
TAG 54:0	503441.9 **Ba**	558862.3 **Ba**	847596.6 **Aa**	493716.4 **Aa**	532073.1 **Aa**	423215.0 **Ab**	524332.3 **Aa**	382027.3 **Aa**	422361.5 **Ab**	578843.3 **Aa**	468644.7 **Aa**	443243.3 **Ab**
TAG 54:1	3709227.8 **Ac**	3561555.2 **Aa**	5001958.1 **Aa**	8873826.7 **Ab**	5668852.7 **Ba**	4845678.4 **Ba**	11579459.0 **Ab**	3635071.8 **Ba**	3714112.4 **Bab**	15541297.2 **Aa**	3544617.1 **Ba**	1616719.3 **Bb**
TAG 54:2	17740917.9**Ad**	15997337.5**Aa**	20616523.9**Aa**	43190028.4 **Ac**	23542257.6**Ba**	22026415.7**Ba**	60771152.1 **Ab**	15740862.1**Ba**	18252550.0**Ba**	88296987.2 **Aa**	23630665.2 **Ba**	3083361.6 **Cb**
TAG 54:3	36299528.2**Ad**	33609822.7**Abc**	40846117.4**Aa**	107568204.4**Ac**	45806671.3**Bb**	38658341.8**Ba**	140635558.2**Ab**	20329440.8**Bc**	24385322.9**Bab**	180690721.4**Aa**	90122582.2 **Ba**	3464413.1 **Cb**
TAG 54:4	25315481.3**Ad**	22036881.8**Ab**	26942843.0**Aa**	69181307.6 **Ac**	25195600.7**Bb**	21055770.5**Ba**	96620856.7 **Ab**	9097661.5 **Bb**	11754056.2**Bab**	151509823.3**Aa**	61491036.1 **Ba**	1867451.7 **Cb**
TAG 54:5	19552413.4**Ad**	16258965.0**Ab**	20051611.5**Aa**	60287624.5 **Ac**	20591883.6**Bb**	16727907.8**Bab**	85761404.6 **Ab**	6059117.0 **Bb**	8206976.1 **Bab**	149210793.5**Aa**	57669884.4 **Ba**	1397476.3 **Cb**
TAG 54:6	7140935.8 **Ad**	5475851.2 **Ab**	6434756.4 **Aa**	16328794.1 **Ac**	5340279.5 **Bb**	4246027.7 **Ba**	24168695.2 **Ab**	1556368.9 **Bb**	2147908.6 **Ba**	52419794.2 **Aa**	19621532.7 **Ba**	811393.9 **Ca**
TAG 54:7	2433494.7 **Ad**	1713174.7 **Ab**	2012022.1 **Aa**	7184022.4 **Ac**	1739616.6 **Bb**	1347273.8 **Ba**	10172305.0 **Ab**	343550.5 **Bb**	532705.9 **Ba**	26807372.1 **Aa**	8028893.2 **Ba**	92076.1 **Ca**
TAG 54:8	592505.1 **Ac**	392241.5 **Ab**	453694.0 **Aa**	759122.0 **Abc**	331049.5 **Bb**	274887.1 **Ba**	1134024.8 **Ab**	95504.2 **Bb**	159518.7 **Ba**	3016191.2 **Aa**	1205998.9 **Ba**	36477.7 **Ca**
TAG 54:9	118175.1 **Ac**	73917.6 **Ab**	86068.8 **Aa**	144536.4 **Abc**	77853.9 **Bb**	66762.6 **Bab**	197431.7 **Ab**	23296.8 **Bb**	42463.7 **Bab**	526566.6 **Aa**	145616.4 **Ba**	10851.3 **Cb**
TAG 56:0	67360.0 **Ab**	71197.8 **Aa**	74652.5 **Aa**	66273.2 **Ab**	58587.5 **Aab**	52219.6 **Ab**	84256.5 **Aab**	41189.9 **Bb**	46755.1 **Bb**	94392.2 **Aa**	62279.8 **Ba**	40073.5 **Cb**
TAG 56:1	309815.7 **Ad**	291253.1 **Aa**	385675.9 **Aa**	1182046.1 **Ac**	402264.4 **Ba**	335853.9 **Bab**	1628979.4 **Ab**	228537.4 **Ba**	236898.4 **Bab**	2793894.4 **Aa**	477795.3 **Ba**	70904.5 **Cb**
TAG 56:2	873864.6 **Ad**	783591.0 **Aa**	1042353.9 **Aab**	3625558.0 **Ac**	1751367.4 **Ba**	1594907.2 **Ba**	5379910.5 **Ab**	1092329.2 **Ba**	1238837.3 **Ba**	8067711.0 **Aa**	1663510.5 **Ba**	156414.7 **Cb**
TAG 56:3	1391172.6 **Ad**	1183395.8 **Ac**	1463979.6 **Aab**	4523077.9 **Ac**	2874528.5 **Bab**	2569080.3 **Ba**	6685213.6 **Ab**	1662966.7 **Bbc**	2123116.2 **Ba**	10845990.8 **Aa**	3783450.7 **Ba**	170960.1 **Cb**
TAG 56:4	878878.1 **Ad**	705594.3 **Abc**	799128.1 **Aa**	1717015.9 **Ac**	1197497.7 **Bab**	1100487.2 **Ba**	2311050.3 **Ab**	632139.1 **Bc**	873433.1 **Ba**	3345887.9 **Aa**	1568264.0 **Ba**	109969.3 **Cb**
TAG 56:5	618553.4 **Ad**	467374.2 **Abc**	520210.9 **Aab**	1302588.5 **Ac**	910071.0 **ABab**	764644.9 **Ba**	1961910.6 **Ab**	353350.3 **Bc**	481847.0 **Bab**	3679256.9 **Aa**	1374297.5 **Ba**	45854.7 **Cb**
TAG 56:6	259884.3 **Ac**	175140.6 **Abc**	175286.0 **Aa**	298734.6 **Abc**	224076.5 **Ab**	187057.5 **Aa**	413045.6 **Ab**	86626.4 **Bc**	123046.5 **Bab**	676446.3 **Aa**	371125.7 **Ba**	16124.3 **Cb**
TAG 56:7	91637.4 **Ab**	52639.9 **Ab**	52641.2 **Aab**	100760.4 **Ab**	68451.6 **ABb**	54848.1 **Ba**	131052.4 **Ab**	24418.6 **Bb**	33810.6 **Bab**	271268.0 **Aa**	121216.2 **Ba**	4677.6 **Cb**
TAG 58:0	26435.4 **Ac**	26795.7 **Aa**	29041.8 **Aa**	27578.8 **Abc**	24092.9 **Aa**	22349.3 **Aab**	37410.6 **Aab**	19371.5 **Ba**	21607.4 **Bab**	39003.6 **Aa**	24251.2 **Ba**	15004.5 **Cb**
TAG 58:1	357189.5 **Ad**	307135.6 **Aab**	375174.2 **Aa**	1108181.9 **Ac**	346099.8 **Bab**	299174.5 **Ba**	1490783.4 **Ab**	184385.8 **Bb**	193721.1 **Ba**	3516646.5 **Aa**	659514.4 **Ba**	27610.0 **Ca**
TAG 58:2	343999.1 **Ad**	317627.5 **Aa**	420722.4 **Aa**	1283982.9 **Ac**	522976.7 **Ba**	456276.7 **Ba**	1849341.8 **Ab**	287091.8 **Ba**	325001.5 **Ba**	3271521.4 **Aa**	578708.0 **Ba**	59803.1 **Ca**
TAG 58:3	210152.4 **Ad**	186644.7 **Ab**	248231.5 **Aa**	500653.7 **Ac**	311903.5 **Bab**	273802.8 **Ba**	689968.8 **Ab**	189967.6 **Bb**	225817.7 **Ba**	1074998.8 **Aa**	334608.2 **Ba**	64503.2 **Cb**
TAG 58:4	136280.3 **Ad**	130615.9 **Aab**	181090.3 **Aa**	376502.4 **Ac**	180374.3 **Bab**	144503.6 **Bab**	539429.9 **Ab**	96383.6 **Bb**	111329.3 **Bab**	947752.2 **Aa**	231286.9 **Ba**	63420.3 **Cb**
TAG 58:5	105904.2 **Ac**	76316.2 **Aab**	91645.9 **Aa**	268736.1 **Ab**	69024.2 **Bb**	58990.4 **Bab**	279043.3 **Ab**	35236.0 **Bb**	39674.5 **Bab**	452003.6 **Aa**	134809.7 **Ba**	7184.3 **Cb**
TAG 58:6	98342.5 **Ad**	66582.2 **Aab**	74915.3 **Aa**	310268.1 **Ac**	57915.3 **Bb**	51436.1 **Ba**	413016.8 **Ab**	30584.4 **Bb**	35983.7 **Ba**	1013484.3 **Aa**	140067.2 **Ba**	7546.2 **Ca**
TAG 60:0	7894.4 **Aa**	8560.5 **Aa**	8325.9 **Aa**	6506.5 **Ab**	5842.6 **Ab**	5357.8 **Ab**	6690.7 **Ab**	5076.3 **Ab**	5787.9 **Ab**	7770.4 **Ab**	6211.5 **Ab**	4862.5 **Ab**

SI Table 10. *(continuation)* Relative abundance of lipid of *Chlorella vulgaris* BR017 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 60:1	202851.3 **Ad**	161486.2 **Aa**	191758.0 **Aab**	412696.8 **Ac**	271373.5 **Aa**	247301.7 **Aa**	720581.7 **Ab**	186962.4 **Ba**	186865.7 **Bab**	1586685.2 **Aa**	329233.1 **Ba**	15429.0 **Cb**
TAG 60:2	410578.4 **Ad**	351665.8 **Aa**	427648.7 **Aab**	1272916.5 **Ac**	619628.2 **Ba**	578031.7 **Ba**	1851553.8 **Ab**	392423.4 **Ba**	438557.5 **Bab**	3926753.0 **Aa**	737289.8 **Ba**	28847.3 **Cb**
TAG 60:3	216803.7 **Ad**	179147.1 **Aa**	238028.4 **Aa**	478530.7 **Ac**	290662.7 **Ba**	266063.2 **Ba**	709249.4 **Ab**	165358.6 **Ba**	194663.3 **Bab**	1696891.7 **Aa**	332536.1 **Ba**	21529.1 **Cb**
TAG 60:4	172174.2 **Ad**	137517.7 **Ab**	174123.1 **Aa**	427842.7 **Ac**	171950.3 **Bab**	140289.0 **Bab**	570890.6 **Ab**	77805.8 **Bb**	93469.4 **Bab**	1350795.0 **Aa**	297610.4 **Ba**	22728.7 **Cb**
TAG 60:5	64578.3 **Ac**	44805.6 **Aab**	50788.0 **Aa**	77631.2 **Ac**	48979.5 **Ba**	39056.4 **Bab**	106010.9 **Ab**	23774.1 **Bb**	25687.5 **Bbc**	237999.8 **Aa**	56344.6 **Ba**	2989.2 **Cc**
TAG 60:5.1	72992.7 **Ac**	54033.2 **Aab**	56174.2 **Aa**	94859.8 **Ac**	54741.5 **Bab**	47408.3 **Ba**	151847.5 **Ab**	39145.5 **Bb**	42390.8 **Ba**	313095.2 **Aa**	84532.1 **Ba**	3178.0 **Cb**
TAG 60:6	66286.5 **Ad**	45809.5 **Aab**	53328.8 **Aa**	110806.9 **Ac**	50297.2 **Bab**	44252.7 **Bab**	216644.3 **Ab**	35445.9 **Bb**	40925.4 **Bab**	489450.4 **Aa**	87198.3 **Ba**	3393.3 **Cb**
FA 16:0	20636253.7**Aa**	17710665.5**ABa**	16331792.9**Ba**	12948602.5 **Ab**	8382244.5 **Bc**	6721828.4 **Bb**	13351159.2 **Ab**	5806816.6 **Bc**	5310828.5 **Bb**	13516054.2 **Ab**	13004349.4 **Ab**	5253280.1 **Bb**
FA 16:1	4997056.4 **Aa**	3698916.1 **Ba**	3554346.4 **Ba**	1811323.1 **Abc**	1266254.9 **ABbc**	1008963.9 **Bb**	1958664.5 **Ab**	785024.8 **Bc**	721746.1 **Bbc**	1205918.1 **Ac**	1497609.9 **Ab**	278634.8 **Bc**
FA 16:2	21972712.7**Aa**	17388833.9**Ba**	15503999.7**Ba**	7822142.2 **Ab**	7899901.6 **Abc**	6909944.1 **Ab**	6989481.8 **Ab**	5562229.2 **Ac**	5774220.0 **Ab**	6201160.9 **Bb**	9815503.4 **Ab**	5338532.3 **Bb**
FA 16:3	16379564.9**Aa**	12883778.5**Ba**	11514779.0**Ba**	9273510.2 **Ab**	8140565.3 **Bb**	6840959.4 **Bb**	8707017.5 **Ab**	5200187.2 **Bb**	5328047.1 **Bb**	8053318.4 **Ab**	8556370.0 **Bb**	5958048.3 **Bb**
FA 16:4	247396.8 **ABa**	282887.1 **Aa**	220483.2 **Ba**	158390.0 **Ab**	57391.7 **Bb**	52079.9 **Bb**	112374.8 **Ab**	31243.6 **Bb**	29123.4 **Bb**	62706.5 **Ac**	48731.1 **Ab**	29639.2 **Ab**
FA 16:4.1	27832.3 **Aa**	24880.0 **Ba**	19257.0 **Ba**	12813.3 **Ab**	6187.9 **Bb**	6555.4 **Bb**	7262.0 **Ac**	3243.1 **Bc**	4103.6 **Bc**	6758.8 **Ac**	5197.4 **Bc**	NA **Bc**
FA 18:0	4401820.6 **Aa**	4245789.5 **Aa**	3951164.7 **Aa**	3747288.4 **Aa**	3429269.0 **ABab**	2755200.5 **Bb**	3776147.4 **Aa**	2759415.2 **Bb**	2431093.9 **Bb**	3531775.1 **Aa**	2651764.4 **Bb**	831270.2 **Cc**
FA 18:1	56648140.8**Aa**	49556840.9**Aa**	35986808.3**Ba**	34434277.2 **Ab**	25063398.8**ABc**	20238401.4**Bb**	40335716.2 **Ab**	15386562.7**Bc**	14672220.6**Bbc**	42861977.9 **Ab**	37389010.7 **Ab**	6113248.3 **Bc**
FA 18:2	50829359.0**Aa**	48379398.7**ABa**	42392638.5**Ba**	22650486.9 **Ab**	23199884.9**Ac**	17563775.2**Ab**	24471377.8 **Ab**	15515773.9**Bc**	15878104.5**Bb**	23968424.4 **Bb**	31486900.9 **Ab**	18821488.3**Bb**
FA 18:3	61653932.3**Aa**	55121928.7**Ba**	50196068.1**Ba**	34345074.7 **Abc**	32387728.4**Bbc**	27389789.5**Bbc**	36828949.4 **Ac**	20710261.4**Bc**	21259256.0**Bc**	36780502.6 **Ab**	35820810.6 **Bb**	30387627.3**Bb**
FA 18:4	NA **Bb**	NA **Bc**	35649.7 **Aa**	16869.2 **Aa**	17945.4 **Aa**	NA **Bd**	17071.4 **Aa**	10402.8 **Bb**	10224.9 **Bc**	13817.8 **Aa**	14324.2 **Aa**	14389.9 **Ab**
FA 20:0	181813.5 **Aa**	186791.2 **Aa**	148994.7 **Aa**	153721.3 **Aa**	172745.6 **Aa**	129523.3 **Aa**	194524.3 **Aa**	122755.4 **Bb**	121329.3 **Ba**	170410.0 **Aa**	143973.2 **Aab**	41940.1 **Bb**
FA 20:1	1428134.9 **Aa**	1334376.8 **Aa**	718297.8 **Ba**	654563.1 **Ab**	539480.5 **ABb**	422904.1 **Bb**	495526.5 **Abc**	NA **Bc**	NA **Bc**	382013.4 **Ac**	NA **Bc**	NA **Bc**
FA 20:3	168218.6 **Aa**	138979.3 **Ba**	129785.3 **Ba**	49422.2 **Bb**	79564.3 **Ab**	69789.3 **ABb**	48801.4 **Ab**	52073.4 **Ac**	52701.7 **Ab**	42500.8 **Bb**	80945.4 **Ab**	68234.7 **Ab**
FA 22:0	82678.8 **Aa**	87288.6 **Aa**	61311.3 **Ba**	69048.6 **Aa**	69986.6 **Aab**	59642.6 **Aa**	70488.3 **Aa**	57749.8 **ABbc**	50111.0 **Ba**	63649.2 **Aa**	42999.6 **Bc**	23558.5 **Cb**
FA 22:1	127831.5 **Ba**	191412.3 **Aa**	113547.7 **Ba**	74512.0 **Ab**	66947.6 **Ab**	40681.1 **Bb**	44903.6 **Ac**	35032.7 **Ac**	32225.9 **Ab**	40743.8 **Ac**	27569.6 **Ac**	30012.5 **Ab**
FA 24:1	10213.5 **Aa**	10274.2 **Aa**	8986.5 **Aa**	5238.9 **Ab**	4143.2 **Ab**	3455.8 **Ab**	3112.1 **Ac**	2988.8 **Ab**	3422.3 **Ab**	2574.3 **Bc**	3092.5 **ABb**	4747.7 **Ab**
FA 26:0	583326.0 **Aa**	575573.3 **Aa**	335104.8 **Ba**	162204.7 **Bbc**	246521.6 **Ab**	206853.9 **ABb**	177548.2 **Bb**	252259.2 **Ab**	204429.1 **ABb**	104692.4 **Bc**	203389.5 **Ab**	125198.2 **Bc**
FA 28:0	225265.9 **Aa**	221953.2 **Aa**	65048.3 **Bab**	145813.1 **Ab**	107807.4 **Ab**	108706.7 **Aa**	72478.6 **Ac**	82300.3 **Ab**	NA **Bc**	123924.4 **Abc**	70509.2 **ABb**	31791.2 **Bbc**
FA 30:0	65153.7 **Aa**	63129.7 **Aa**	8771.9 **Bab**	58614.7 **Aa**	31547.4 **Bb**	34560.7 **Ba**	21597.4 **Ab**	28995.1 **Ab**	8578.6 **Ab**	46257.9 **Aab**	12394.9 **Bb**	3590.1 **Bb**
FA 30:1	4439.0 **Aa**	4389.5 **Aa**	5223.3 **Aa**	2498.9 **Ab**	2837.9 **Ab**	1981.9 **Ab**	3230.8 **Ab**	1744.4 **Bb**	1449.3 **Bb**	2823.5 **Ab**	2085.6 **ABb**	1351.5 **Bb**

SI Table 11. Relative abundance of lipid of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
DAG 34:1	NA	Bd	24344.3	Aa	25191.6	Aa	18790.9	Bc	18880.7	Bab	28273.9	Aa	39270.7	Ab	14309.5	Bb	13355.7	Bb	69659.8	Aa	NA	Bc	2419.8	Bc
DAG 34:2	19734.9	Bc	20103.1	Bb	26947.6	Aa	17450.4	ABc	14704.0	Bbc	22398.8	Aa	39019.8	Ab	11300.6	Bc	12938.5	Bb	70460.1	Aa	35432.5	Ba	5379.2	Cc
DAG 34:3	39741.5	Bc	39612.8	Bab	55915.5	Aa	24106.5	Bc	23997.2	Bbc	41271.7	Aa	73330.2	Ab	16711.6	Bc	19991.3	Bb	118279.7	Aa	55784.8	Ba	5476.7	Cb
DAG 34:4	45446.9	Bc	47125.0	Bab	69652.0	Aa	33044.3	Ac	26474.1	Abc	43193.1	Ab	76515.1	Ab	20216.7	Bc	22317.8	Bc	132022.3	Aa	63778.1	Ba	4948.3	Cc
DAG 34:5	51926.2	Ac	53663.4	Ab	83677.5	Aa	48552.4	Ac	45830.2	Ab	76760.6	Aab	129351.1	Ab	37925.7	Bb	41583.3	Bbc	194072.5	Aa	103033.6	Ba	14373.9	Cc
DAG 34:6	36686.8	Ac	37167.3	Ab	53100.1	Aab	46803.2	Ac	39062.1	Ab	67495.2	Aa	179756.4	Ab	35988.5	Bb	38653.1	Bab	261614.1	Aa	99807.2	Ba	20818.5	Cb
DAG 36:1	710.3	Ad	1134.7	Aab	926.8	Aab	1879.7	Ac	766.7	Bb	1357.6	ABa	3066.9	Ab	898.8	Bb	1111.9	Ba	7055.2	Aa	1923.7	Ba	NA	Cb
DAG 36:2	54547.3	Ac	48589.7	Ab	52798.3	Aa	82043.4	Ac	51738.1	Ab	75481.3	Aa	190687.6	Ab	28925.0	Bb	45497.4	Ba	323815.7	Aa	187122.9	Ba	5870.7	Cb
DAG 36:3	60497.3	Ac	56497.8	Ab	73323.0	Aa	75674.4	Ac	52909.4	Ab	82863.7	Aa	266580.3	Ab	28229.6	Bb	37920.9	Bab	328324.4	Aa	256583.1	Ba	6597.6	Cb
DAG 36:4	NA	Ad	NA	Ab	NA	Ab	60373.6	Ac	NA	Bb	57202.5	Aa	180559.8	Ab	22665.8	Bb	22386.2	Bb	310806.1	Aa	175609.3	Ba	NA	Cb
DAG 36:5	38156.8	Ac	44738.8	Aab	51566.2	Aab	41613.5	Ac	40622.0	Ab	58504.7	Aa	92251.8	Ab	26154.1	Bb	30565.0	Bbc	156327.4	Aa	70641.3	Ba	15740.2	Cc
DAG 36:6	15571.3	Ac	14448.5	Ab	24014.0	Aab	18424.0	Bc	22323.8	ABab	37597.4	Aa	53021.4	Ab	17233.3	Bb	21050.6	Bab	92128.4	Aa	35097.4	Ba	8961.5	Cb
DGDG 32:0	112482.2	Aa	111202.4	Aa	113578.7	Aa	92547.1	Ab	64544.7	Bc	65083.9	Bb	94891.9	Ab	67322.3	Bc	62214.1	Bb	113930.2	Aa	89025.0	Bb	70710.3	Cb
DGDG 32:1	24193.9	Ba	25179.2	Aa	26657.3	Aa	9842.5	Bb	19778.9	Ab	19705.8	Ab	14281.4	Bb	18340.5	Ab	18719.4	Ab	13650.9	Bb	17165.4	Ab	20488.4	Ab
DGDG 32:2	46921.2	Aa	49656.2	Aa	55042.2	Aab	15517.3	Bb	50610.0	Aa	46747.8	Abc	23412.0	Bb	35219.6	Ab	38735.4	Ac	19157.9	Cb	31851.0	Bb	62504.2	Aa
DGDG 32:3	60145.8	Aa	NA	Bb	84908.2	Ab	20929.2	Bb	NA	Bb	98094.4	Ab	39491.7	Bab	NA	Cb	78902.7	Ab	30323.9	Bab	53169.9	Ba	182683.1	Aa
DGDG 32:3.1	16484.0	Aab	19348.8	Ab	21731.7	Ac	9728.6	Bb	37568.6	Aa	41091.9	Ab	20915.3	Bab	32491.0	ABab	36861.0	Ab	27539.7	Ba	29701.0	Bab	61640.4	Aa
DGDG 34:0	114285.2	Aa	113700.1	Aa	129243.0	Aa	67344.3	Bb	81207.6	ABb	97304.2	Ab	64687.0	Bb	97001.2	Aab	102761.8	Ab	69499.7	Bb	54615.0	Bc	89587.7	Ab
DGDG 34:1	1727995.6	Ba	1735292.3	Aa	1983658.0	Aa	895842.1	Bb	1697280.3	Ab	1823910.0	Ab	1251740.8	Bab	1730526.5	Aab	1809117.4	Aab	1196551.3	Bb	1680544.7	Ab	1510060.9	Ab
DGDG 34:2	1757000.9	Aa	1815543.4	Aa	2065630.2	Aa	499717.3	Bb	1887310.5	Aa	1945742.3	Aa	861006.4	Bb	1656932.3	Aab	1778127.2	Aa	680822.5	Bb	1366042.5	Ab	1729209.7	Aa
DGDG 34:3	640471.9	Aab	755701.5	Ac	830144.4	Ab	392085.6	Bb	1826814.3	Aa	1920851.3	Aa	773462.2	Ba	1602682.7	Aab	1765520.2	Aa	820021.9	Ca	1254786.6	Bb	1952225.6	Aa
DGDG 34:4	1078677.9	Ba	1315267.6	ABa	1489562.7	Aa	177106.8	Bb	1553853.6	Aa	1690312.6	Aa	170190.9	Bb	1308905.6	Aa	1537520.3	Aa	109583.6	Cb	869101.9	Bb	1498860.9	Aa
DGDG 34:5	1283971.6	Aa	1445721.4	Ac	1768604.1	Ab	571595.4	Bb	2928173.1	Aa	3086039.8	Aa	916285.8	Bab	2548202.6	Aab	2885313.3	Aa	543897.9	Cb	2103782.7	Bb	3303541.4	Aa
DGDG 34:6	1386282.2	Aab	1546367.7	Ab	1942844.7	Ac	719919.9	Bb	3317940.8	Aa	3746241.1	Aab	1616634.7	Ba	3115804.1	Aa	3482189.6	Ab	1198690.5	Cab	2800703.9	Ba	4378153.4	Aa
DGDG 36:2	35233.0	Ba	48960.1	Aa	46630.3	ABab	10400.8	Bb	43499.6	Aa	48848.1	Aab	14874.0	Cb	46428.8	Ba	58355.9	Aa	12427.6	Bb	20885.8	Bb	37345.0	Ab
DGDG 36:3	62222.5	Ba	67981.6	ABab	82226.3	Aa	8633.8	Bb	71608.8	Aa	70571.9	Aab	13638.1	Bb	49382.8	Abc	50453.7	Ab	9375.6	Bb	30200.3	Ac	NA	Bc
DGDG 36:4	87998.9	Aa	108915.2	Aa	112024.8	Ab	NA	Bb	134863.2	Aa	136946.4	Ab	NA	Bb	111292.6	Aa	125930.2	Ab	NA	Cb	50463.0	Bb	257016.1	Aa
DGDG 36:5	137644.1	Aa	172689.3	Ab	195343.5	Ac	28311.3	Bb	430017.8	Aa	439344.3	Ab	42256.1	Bab	338254.6	Aa	386250.3	Ab	24392.6	Cb	158758.3	Bb	583108.1	Aa
DGDG 36:6	102468.2	Aa	134733.3	Ac	151540.9	Ac	45038.1	Ba	493136.7	Aa	496678.7	Ab	81100.6	Ca	423910.5	Ba	499043.9	Ab	56459.2	Ca	217871.3	Bb	998698.8	Aa

SI Table 11. *(continuation)* Relative abundance of lipid of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
LysoPC 16:0	56023.0	**Bbc**	76283.4	**ABa**	80968.9	**Aa**	53835.0	**Bc**	82157.2	**Aa**	89131.5	**Aa**	82427.6	**Ab**	97477.8	**Aa**	103355.7	**Aa**	129231.6	**Aa**	71970.3	**Ba**	105833.6	**Aa**
LysoPC 16:0.1	4727.9	**Ab**	6179.5	**Aab**	6066.2	**Ab**	4628.6	**Bb**	6612.2	**ABab**	7815.6	**Aab**	6646.7	**Bb**	8038.2	**ABa**	8760.0	**Aa**	10468.9	**Ba**	5376.6	**Ab**	9120.3	**Aa**
LysoPC 16:2	13449.9	**Aa**	26449.0	**Ab**	33993.9	**Ad**	20343.6	**Ba**	103166.2	**Aa**	91813.8	**Ac**	35818.0	**Ba**	131894.4	**Aa**	152002.9	**Ab**	35782.0	**Ba**	52138.8	**Bb**	418833.6	**Aa**
LysoPC 16:3	6011.3	**Aa**	12567.7	**Ab**	18075.3	**Ac**	13528.9	**Ba**	80693.7	**Aab**	76899.9	**Ac**	30778.1	**Ba**	131789.3	**Aa**	165136.1	**Ab**	29493.5	**Ba**	60797.2	**Bb**	702339.9	**Aa**
LysoPC 18:2	38108.9	**Aa**	66112.5	**Ab**	80888.0	**Ac**	72848.6	**Ba**	594513.2	**Aa**	433210.5	**Ab**	116951.8	**Ba**	634484.8	**Aa**	603003.0	**Ab**	137231.8	**Ba**	295462.7	**Bb**	2334589.2	**Aa**
LysoPC 18:3	17791.3	**Aa**	28122.9	**Ab**	36567.2	**Ac**	29336.6	**Ba**	237753.7	**Aa**	217022.6	**Ab**	63616.8	**Ba**	292780.3	**Aa**	376297.3	**Ab**	88287.1	**Ba**	177424.1	**Bab**	1716247.8	**Aa**
MGDG 34:1	133410.2	**Ba**	182953.5	**Ab**	199979.0	**Ab**	34828.2	**Bb**	258734.0	**Aa**	293102.1	**Aa**	NA	**Cb**	158849.7	**Bb**	231177.9	**Ab**	NA	**Bb**	94240.7	**Ac**	106560.5	**Ac**
MGDG 34:2	84123.2	**Aa**	106305.3	**Aa**	122971.2	**Aa**	5996.8	**Bb**	124092.8	**Aa**	161907.7	**Aa**	8611.8	**Cb**	92406.2	**Ba**	157623.4	**Aa**	8154.6	**Bb**	23298.4	**Bb**	64839.0	**Ab**
MGDG 34:2.1	81492.1	**Ba**	98367.1	**ABb**	128838.5	**Ab**	16091.4	**Cb**	219813.3	**Ba**	268131.1	**Aa**	29885.3	**Cb**	115491.1	**Bb**	173585.3	**Ab**	21498.6	**Bb**	42117.9	**Bc**	171111.0	**Ab**
MGDG 34:3	2155.4	**Aa**	2692.7	**Ab**	3097.5	**Ab**	NA	**Cb**	5390.5	**Ba**	6757.3	**Aa**	NA	**Cb**	5226.9	**Ba**	7501.0	**Aa**	NA	**Bb**	NA	**Bc**	4070.8	**Ab**
MGDG 34:4	165640.7	**Aa**	257371.3	**Ab**	303319.7	**Ad**	13914.6	**Ba**	632711.4	**Aa**	791254.9	**Ac**	22275.9	**Ca**	611571.2	**Ba**	1075986.2	**Ab**	21038.4	**Ba**	150291.3	**Bb**	1611472.4	**Aa**
MGDG 34:5	133120.7	**Aa**	217463.9	**Ab**	262701.7	**Ad**	20718.8	**Ca**	865954.5	**Ba**	1073759.4	**Ac**	36275.2	**Ca**	891039.9	**Ba**	1332932.0	**Ab**	32951.7	**Ca**	256096.6	**Bb**	2415936.3	**Aa**
MGDG 34:6	NA	**Aa**	NA	**Aa**	NA	**Ac**	17000.7	**Aa**	NA	**Aa**	NA	**Ac**	NA	**Ba**	NA	**Ba**	1481964.8	**Ab**	22133.3	**Ba**	NA	**Ba**	3164081.7	**Aa**
MGDG 36:5	NA	**Aa**	NA	**Ab**	NA	**Ac**	NA	**Ba**	98558.1	**Aa**	124310.8	**Ab**	NA	**Ba**	NA	**Bb**	109975.0	**Ab**	NA	**Ba**	NA	**Bb**	303630.7	**Aa**
MGDG 36:6	13313.2	**Aa**	21746.9	**Aa**	26797.3	**Ac**	3294.8	**Ba**	70220.9	**Aa**	87120.8	**Abc**	4989.5	**Ba**	60209.2	**Ba**	126542.2	**Ab**	3283.9	**Ba**	12705.0	**Ba**	616385.0	**Aa**
PC 32:0	27884.4	**Ba**	33228.7	**Aa**	33373.0	**Aa**	18829.4	**Ac**	14986.6	**Ab**	18691.7	**Ab**	20125.1	**Abc**	13975.4	**Bb**	14926.6	**Bbc**	25597.2	**Aab**	14456.8	**Bb**	11727.0	**Bc**
PC 32:1	37019.2	**Cb**	48996.0	**Bb**	47631.5	**Ab**	21145.6	**Cc**	32765.9	**Bc**	42177.9	**Ac**	30623.9	**Cb**	42601.6	**Bb**	51938.7	**Ab**	43737.5	**Ca**	50077.8	**Ba**	70931.1	**Aa**
PC 32:1.1	19640.3	**Aa**	23350.5	**Ab**	26956.0	**Ac**	13257.0	**Ba**	29784.5	**Aab**	33757.7	**Ac**	17363.3	**Ba**	36786.8	**Aa**	45621.5	**Ab**	21942.9	**Ba**	25686.9	**Bab**	89648.7	**Aa**
PC 32:2	36162.2	**Aa**	60144.5	**Ab**	80527.4	**Ac**	28031.2	**Ba**	206907.0	**Aa**	203240.7	**Ab**	51279.5	**Ba**	223860.9	**Aa**	311713.2	**Ab**	86150.6	**Ba**	116767.7	**Bab**	862977.5	**Aa**
PC 32:3	28309.1	**Aa**	51098.5	**Aa**	70093.4	**Ab**	19171.1	**Aa**	173576.6	**Aa**	170847.2	**Ab**	34725.1	**Ba**	206818.0	**ABa**	304590.6	**Ab**	58467.7	**Ba**	127115.5	**Ba**	1400996.5	**Aa**
PC 32:4	1475.1	**Aa**	3321.2	**Aa**	3960.3	**Ac**	1137.4	**Aa**	7899.4	**Aa**	8493.5	**Abc**	1285.1	**Ba**	10750.2	**Aa**	14495.6	**Ab**	938.6	**Ba**	3578.3	**Ba**	77157.1	**Aa**
PC 34:1	157716.3	**Aa**	NA	**Ba**	NA	**Ba**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Aa**
PC 34:2	NA	**Aa**	NA	**Ac**	NA	**Ac**	NA	**Ba**	474158.4	**Ab**	NA	**Bc**	NA	**Ba**	731286.7	**Aa**	889451.0	**Ab**	NA	**Ba**	NA	**Bc**	2317233.4	**Aa**
PC 34:3	112093.4	**Aa**	152579.6	**Ab**	178609.7	**Ac**	55165.6	**Ba**	348940.2	**Aab**	370404.2	**Ac**	96870.5	**Ba**	441842.2	**Aa**	648680.8	**Ab**	145512.7	**Ba**	211706.2	**Bab**	1913221.4	**Aa**
PC 34:4	22843.8	**Aa**	38233.6	**Ab**	52463.4	**Ac**	10762.2	**Ba**	133196.5	**Aab**	129268.9	**ABbc**	16688.4	**Ba**	192855.4	**Aa**	261005.7	**Ab**	18021.0	**Ba**	53878.5	**Bb**	967318.1	**Aa**
PC 34:5	14725.0	**Aa**	23353.2	**Aa**	35820.0	**Ab**	11715.1	**Aa**	145439.2	**Aa**	147789.4	**Ab**	17685.5	**Ba**	242849.1	**ABa**	352547.6	**Ab**	17308.3	**Ba**	65592.9	**Ba**	1850434.6	**Aa**
PC 34:6	7388.3	**Aa**	11616.6	**Aa**	15522.5	**Ab**	NA	**Aa**	97877.4	**Aa**	105040.7	**Ab**	11257.4	**Ba**	249605.8	**ABa**	377081.9	**Ab**	10400.5	**Ba**	45869.9	**Ba**	2021642.8	**Aa**
PC 36:1	151921.9	**Aab**	200534.6	**Ac**	205906.7	**Ab**	89286.7	**Bb**	202994.0	**Ac**	254422.1	**Aab**	136472.3	**Bab**	295856.5	**Ab**	330892.2	**Aa**	172200.5	**Ca**	433731.7	**Aa**	254819.0	**Bab**
PC 36:2	2424013.6	**Aa**	3222168.7	**Ac**	3223319.3	**Ac**	1133915.9	**Ba**	3933832.4	**Ac**	4875504.8	**Ac**	2070216.4	**Ba**	6770862.7	**Ab**	7570075.4	**Ab**	2713487.8	**Ba**	10469420.0	**Aa**	10216540.5	**Aa**

SI Table 11. *(continuation)* Relative abundance of lipid of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
PC 36:3	82068.5	**Aa**	108467.1	**Ac**	144721.0	**Ac**	69071.4	**Ba**	382712.0	**Aa**	351101.4	**Ab**	73750.4	**Ba**	322707.5	**Aab**	405843.5	**Ab**	110152.5	**Ba**	177649.3	**Bbc**	1238630.0	**Aa**
PC 36:3.1	12747.8	**Aa**	17141.8	**Ac**	18354.5	**Ac**	7427.5	**Aa**	71604.6	**Ac**	90674.9	**Ac**	8886.2	**Ba**	290832.2	**Ab**	246327.9	**Ab**	11871.1	**Ca**	714374.5	**Aa**	519806.4	**Ba**
PC 36:5	35581.3	**Aa**	53479.7	**Aa**	76277.6	**Ab**	36152.3	**Aa**	497941.5	**Aa**	492896.3	**Ab**	61098.5	**Aa**	514515.8	**Aa**	706339.1	**Ab**	87362.8	**Ba**	260830.7	**Ba**	4592256.4	**Aa**
PC 36:6	35917.1	**Aa**	45937.5	**Aa**	57886.9	**Ab**	26794.4	**Aa**	246015.0	**Aa**	267044.5	**Ab**	39682.7	**Aa**	292242.1	**Aa**	417840.5	**Ab**	51240.3	**Ba**	149341.1	**Ba**	3631978.9	**Aa**
PC 38:2	36216.5	**Aa**	43612.1	**Ac**	49434.1	**Ac**	19152.4	**Ba**	54963.1	**Abc**	71021.4	**Ab**	31582.6	**Ba**	74308.9	**Aab**	83852.0	**Aab**	35337.8	**Ba**	86284.5	**Aa**	95886.2	**Aa**
PC 38:3	2761.9	**Aa**	4185.3	**Ab**	6156.7	**Ac**	NA	**Ba**	15344.1	**Aa**	15611.7	**Ab**	NA	**Ca**	15546.5	**Ba**	23264.1	**Aa**	NA	**Ba**	2869.2	**Bb**	23759.3	**Aa**
PC 38:3.1	NA	**Bc**	1579.4	**Ab**	1058.0	**Ab**	NA	**Bc**	1447.2	**Ab**	1597.1	**Ab**	1411.2	**Ab**	2232.9	**Ab**	2109.4	**Ab**	2486.2	**Ca**	5788.8	**Aa**	3846.4	**Ba**
PE 32:1	131215.1	**Aa**	184828.8	**Abc**	185159.9	**Ac**	62074.4	**Ba**	263011.3	**Aab**	307970.6	**Ab**	71836.3	**Ca**	300670.5	**Ba**	512768.4	**Aa**	55849.4	**Aa**	91851.6	**Ac**	40064.5	**Ad**
PE 34:1	286740.6	**Aa**	379462.2	**Aa**	386596.6	**Aa**	132233.1	**Bb**	328350.1	**Aa**	378770.2	**Aa**	168347.2	**Cab**	305234.3	**Ba**	435975.0	**Aa**	144228.7	**Ab**	161711.0	**Ab**	132246.4	**Ab**
PE 34:2	871033.5	**Aa**	1196325.0	**Ac**	1225440.4	**Ac**	419864.2	**Ba**	1966824.7	**Aab**	2158304.7	**Ab**	601480.2	**Ba**	2350497.8	**Aa**	2913842.6	**Aa**	475654.8	**Ba**	1555236.6	**Abc**	1687281.1	**Abc**
PE 34:3	19545.4	**Aa**	26579.5	**Aa**	27573.6	**Ac**	9300.6	**Ba**	29764.3	**ABa**	32216.3	**Abc**	13649.6	**Ba**	38814.4	**Aa**	52716.1	**Ab**	13393.3	**Ba**	21809.2	**Ba**	86205.2	**Aa**
PE 34:4	1869.8	**Aa**	2444.7	**Aa**	2560.3	**Ac**	996.7	**Aa**	8117.6	**Aa**	8770.3	**Abc**	NA	**Ba**	12206.7	**ABa**	17361.1	**Ab**	1795.3	**Ba**	4290.7	**Ba**	46945.3	**Aa**
PE 34:5	1066.8	**Aa**	798.5	**Aa**	1401.5	**Ab**	NA	**Aa**	4939.0	**Aa**	5816.1	**Ab**	820.0	**Ba**	13224.9	**ABa**	19720.2	**Ab**	NA	**Ba**	3763.2	**Ba**	61138.2	**Aa**
PE 34:6	NA	**Aa**	NA	**Aa**	NA	**Ab**	NA	**Aa**	3494.5	**Aa**	4406.9	**Ab**	NA	**Aa**	9353.5	**Aa**	13502.4	**Ab**	NA	**Ba**	2181.2	**Ba**	49118.8	**Aa**
PE 36:1	27913.8	**Ba**	49499.6	**Ab**	55398.3	**Ac**	20114.0	**Ca**	117607.5	**Ba**	145657.5	**Aa**	25769.6	**Ba**	97179.1	**Aa**	114324.6	**Ab**	18806.7	**Ba**	44072.1	**Ab**	42635.5	**Ac**
PE 36:2	1451105.4	**Aa**	1880464.6	**Ab**	2160134.5	**Ab**	710240.0	**Ba**	3711302.4	**Aa**	4214377.3	**Aa**	1076351.4	**Ba**	3838578.7	**Aa**	4005914.3	**Aa**	688853.3	**Ba**	2055708.1	**Ab**	2351022.6	**Ab**
PE 36:3	NA	**Aa**	NA	**Ac**	NA	**Ad**	NA	**Ca**	272180.1	**Ba**	331042.8	**Aa**	NA	**Ba**	232494.2	**Aa**	219152.9	**Ab**	NA	**Ba**	97228.5	**Ab**	92494.5	**Ac**
PE 36:3.1	32156.6	**Aa**	47593.3	**Aa**	45856.9	**Ab**	20916.5	**Aa**	49687.6	**Aa**	50647.4	**Ab**	34768.6	**Aa**	50818.8	**Aa**	63733.9	**Ab**	36449.9	**Ba**	36795.3	**Ba**	113584.9	**Aa**
PE 36:4	124470.7	**Ba**	158073.1	**Aa**	141715.8	**ABb**	51051.5	**Bb**	84148.6	**Ac**	113084.3	**Ab**	55239.1	**Cb**	117033.1	**Bb**	187643.9	**Aa**	47879.2	**Ab**	68751.8	**Ac**	40851.4	**Ac**
PE 36:4.1	59934.0	**Aa**	86576.0	**Aa**	84757.1	**Ab**	31187.3	**Aa**	79709.7	**Aa**	85095.0	**Ab**	57234.0	**Aa**	86237.9	**Aa**	113138.6	**Ab**	49203.5	**Ba**	67878.0	**Ba**	229778.3	**Aa**
PE 36:5	NA	**Aa**	5680.4	**Aa**	7674.7	**Ab**	NA	**Aa**	20640.5	**Aa**	25118.3	**Ab**	4064.6	**Aa**	48554.0	**Aa**	75943.1	**Ab**	2858.2	**Ba**	27420.1	**Ba**	235537.3	**Aa**
PE 36:6	5184.1	**Aa**	6595.2	**Aa**	8471.5	**Ab**	2729.1	**Aa**	12516.5	**Aa**	15576.0	**Ab**	4640.5	**Ba**	30053.7	**ABa**	45005.3	**Ab**	2950.2	**Ba**	13159.4	**Ba**	104568.2	**Aa**
PE 38:2	6242891.2	**Aa**	7047089.0	**Aa**	6907199.4	**Aa**	2274124.9	**Bb**	4078555.7	**Ab**	4835564.0	**Ab**	2902682.3	**Bb**	4867679.3	**Ab**	5859939.0	**Aab**	2513543.0	**Cb**	4247767.3	**Bb**	7132857.2	**Aa**
PE 38:3	2724.7	**Aa**	4499.8	**Ad**	3412.8	**Ac**	NA	**Ca**	20895.9	**Bb**	27938.8	**Ab**	NA	**Ca**	27948.4	**Ba**	36102.3	**Aa**	NA	**Ca**	14058.9	**Bc**	23272.0	**Ab**
PE 40:1	7589.8	**Ba**	11061.9	**Aa**	9970.2	**Aa**	5309.6	**Ba**	9250.3	**Aa**	10401.9	**Aa**	6554.0	**Ba**	10447.8	**Aa**	12134.0	**Aa**	7109.7	**Ba**	11911.1	**Aa**	12173.5	**Aa**
PE 40:2	86767.3	**Aab**	119096.7	**Ac**	123743.5	**Ab**	53298.3	**Bb**	134428.2	**Ac**	158775.4	**Ab**	98465.1	**Bab**	217443.0	**Ab**	252072.9	**Aa**	134223.7	**Ba**	287294.0	**Aa**	265366.9	**Aa**
PE 40:3	NA	**Aa**	NA	**Aa**	NA	**Ac**	NA	**Aa**	NA	**Aa**	NA	**Ac**	NA	**Ba**	NA	**Ba**	18955.6	**Aa**	NA	**Ba**	NA	**Ba**	12842.6	**Ab**

SI Table 11. *(continuation)* Relative abundance of lipid of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N		0h_3.5N		0h_7N		10h_0N		10h_3.5N		10h_7N		18h_0N		18h_3.5N		18h_7N		36h_0N		36h_3.5N		36h_7N	
PG 32:0	17670.5	Ba	21555.9	Aa	22271.1	Aa	7074.2	Bb	13550.9	Ab	15223.4	Ac	7973.2	Bb	14315.3	Ab	17255.3	Abc	9265.4	Bb	7748.1	Bc	19732.4	Aab
PG 32:1	8070.9	Aa	11917.3	Ab	10094.8	Ac	2829.4	Ba	22116.1	Aa	26715.1	Ac	3351.1	Ca	24776.2	Ba	38775.9	Aa	4377.8	Aa	4223.5	Ab	5682.6	Ac
PG 34:2	184381.7	Aa	240030.8	Abc	224668.6	Ac	71458.7	Bb	305645.6	Aab	349012.5	Ab	94591.7	Cab	343486.2	Ba	470463.6	Aa	73022.0	Cb	183646.5	Bc	361435.4	Ab
PG 34:3	8534.2	Aa	11554.4	Ab	10945.3	Ad	2778.6	Ba	83369.7	Aa	90949.8	Ac	5517.1	Ca	66469.9	Ba	115756.8	Ab	NA	Ba	NA	Bb	337365.1	Aa
PG 34:4	13002.9	Aa	17705.5	Aab	17288.9	Ac	6719.8	Ba	34254.2	Aab	41264.2	Abc	10699.3	Ba	39489.7	Aa	54252.3	Ab	7698.7	Ba	14623.4	Bb	248660.8	Aa
PG 36:1	39491.8	Ba	63451.9	Ab	65217.8	Ab	20720.1	Cb	78766.1	Bab	98271.1	Aa	31793.9	Cab	91671.7	Ba	109445.6	Aa	27115.7	Cab	62289.4	Ab	42860.2	Bc
PG 36:2	741250.4	Aa	854641.0	Ab	875780.4	Abc	335002.2	Bb	1064538.3	Aab	1199581.9	Aab	459584.7	Bab	1296144.4	Aa	1476250.4	Aa	354989.0	Bb	858240.1	Ab	849915.6	Ac
SQDG 32:0	2539144.4	Aa	2391220.6	Aa	2352230.1	Aa	1264434.9	Ab	1230777.6	Ab	1359238.2	Ac	1296701.9	Ab	1364238.0	Ab	1318269.5	Ac	1348807.1	Bb	1214316.3	Bb	1865231.7	Ab
SQDG 32:1	17551.5	Aa	21418.9	Aa	20815.4	Ab	5294.7	Bb	14011.2	Ab	NA	Cc	6824.7	Bb	12583.2	Abc	15608.2	Ab	5690.2	Bb	7141.8	Bc	70516.6	Aa
SQDG 32:2	17158.4	Ba	21341.7	ABa	23031.0	Ab	4769.6	Bb	17940.3	Aa	20477.2	Ab	NA	Bb	20556.5	Aa	25598.9	Ab	NA	Cb	11255.8	Bb	78123.1	Aa
SQDG 32:3	28468.2	Aa	32884.2	Aa	35430.7	Ab	7235.8	Bb	26174.8	Aab	30582.6	Ab	10183.4	Bb	32441.3	Aa	38177.0	Ab	7949.9	Cb	18360.7	Bb	106814.6	Aa
SQDG 34:0	216113.8	Ba	248959.3	Aa	255674.9	Aa	105276.0	Bb	208118.8	Ab	NA	Cb	102222.7	Bb	243842.5	Aa	266379.9	Aa	99985.9	Ab	104485.3	Ac	NA	Bb
SQDG 34:1	777089.1	Ba	857427.4	ABa	950796.0	Aa	NA	Cb	616580.9	Bb	729103.5	Ab	NA	Cb	542894.8	Bb	681504.5	Ab	NA	Bb	337883.5	Ac	394033.6	Ac
SQDG 34:2	1258210.1	Aa	1360644.8	Aa	1460863.6	Ab	230783.9	Bb	1182646.9	Aa	1359259.6	Ab	314798.0	Bb	1129577.1	Aa	1321994.1	Ab	242532.9	Cb	626941.8	Bb	2622296.0	Aa
SQDG 34:3	526615.6	Aa	630123.5	Ab	691578.7	Ac	284195.2	Bb	1257292.7	Aa	1419209.7	Ab	473618.6	Bab	1117847.8	Aa	1284151.7	Ab	402144.6	Cab	803870.9	Bb	2690696.1	Aa
SQDG 34:4	14342.9	Ba	17322.4	ABa	21183.6	Abc	2829.5	Bb	16515.4	Aa	18187.5	Ac	3731.7	Bb	19545.1	Aa	24205.6	Ab	1591.3	Cb	6959.2	Bb	43158.2	Aa
SQDG 34:5	9309.4	Aa	11366.7	Aab	13554.5	Ac	1320.6	Bab	13530.3	Aab	16698.7	Abc	1636.6	Bab	17642.4	Aa	23613.7	Ab	NA	Bb	6661.4	Bb	53613.3	Aa
SQDG 34:6	6484.9	Aa	7086.0	Aa	9242.9	Ac	1520.4	Ba	12267.5	ABa	13916.8	Abc	1253.0	Ba	18416.4	Aa	25721.8	Ab	NA	Ba	5575.8	Ba	78471.3	Aa
TAG 42:0	16191.1	ABb	15312.8	Ba	20697.6	Aa	16764.3	Ab	9371.9	Bb	12765.0	ABb	20950.6	Aab	9386.5	Bb	9719.5	Bb	24015.9	Aa	18386.5	Ba	8148.0	Cb
TAG 42:1	15201.5	Ac	13741.1	Ab	15488.1	Aa	19625.1	Ac	9446.6	Bb	12217.5	Bab	27539.1	Ab	8790.0	Bb	8687.2	Bb	34386.0	Aa	21718.7	Ba	8544.1	Cb
TAG 42:2	2868.9	Ac	2663.3	Ab	2954.7	Aa	3325.9	Ac	1601.0	Bb	2121.2	Ba	6525.1	Ab	1957.3	Bb	1767.6	Ba	9205.5	Aa	4406.2	Ba	1876.4	Ca
TAG 44:0	55651.4	ABbc	48365.9	Ba	67429.6	Aa	43590.5	Ac	30114.6	Bb	38451.4	ABb	61834.5	Ab	28865.0	Bb	29474.3	Bb	82772.0	Aa	51593.1	Ba	29107.6	Cb
TAG 44:1	31080.8	Ac	28492.6	Ab	37659.8	Aa	43641.1	Ac	26844.7	Bb	32831.2	ABa	106431.2	Ab	29592.9	Bb	25534.9	Ba	149687.8	Aa	49441.4	Ba	27315.6	Ca
TAG 44:2	19411.5	Ac	16430.0	Aa	17377.0	Aa	22894.2	Abc	11282.4	Bb	13683.3	Bb	23988.8	Ab	10278.8	Bb	11184.6	Bbc	39523.0	Aa	19274.0	Ba	9862.7	Cc
TAG 46:0	236183.8	ABbc	204126.7	Bab	285094.2	Aa	201126.0	Ac	164135.4	Ab	199127.8	Ab	271919.6	Ab	159741.7	Bb	170111.6	Bb	360621.0	Aa	229444.6	Ba	156315.5	Cb
TAG 46:1	149078.4	Ac	149057.5	Ab	175569.2	Aa	245729.1	Ac	164668.3	Ab	202041.0	Aa	748491.9	Ab	182278.3	Bb	116289.7	Ba	1165697.5	Aa	370353.6	Ba	69345.5	Ca
TAG 46:2	30326.0	Ac	30291.2	Ab	33494.7	Aa	40682.3	Ac	24059.1	Bb	30886.4	ABa	88404.1	Ab	NA	Cc	22269.1	Ba	169169.1	Aa	66465.3	Ba	NA	Cb
TAG 46:2.1	23773.6	Bc	22035.5	Bb	38460.7	Aa	25809.6	Ac	27056.0	Ab	30982.1	Aab	45970.0	Ab	23383.6	Bb	27020.4	Bab	58787.8	Aa	39965.2	Ba	24357.6	Cb
TAG 46:3	9576.3	Ad	10259.6	Ab	11139.4	Aa	20464.9	Ac	4829.3	Bb	7565.3	Ba	55531.4	Ab	5443.3	Bb	6095.7	Ba	140871.3	Aa	36711.9	Ba	3071.3	Ca

SI Table 11. *(continuation)* Relative abundance of lipid of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 46:3.1	3236.3 **Bc**	4405.8 **Bab**	6086.5 **Aa**	5824.5 **Ab**	2615.8 **Bc**	3296.8 **Bb**	8977.5 **Aa**	2941.2 **Bbc**	2877.2 **Bb**	NA **Cd**	5580.4 **Aa**	2194.3 **Bb**
TAG 46:4	2607.6 **Ac**	1505.2 **Ab**	2755.6 **Aa**	3768.2 **Ac**	1081.6 **Bb**	1688.5 **Bab**	9505.9 **Ab**	1185.3 **Bb**	1918.2 **Ba**	23630.4 **Aa**	7192.4 **Ba**	NA **Cb**
TAG 48:0	1241367.1 **Ab**	988385.8 **Aab**	1299294.3 **Aa**	1312868.1 **Ab**	748323.5 **Bb**	908115.4 **Bb**	1559941.8 **Ab**	763916.6 **Bb**	785866.6 **Bb**	2866354.8 **Aa**	1180491.3 **Ba**	672321.8 **Cb**
TAG 48:1	1186716.7 **Ac**	986810.6 **Ab**	1235582.8 **Aa**	1739235.8 **Ac**	1057210.3 **Ab**	1284093.0 **Aa**	4099633.0 **Ab**	1063499.2 **Bb**	820813.1 **Ba**	7322018.4 **Aa**	2055695.1 **Ba**	557848.2 **Ca**
TAG 48:2	765537.4 **Ac**	602835.2 **Ab**	683506.0 **Aa**	1039434.5 **Ac**	317636.5 **Bb**	455073.6 **Ba**	2561950.0 **Ab**	323278.2 **Bb**	310876.4 **Ba**	5110691.4 **Aa**	2204865.5 **Ba**	143238.9 **Ca**
TAG 48:3	623718.9 **Ac**	509614.2 **Ab**	638842.6 **Aa**	973314.2 **Ac**	302897.8 **Ab**	466035.2 **Aa**	3496855.2 **Ab**	291509.1 **Bb**	293449.0 **Ba**	8311221.9 **Aa**	3196786.6 **Ba**	45208.7 **Ca**
TAG 48:4	122425.9 **Ac**	96813.6 **Ab**	114069.1 **Aa**	266846.0 **Ac**	50884.7 **Bb**	81218.1 **Ba**	718303.3 **Ab**	39571.4 **Bb**	45028.3 **Ba**	1267038.1 **Aa**	731689.9 **Ba**	5740.1 **Ca**
TAG 48:5	61369.6 **Ac**	52301.0 **Ab**	66466.6 **Aa**	154471.8 **Ac**	37222.9 **Ab**	59382.9 **Aa**	577863.4 **Ab**	29945.6 **Bb**	32773.6 **Ba**	1147492.0 **Aa**	551235.9 **Ba**	6227.7 **Ca**
TAG 48:6	19164.8 **Ac**	15346.9 **Ab**	21024.9 **Aa**	59021.0 **Ac**	14178.4 **Ab**	23748.3 **Aa**	312378.1 **Ab**	11366.6 **Bb**	13086.6 **Ba**	677422.1 **Aa**	380780.1 **Ba**	3210.3 **Ca**
TAG 48:7	4145.8 **Ac**	3694.4 **Ab**	4838.7 **Aa**	10574.0 **Ac**	4528.6 **Ab**	7667.6 **Aa**	46620.7 **Ab**	3343.6 **Bb**	3624.6 **Ba**	63965.8 **Aa**	64873.6 **Aa**	1273.1 **Ba**
TAG 48:8	1583.9 **Ac**	1410.8 **Ab**	2253.4 **Aa**	NA **Ac**	2619.9 **Ab**	4390.7 **Aa**	25752.1 **Ab**	2150.3 **Bb**	2590.7 **Ba**	44677.4 **Aa**	33620.4 **Ba**	1601.0 **Ca**
TAG 48:9	NA **Ac**	NA **Ab**	NA **Aa**	NA **Ac**	NA **Ab**	795.8 **Aa**	6465.0 **Ab**	NA **Bb**	NA **Ba**	15433.7 **Aa**	7762.0 **Ba**	NA **Ca**
TAG 50:0	1906605.2 **Aab**	1485302.3 **Aa**	2003817.4 **Aa**	1524535.2 **Ab**	1344440.6 **Aa**	1533694.1 **Aa**	1691225.3 **Ab**	1339100.7 **Aa**	1340854.9 **Aa**	2415313.6 **Aa**	1483048.7 **Ba**	1335050.6 **Ba**
TAG 50:1	16896342.3 **Ac**	12944730.0 **Ab**	14906593.9 **Aa**	29312773.0 **Ac**	8054319.7 **Bb**	11077871.2 **Ba**	57515413.5 **Ab**	7529455.1 **Bb**	6829483.1 **Ba**	105188799.7 **Aa**	44203863.5 **Ba**	2776860.4 **Ca**
TAG 50:2	4195603.0 **Ac**	3345349.3 **Ab**	3941622.3 **Aa**	6460943.9 **Ac**	2620844.3 **Ab**	3573912.8 **Aa**	16973608.7 **Ab**	2580299.3 **Bb**	2334733.6 **Ba**	36737527.4 **Aa**	13967175.6 **Ba**	1196668.2 **Ca**
TAG 50:3	3134061.6 **Ac**	2385943.8 **Ab**	2959700.1 **Aa**	6837116.2 **Ac**	1642109.3 **Bb**	2485034.1 **Ba**	16003231.2 **Ab**	1139327.5 **Bb**	1251641.2 **Ba**	28921610.3 **Aa**	14961759.4 **Ba**	160472.5 **Ca**
TAG 50:4	2382423.9 **Ac**	1842915.6 **Ab**	2226128.1 **Aa**	5511708.5 **Ac**	977611.4 **Bb**	1598821.4 **ABa**	18939887.4 **Ab**	646230.3 **Bb**	734473.7 **Ba**	39459695.8 **Aa**	16298232.5 **Ba**	65547.4 **Ca**
TAG 50:5	1032049.7 **Ac**	785858.1 **Ab**	1059566.7 **Aa**	3201061.5 **Ac**	400885.8 **Bb**	671626.7 **ABa**	11988588.2 **Ab**	330729.2 **Bb**	335549.1 **Ba**	21657677.3 **Aa**	8462311.0 **Ba**	49135.7 **Ca**
TAG 50:6	1895.9 **Aa**	NA **Ba**	NA **Ba**	NA **Ab**	NA **Aa**	NA **Aa**	NA **Ab**	NA **Aa**	NA **Aa**	NA **Ab**	NA **Aa**	NA **Aa**
TAG 50:6.1	503956.4 **Ac**	394323.6 **Ab**	526871.3 **Aa**	2236567.3 **Ac**	224397.9 **Ab**	374186.7 **Aa**	12365702.7 **Ab**	232337.4 **Bb**	223246.9 **Ba**	26014107.3 **Aa**	7013747.2 **Ba**	46865.5 **Ca**
TAG 50:7	85652.8 **Ac**	71709.8 **Ab**	103307.4 **Aa**	338819.8 **Ac**	63034.0 **Ab**	98513.8 **Aa**	1597224.4 **Ab**	44610.9 **Bb**	51524.3 **Ba**	2897404.9 **Aa**	1385797.7 **Ba**	15167.9 **Ca**
TAG 50:8	34348.3 **Ac**	28767.1 **Ab**	45192.2 **Aa**	169884.9 **Ac**	40345.4 **Ab**	63409.5 **Aa**	944001.1 **Ab**	31332.9 **Bb**	39876.4 **Ba**	1941959.7 **Aa**	648574.3 **Ba**	18275.7 **Ca**
TAG 50:9	11510.9 **Ac**	8918.4 **Ab**	13692.6 **Aa**	77694.6 **Ac**	19265.4 **Ab**	29776.9 **Aa**	561346.8 **Ab**	16547.4 **Bb**	21143.3 **Ba**	1324415.7 **Aa**	326326.5 **Ba**	14230.9 **Ca**
TAG 52:0	1680212.0 **Aa**	1333448.8 **Aa**	1760973.6 **Aa**	1257012.3 **Aa**	1244599.4 **Aa**	1442794.2 **Aa**	1333460.5 **Aa**	1180284.0 **Aa**	1291024.2 **Aa**	1780744.1 **Aa**	1236093.9 **Aa**	1232040.6 **Aa**
TAG 52:1	7752771.6 **Ac**	6156442.8 **Ab**	7930367.8 **Aa**	11108715.0 **Ac**	5402514.7 **Bb**	7201396.1 **ABa**	22930456.6 **Ab**	5804238.6 **Bb**	5531231.0 **Ba**	46389107.1 **Aa**	12095141.0 **Ba**	3661678.4 **Ca**
TAG 52:2	27927774.3 **Ad**	21049253.6 **Ab**	27277934.5 **Aa**	57312401.9 **Ac**	18636139.4 **Bb**	27288574.5 **Ba**	111481451.5 **Ab**	15455117.3 **Bb**	17278103.4 **Ba**	164276251.3 **Aa**	90894194.2 **Ba**	6489171.0 **Ca**
TAG 52:3	9823653.8 **Ac**	7341192.3 **Ab**	9398841.9 **Aa**	22983090.8 **Ac**	6060603.9 **Bb**	8924754.8 **Ba**	59244688.3 **Ab**	4602482.3 **Bb**	4954195.6 **Ba**	109361793.9 **Aa**	46640910.4 **Ba**	1595831.7 **Ca**
TAG 52:4	4619068.1 **Ac**	3534704.6 **Ab**	4648218.4 **Aa**	13474444.7 **Ac**	2927308.8 **Bb**	4364138.7 **ABa**	43970594.4 **Ab**	2088431.2 **Bb**	2326419.0 **Ba**	87663219.6 **Aa**	38328708.0 **Ba**	866964.1 **Ca**
TAG 52:5	1977478.5 **Ac**	1468403.3 **Ab**	2080624.6 **Aa**	7357131.9 **Ac**	1192228.6 **Bb**	1955646.1 **Ba**	23682289.3 **Ab**	599276.3 **Bb**	785713.8 **Ba**	43842876.1 **Aa**	18121112.1 **Ba**	NA **Ca**

SI Table 11. *(continuation)* Relative abundance of lipid of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 52:5.1	3036.0 **Ab**	2510.8 **Aa**	NA **Aa**	NA **Ab**	3101.8 **Aa**	4042.4 **Aa**	NA **Ab**	1425.8 **Aa**	NA **Aa**	135382.8 **Aa**	22968.2 **Ba**	NA **Ba**
TAG 52:6	937502.5 **Ac**	732887.1 **Ab**	1010398.3 **Aa**	5519830.3 **Ac**	582392.5 **Bb**	922726.0 **Ba**	20155286.3 **Ab**	300524.4 **Bb**	403206.4 **Ba**	37038774.4 **Aa**	14977956.9 **Ba**	58071.5 **Ca**
TAG 52:6.1	NA **Aa**	NA **Aa**	NA **Aa**	NA **Aa**	1800.2 **Aa**	2455.0 **Aa**	17106.6 **Aa**	834.3 **Aa**	NA **Aa**	NA **Aa**	28976.5 **Aa**	2568.2 **Aa**
TAG 52:7	394226.5 **Ac**	305754.3 **Ab**	440568.1 **Aa**	3307141.3 **Ac**	273968.4 **Ab**	484951.4 **Aa**	18633981.1 **Ab**	165700.7 **Bb**	213987.9 **Ba**	37530906.6 **Aa**	10994292.6 **Ba**	57216.5 **Ca**
TAG 52:8	93796.9 **Ac**	76920.8 **Ab**	118774.2 **Aa**	578599.0 **Ac**	129108.6 **Ab**	212312.8 **Aa**	3747984.7 **Ab**	90427.7 **Bb**	119240.2 **Ba**	5897422.7 **Aa**	2015061.3 **Ba**	56193.9 **Ca**
TAG 52:9	23018.9 **Ac**	19366.1 **Ab**	31914.7 **Aa**	220181.0 **Ac**	56533.7 **Ab**	91541.5 **Aa**	1873845.1 **Ab**	45208.0 **Bb**	59339.9 **Ba**	2882543.3 **Aa**	707004.1 **Ba**	43110.8 **Ca**
TAG 54:0	559345.4 **ABa**	479528.8 **Ba**	626917.5 **Aa**	431656.2 **Ab**	419673.4 **Aa**	492015.9 **Ab**	490247.9 **Aab**	430777.5 **Aa**	446196.0 **Ab**	591734.1 **Aa**	444174.3 **Ba**	450005.4 **Bb**
TAG 54:1	2554377.8 **Ac**	2148596.7 **Aa**	2812659.7 **Aa**	2615892.2 **Ac**	1871236.9 **Aa**	2316886.5 **Aa**	3690225.1 **Ab**	1905503.2 **Ba**	1997861.6 **Ba**	7376905.5 **Aa**	2491490.7 **Ba**	1719520.6 **Ba**
TAG 54:2	7685421.1 **Ac**	6167656.1 **Ab**	8010122.9 **Aab**	9337202.7 **Ac**	6399412.8 **Ab**	9450557.0 **Aa**	18247898.9 **Ab**	6024388.9 **Bb**	7030580.6 **Bab**	41170551.5 **Aa**	13612463.7 **Ba**	3232132.0 **Cb**
TAG 54:3	13755443.6 **Ad**	10288304.3 **Ab**	14026944.0 **Aa**	37974608.9 **Ac**	10974485.6 **Bb**	16674830.9 **Ba**	81780290.7 **Ab**	6766966.7 **Bb**	8839411.8 **Ba**	137699455.2 **Aa**	62519786.3 **Ba**	3365679.1 **Ca**
TAG 54:4	6738090.1 **Ad**	5419855.4 **Ab**	7168378.8 **Aa**	21064933.7 **Ac**	5187259.3 **Bb**	7605833.9 **Ba**	55567940.8 **Ab**	3134646.8 **Bb**	3824352.7 **Ba**	99255567.4 **Aa**	40723085.8 **Ba**	1858864.9 **Ca**
TAG 54:5	4136976.0 **Ac**	3261787.1 **Ab**	4490053.0 **Aa**	14405321.5 **Ac**	3323970.7 **Bb**	4626480.3 **ABa**	46677911.4 **Ab**	2139945.6 **Bb**	2569499.2 **Ba**	92987177.3 **Aa**	40148284.6 **Ba**	1412770.8 **Ca**
TAG 54:6	1711580.7 **Ac**	1355546.2 **Ab**	1874929.0 **Aa**	4106121.7 **Ac**	1232842.8 **Ab**	1713827.7 **Aa**	15632307.4 **Ab**	922517.7 **Bb**	1087411.6 **Ba**	29769606.1 **Aa**	13074367.2 **Ba**	772660.5 **Ca**
TAG 54:7	243028.7 **Ac**	208725.7 **Ab**	294825.8 **Aa**	1001063.0 **Ac**	207053.1 **Ab**	321778.8 **Aa**	5488184.3 **Ab**	129890.7 **Bb**	178461.6 **Ba**	12558807.2 **Aa**	4938362.5 **Ba**	79028.3 **Ca**
TAG 54:8	44589.3 **Ac**	38909.7 **Ab**	56462.8 **Aa**	127034.8 **Ac**	51950.4 **Ab**	84689.0 **Aa**	767992.6 **Ab**	36767.4 **Bb**	54433.3 **Ba**	1393423.4 **Aa**	687961.2 **Ba**	26266.5 **Ca**
TAG 54:9	5112.6 **Ac**	5728.8 **Ab**	8856.6 **Aa**	15885.6 **Ac**	10769.5 **Ab**	18459.9 **Aa**	90159.0 **Ab**	9346.0 **Bb**	14007.7 **Ba**	202311.4 **Aa**	78327.0 **Ba**	7672.0 **Ca**
TAG 56:0	53902.4 **Bbc**	44281.9 **Ba**	67413.6 **Aa**	41832.8 **Ac**	33255.3 **Aa**	42068.8 **Ab**	58508.7 **Ab**	34555.9 **Ba**	36189.2 **Bb**	90113.8 **Aa**	43913.3 **Ba**	31046.9 **Cb**
TAG 56:1	140187.2 **Ac**	124929.3 **Ab**	166127.4 **Aa**	270516.9 **Ac**	97381.9 **Bb**	133838.5 **Ba**	603386.5 **Ab**	99675.6 **Bb**	102987.8 **Ba**	1490866.9 **Aa**	330376.8 **Ba**	69943.0 **Ca**
TAG 56:2	470089.0 **Ad**	408399.7 **Ab**	532160.4 **Aa**	1053602.4 **Ac**	424010.7 **Bb**	654518.8 **ABa**	2263472.7 **Ab**	402071.0 **Bb**	481158.6 **Ba**	5004199.7 **Aa**	1198530.7 **Ba**	165702.7 **Ca**
TAG 56:3	1080335.1 **Ac**	881080.9 **Ab**	1124672.5 **Aab**	2592820.7 **Ac**	1359613.2 **Ab**	2105827.4 **Aa**	6185583.1 **Ab**	870360.1 **Bb**	1236171.8 **Bab**	12182940.2 **Aa**	3187102.2 **Ba**	169704.0 **Cb**
TAG 56:4	445574.7 **Ac**	376769.2 **Ab**	478531.2 **Aab**	829479.5 **Ac**	551284.0 **Ab**	777019.6 **Aa**	1899871.8 **Ab**	350098.4 **Bb**	447289.8 **Bab**	2546509.8 **Aa**	1199403.6 **Ba**	102221.7 **Cb**
TAG 56:5	186787.2 **Ac**	164139.3 **Ab**	204708.8 **Aab**	442050.6 **Ac**	228808.8 **Ab**	353650.1 **Aa**	1355773.2 **Ab**	158210.6 **Bb**	184220.9 **Bab**	2594560.3 **Aa**	1025133.9 **Ba**	42661.4 **Cb**
TAG 56:6	39840.6 **Ac**	40218.9 **Ab**	44487.0 **Aab**	84532.7 **Ac**	53031.5 **Ab**	88149.3 **Aa**	312324.6 **Ab**	38665.4 **Bb**	41485.0 **Bab**	486071.6 **Aa**	271058.7 **Ba**	11437.7 **Cb**
TAG 56:7	7887.7 **Ac**	8950.5 **Ab**	9865.7 **Aa**	18765.8 **Ac**	9205.8 **Ab**	18048.5 **Aa**	76189.2 **Ab**	8296.4 **Bb**	8686.1 **Ba**	154873.5 **Aa**	80699.9 **Ba**	2623.4 **Ca**
TAG 58:0	29926.2 **Ab**	24129.6 **Ba**	32197.5 **Aa**	24130.3 **Ac**	14952.5 **Bb**	20280.0 **Ab**	32464.1 **Ab**	14925.9 **Bb**	16379.0 **Bbc**	52511.4 **Aa**	21141.0 **Ba**	12651.8 **Cc**
TAG 58:1	98693.0 **Ac**	84475.0 **Ab**	110384.1 **Aa**	150477.1 **Ac**	56025.9 **Ab**	81741.2 **Aa**	464592.4 **Ab**	55674.2 **Bb**	53197.3 **Ba**	1359891.9 **Aa**	312298.8 **Ba**	24391.4 **Ca**
TAG 58:2	147794.3 **Ac**	124282.8 **Ab**	168873.5 **Aa**	280597.4 **Ac**	119509.1 **Bb**	177835.3 **ABa**	674419.1 **Ab**	116548.4 **Bb**	124347.1 **Ba**	1533567.2 **Aa**	390706.7 **Ba**	60629.3 **Ca**
TAG 58:3	184411.1 **Ac**	152137.1 **Ab**	215380.7 **Aab**	398411.7 **Ac**	176898.5 **Bb**	309206.4 **ABa**	934390.6 **Ab**	135448.4 **Bb**	167878.8 **Bab**	1951669.2 **Aa**	455769.9 **Ba**	71324.2 **Cb**
TAG 58:4	110283.4 **Ac**	96172.4 **Ab**	124084.3 **Aa**	119485.3 **Ac**	83059.8 **Ab**	111350.3 **Aab**	240094.9 **Ab**	76839.3 **Bb**	84592.0 **Bab**	486886.6 **Aa**	191817.0 **Ba**	62967.4 **Cb**

SI Table 11. *(continuation)* Relative abundance of lipid of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
TAG 58:5	22231.9 **Ac**	18325.4 **Ab**	22999.9 **Aa**	30583.7 **Ac**	13492.3 **Ab**	17194.9 **Aa**	107978.9 **Ab**	13239.6 **Bb**	12079.6 **Ba**	213318.7 **Aa**	75034.0 **Ba**	NA **Ca**
TAG 58:6	14538.4 **Ac**	12543.8 **Ab**	16334.3 **Aa**	25042.4 **Ac**	9731.1 **Ab**	14259.6 **Aa**	167896.9 **Ab**	10529.6 **Bb**	10975.5 **Ba**	360060.7 **Aa**	80751.2 **Ba**	8203.1 **Ca**
TAG 60:0	63303.8 **Ac**	52027.1 **Aa**	67770.1 **Aa**	64320.9 **Ac**	21444.1 **Bb**	27863.8 **Bb**	98957.4 **Ab**	18576.0 **Bb**	18645.5 **Bb**	170768.9 **Aa**	55650.8 **Ba**	22176.5 **Cb**
TAG 60:1	90007.9 **Ac**	72309.4 **Ab**	91642.2 **Aa**	NA **Bd**	38731.1 **Ab**	66179.5 **Aab**	234941.0 **Ab**	40984.2 **Bb**	40038.8 **Bbc**	651955.0 **Aa**	144439.2 **Ba**	NA **Cc**
TAG 60:2	120731.3 **Ac**	101033.1 **Ab**	133377.4 **Aa**	162382.4 **Ac**	89807.5 **Ab**	144152.6 **Aa**	532792.2 **Ab**	88012.7 **Bb**	89401.0 **Ba**	1440780.2 **Aa**	344061.4 **Ba**	24225.3 **Ca**
TAG 60:3	64752.3 **Ac**	55459.4 **Ab**	72627.3 **Aa**	83038.2 **Ac**	51816.1 **Ab**	81421.7 **Aa**	260610.6 **Ab**	49020.4 **Bb**	49664.7 **Bab**	645100.3 **Aa**	175517.4 **Ba**	20322.3 **Cb**
TAG 60:4	43927.2 **Ac**	36501.3 **Ab**	49044.0 **Aa**	54900.5 **Ac**	31603.0 **Ab**	44669.2 **Aa**	183063.5 **Ab**	29873.9 **Bb**	31434.8 **Ba**	485217.6 **Aa**	186751.2 **Ba**	21279.7 **Ca**
TAG 60:5	10003.3 **Ac**	8779.9 **Ab**	12437.3 **Aa**	8009.9 **Ac**	7034.2 **Ab**	12225.0 **Aa**	47615.3 **Ab**	7861.5 **Bb**	8606.4 **Bab**	100306.8 **Aa**	36280.4 **Ba**	NA **Cb**
TAG 60:5.1	17238.5 **Ac**	15117.7 **Ab**	17272.4 **Aa**	11532.1 **Ac**	NA **Bc**	NA **Bb**	62684.2 **Ab**	11038.4 **Bbc**	7232.0 **Bab**	129338.1 **Aa**	37740.8 **Ba**	NA **Cb**
TAG 60:6	12476.7 **Ac**	10186.9 **Ab**	12113.5 **Aa**	7832.0 **Ac**	4397.7 **Ab**	7456.3 **Aa**	95538.9 **Ab**	9214.6 **Bb**	6475.8 **Ba**	204118.1 **Aa**	43066.1 **Ba**	2941.8 **Ca**
FA 16:0	14217717.0**Aa**	10736904.7**Ba**	10981843.0**Ba**	9047770.3 **Ab**	6090360.4 **Ab**	7667712.9 **Aab**	10234216.7 **Ab**	5233853.3 **Bb**	5337535.4 **Bb**	12103500.3 **Aab**	10144029.6**Aa**	5186111.2 **Bb**
FA 16:1	2484463.0 **Aa**	2003375.3 **Ba**	1875501.7 **Ba**	NA **Ab**	NA **Ac**	NA **Ab**	NA **Ab**	NA **Ac**	NA **Ab**	NA **Bb**	1007432.3 **Ab**	NA **Bb**
FA 16:2	8813561.9 **Aa**	6798519.5 **Ba**	7257595.8 **ABa**	2341551.1 **Bb**	4355749.7 **Ab**	5502689.4 **Aab**	3510159.3 **Ab**	4107097.8 **Ab**	4068224.8 **Ab**	3213212.4 **Bb**	5897426.3 **Aab**	4324470.3 **ABb**
FA 16:3	4862159.1 **Aa**	4003133.2 **Aa**	3859757.1 **Ab**	2627964.9 **Bb**	4411476.6 **Aa**	5517919.5 **Aab**	4800589.6 **Aa**	4282447.7 **Aa**	4173322.1 **Aab**	4341264.4 **Aab**	5471181.1 **Aa**	5838324.1 **Aa**
FA 16:4	83989.6 **Aa**	61294.6 **Ba**	52923.6 **Ba**	68103.7 **Ab**	24132.8 **Bb**	30285.0 **Bb**	60012.7 **Ab**	30949.0 **Bb**	22667.6 **Bb**	62849.0 **Ab**	41068.5 **Bb**	25613.9 **Bb**
FA 16:4.1	64087.7 **Aa**	71551.4 **Ac**	88648.1 **Ac**	28276.9 **Ca**	229698.2 **Ba**	294466.6 **Ab**	50026.5 **Ba**	169424.3 **Aab**	229274.5 **Ab**	61563.8 **Ba**	119325.2 **Bbc**	495776.5 **Aa**
FA 18:0	2568615.4 **Aa**	2397054.3 **Aa**	2897670.5 **Aa**	1845119.1 **Ab**	1481448.5 **Ab**	1805023.8 **Ab**	2302321.6 **Aab**	1204929.6 **Bb**	1174051.6 **Bbc**	2585658.9 **Aa**	1650999.3 **Bb**	609885.7 **Cc**
FA 18:1	43265499.7**Aa**	37082503.1**Aa**	36898622.9**Aa**	23422480.3**Ab**	25347368.1**Aab**	27611035.7**Aab**	32685352.5 **Aab**	18075340.4**Bb**	16448817.5**Bbc**	37970208.3 **Aa**	31883356.8**Aa**	8856878.1 **Bc**
FA 18:2	26729953.4**Aa**	22136434.0**Aa**	24030402.2**Aa**	10838193.4**Bb**	15490360.8**ABab**	19392589.4**Aab**	16058450.3 **Ab**	13419007.4**Ab**	13489361.0**Ab**	16465761.1 **Ab**	19347950.8**Aab**	16981160.6**Ab**
FA 18:3	21988295.5**Aa**	18387863.2**Aa**	20920774.2**Aab**	12791703.3**Bb**	20553441.1**Aa**	25002083.6**Aab**	22874193.8 **Aa**	18099896.1**Aa**	19051952.8**Ab**	22352859.3 **Aa**	24142355.7**Aa**	28040903.5**Aa**
FA 18:4	1220301.4 **Aa**	1117761.7 **Aa**	1206223.4 **Aa**	523145.3 **Bb**	1063551.8 **Aa**	1281335.8 **Aa**	991991.8 **Aa**	645473.9 **Ab**	632452.0 **Ab**	894561.3 **Aab**	639311.7 **ABb**	479218.4 **Bb**
FA 20:0	111615.0 **Ac**	127027.7 **Aa**	125024.9 **Aa**	123630.6 **Abc**	97494.7 **Bb**	116306.1 **ABa**	140838.6 **Ab**	86673.1 **Bb**	103144.2 **Ba**	178498.5 **Aa**	126426.4 **Ba**	75009.9 **Cb**
FA 20:1	1272475.4 **Abc**	1264717.6 **Aa**	1187853.8 **Aa**	1057908.7 **Ac**	769641.2 **Bb**	935866.4 **ABa**	1440614.1 **Ab**	530802.0 **Bb**	578481.6 **Bb**	1911573.2 **Aa**	1134460.0 **Ba**	345573.5 **Cb**
FA 20:3	27047.7 **Aa**	26421.4 **Aa**	29456.8 **Ab**	11979.2 **Bb**	29619.5 **Aa**	36479.2 **Aab**	21080.9 **Aab**	25155.6 **Aa**	26478.3 **Ab**	20240.8 **Cab**	34173.4 **Ba**	44158.4 **Aa**
FA 22:0	1347365.2 **Aa**	1213354.7 **Ba**	1087273.3 **Ba**	941697.0 **Ab**	755651.2 **Bb**	860010.5 **Bb**	908265.5 **Abc**	639985.3 **Bbc**	686390.6 **Bbc**	831205.0 **Ac**	476004.2 **Bc**	806350.5 **Bc**
FA 22:1	466523.4 **Abc**	471834.0 **Aa**	468034.7 **Aab**	381250.1 **Bc**	424666.2 **ABab**	507735.5 **Aa**	581999.9 **Aab**	339064.7 **Bb**	363087.0 **Bb**	660725.4 **Aa**	439145.7 **Bab**	397289.2 **Bab**
FA 24:1	69257.9 **Aa**	70643.8 **Aa**	70008.0 **Aa**	45986.3 **Ab**	57026.7 **Aab**	61922.1 **Aa**	73941.8 **Aa**	61537.9 **Aab**	60907.1 **Aa**	62882.5 **Aab**	43461.2 **Bb**	57129.3 **ABa**
FA 26:0	131229.2 **Ca**	124305.5 **Ba**	139993.9 **Aa**	55124.9 **Cc**	72784.1 **Bc**	88083.9 **Ac**	74923.3 **Cb**	94751.1 **Bb**	93079.9 **Ab**	62900.2 **Cc**	72840.5 **Bc**	89676.3 **Ac**
FA 28:0	151584.9 **Ba**	159347.7 **Ba**	193106.9 **Aa**	124759.6 **Bab**	155364.5 **Aa**	151087.9 **ABb**	149861.2 **Aa**	106921.9 **Bb**	125911.1 **ABb**	107919.1 **Ab**	78384.0 **Bb**	86795.4 **ABc**

SI Table 11. *(continuation)* Relative abundance of lipid of *Monoraphidium* sp. BR023 strain grown under three different concentrations of nitrogen (0, 3.5 and 7 mM) in four growth time (0h, 10h, 18h and 36h). Different lowercase letters refer to statistical difference between times within the same nitrogen concentration. Capital letter refer to statistical difference between nitrogen concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_0N	0h_3.5N	0h_7N	10h_0N	10h_3.5N	10h_7N	18h_0N	18h_3.5N	18h_7N	36h_0N	36h_3.5N	36h_7N
FA 30:0	25528.1 **Ba**	31117.2 **Ba**	48121.4 **Aa**	25857.5 **Aa**	24583.3 **Aab**	26990.9 **Abc**	26998.4 **Aa**	27376.9 **Aa**	38036.1 **Aab**	17600.5 **Aa**	13995.2 **Ab**	17416.7 **Ac**
FA 30:1	75952.5 **Aa**	80028.8 **Ab**	78880.8 **Ab**	86814.5 **Ba**	135543.0 **Aa**	134596.1 **Aa**	103800.5 **Aa**	116892.1 **Aab**	138860.8 **Aa**	63957.2 **Aa**	38347.4 **Ac**	50622.4 **Ab**

SI Table 12. P-values for significant main effects and interactions between the factors (strain, concentration and time) of three freshwater strains (*C. reinhardtii* CC503, *Chlamydomonas* sp. BR020, and *C. vulgaris* BR017) cultured in TAP medium with 5µM rapamycin and control.

	nº cells	**µ**	**Chl *a***	**Chl *b***	**Chl *a*/*b***	**Carotenoids**	**Starch**
Main effects							
Strain	0.000**	0.000**	0.001**	0.000**	0.000**	0.000**	0.000**
Concentration	0.000**	0.000**	0.002**	0.000**	0.160NS	0.008**	0.000**
Time	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Interaction effects							
Strain x Concentration	0.000**	0.000**	0.692NS	0.000**	0.000**	0.076NS	0.000**
Strain x Time	0.000**	0.000**	0.031**	0.000**	0.000**	0.000**	0.000**
Concentration x Time	0.000**	0.000**	0.004**	0.000**	0.000**	0.001**	0.000**
Strain x Concentration x Time	0.000**	0.000**	0.238NS	0.000**	0.000**	0.286NS	0.000**
R^2	0.9938	0.9959	0.6087	0.8859	0.8766	0.8274	0.9853

** indicate that effect and interaction are significant at *p*-value < 0.05;
NS = not significant;

SI Table 13. Relative abundance of primary metabolites of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Alanine	0.000741057	**Ab**	0.000755865	**Ab**	0.001052607	**Ba**	0.001888299	**Aa**	0.000553517	**Ac**	0.000638368	**Ab**
Asparagine	2.52996E-06	**Ab**	3.66503E-06	**Ab**	6.31093E-06	**Ba**	1.05142E-05	**Aa**	3.87454E-06	**Ab**	3.77859E-06	**Ab**
Aspartate	0.000166968	**Ab**	0.000212635	**Ab**	0.000351804	**Ba**	0.000669829	**Aa**	0.000213945	**Ab**	0.000243736	**Ab**
b-Alanine	3.15234E-05	**Aa**	3.20424E-05	**Aa**	1.61539E-05	**Bb**	3.90516E-05	**Aa**	2.62913E-05	**Aa**	2.18986E-05	**Ab**
Butanoic acid, 2-amino-	6.11946E-06	**Bc**	8.02993E-06	**Ac**	2.13869E-05	**Ba**	2.94525E-05	**Aa**	1.46774E-05	**Bb**	1.65468E-05	**Ab**
Citrulline	2.49464E-07	**Ab**	NA	**Ac**	1.53433E-06	**Ba**	3.05085E-06	**Aa**	1.2477E-06	**Aa**	1.43107E-06	**Ab**
Cysteine	2.97408E-08	**Ab**	3.17626E-08	**Ac**	8.67687E-08	**Ba**	1.60597E-07	**Aa**	8.56038E-08	**Aa**	7.87654E-08	**Ab**
Cysteinyl-glycine	3.33889E-07	**Ac**	3.03455E-07	**Ac**	1.13688E-06	**Aa**	1.51687E-06	**Aa**	7.08522E-07	**Ab**	8.02123E-07	**Ab**
Glutamate	0.000542898	**Ba**	0.000589931	**Ab**	0.000382694	**Bb**	0.000713309	**Aa**	0.000195927	**Ac**	0.000233998	**Ac**
Glutamine	1.84984E-05	**Ab**	2.30039E-05	**Ab**	0.000214415	**Ba**	0.000363009	**Aa**	3.49475E-05	**Ab**	5.22762E-05	**Ab**
Glycine	1.74342E-05	**Aa**	1.85641E-05	**Aa**	6.45173E-05	**Aa**	6.09249E-05	**Aa**	3.47873E-05	**Aa**	7.05194E-05	**Aa**
Histidine	1.50895E-05	**Aa**	1.50395E-05	**Ab**	4.12972E-05	**Ba**	0.00011788	**Aa**	2.47634E-05	**Aa**	3.81471E-05	**Ab**
Hydroxyproline	1.63288E-07	**Ba**	2.12987E-07	**Aa**	1.35562E-07	**Ba**	2.32276E-07	**Aa**	1.12556E-07	**Bb**	1.08775E-07	**Ab**
Isoleucine	3.12619E-05	**Ab**	3.0879E-05	**Ac**	8.72515E-05	**Ba**	0.00014346	**Aa**	7.01511E-05	**Aa**	7.01906E-05	**Ab**
Leucine	7.81102E-05	**Ab**	7.63717E-05	**Ac**	0.000185341	**Ba**	0.000312698	**Aa**	0.000159738	**Aa**	0.000149504	**Ab**
Methionine	1.04657E-05	**Aa**	1.27425E-05	**Aa**	1.39116E-05	**Aa**	3.62477E-05	**Aa**	2.01513E-05	**Aa**	2.44217E-05	**Aa**
O-acetyl-serine	2.1476E-05	**Aa**	2.29752E-05	**Aa**	1.01626E-05	**Bb**	1.87863E-05	**Ab**	5.22955E-06	**Ac**	6.21116E-06	**Ac**
Ornithine	2.05519E-05	**Ab**	2.00369E-05	**Ab**	0.000110365	**Ba**	0.000184177	**Aa**	5.2255E-05	**Ab**	6.43986E-05	**Ab**
Phenylalanine	1.20568E-05	**Ab**	1.2439E-05	**Ac**	4.3262E-05	**Ba**	6.57532E-05	**Aa**	4.61337E-05	**Aa**	5.20998E-05	**Ab**
Proline	0.000194185	**Aa**	0.000255206	**Aa**	0.000102974	**Aa**	0.00031157	**Aa**	0.000111955	**Aa**	0.000139721	**Aa**
Pyroglutamic acid	0.000322738	**Ac**	NA	**Bc**	0.000964576	**Ba**	0.00171211	**Aa**	0.00051468	**Ab**	0.00057389	**Ab**
Serine	9.34108E-05	**Ab**	8.90937E-05	**Ab**	0.000136853	**Ba**	0.000256335	**Aa**	0.000104784	**Aab**	0.000105201	**Ab**
Threonine	3.34612E-05	**Ac**	3.41607E-05	**Ac**	0.000129353	**Ba**	0.000215477	**Aa**	8.33389E-05	**Ab**	7.48743E-05	**Ab**
Tryptophan	NA	**Bb**	1.06657E-05	**Ab**	2.71379E-05	**Ba**	4.06807E-05	**Aa**	2.81196E-05	**Ba**	3.31822E-05	**Aa**
Tyrosine	2.46018E-05	**Ac**	2.44598E-05	**Ab**	0.000112704	**Bb**	0.000175602	**Aa**	0.00015245	**Aa**	0.000176567	**Aa**
Valine	0.000101201	**Ab**	0.00010404	**Ab**	0.00024732	**Ba**	0.000436521	**Aa**	0.000168542	**Aab**	0.000167857	**Ab**

SI Table 13. *(continuation)* Relative abundance of primary metabolites of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Organic acid	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Ascorbic acid	2.16073E-08	**Ab**	NA	**Ab**	1.8137E-06	**Aa**	3.58932E-06	**Aa**	2.04487E-06	**Aa**	2.85334E-06	**Aa**
Benzoate	5.77268E-06	**Aa**	8.97847E-06	**Aa**	3.67198E-06	**Aab**	5.50973E-06	**Aab**	1.65194E-06	**Ab**	2.23128E-06	**Ab**
Capric acid	1.50914E-07	**Ba**	2.32344E-07	**Aa**	1.217E-07	**Ba**	2.6703E-07	**Aa**	1.25284E-07	**Ba**	1.07236E-07	**Aa**
Citramalate	7.54088E-07	**Ab**	1.00921E-06	**Ab**	1.76492E-06	**Ba**	3.04989E-06	**Aa**	4.02942E-07	**Ab**	6.23601E-07	**Ab**
Citrate	4.74958E-05	**Aa**	3.91427E-05	**Ab**	4.49255E-05	**Ba**	8.17115E-05	**Aa**	5.53537E-05	**Aa**	6.52645E-05	**Aa**
Dehydroascorbate	4.97623E-08	**Aa**	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Ba**	4.88017E-07	**Aa**
Docosanoic acid	-1.04108E-08	**Ab**	8.87E-09	**Ab**	8.12303E-08	**Aa**	1.10652E-07	**Aa**	1.44778E-07	**Aa**	1.52289E-07	**Aa**
Eicosanoic acid methyl ester	2.713E-06	**Ba**	3.54965E-06	**Aa**	1.57833E-06	**Bb**	2.75111E-06	**Ab**	8.40084E-07	**Bc**	9.20614E-07	**Ac**
Fumarate	-1.85532E-06	**Bc**	NA	**Ab**	1.07133E-06	**Aa**	NA	**Bb**	3.90383E-07	**Bb**	7.24472E-07	**Aa**
Galacturonic acid	3.02455E-08	**Aa**	NA	**Aa**	1.59854E-07	**Aa**	7.65773E-08	**Aa**	7.51238E-08	**Aa**	9.53884E-08	**Aa**
Gluconate	6.19734E-07	**Ab**	6.14438E-07	**Ab**	2.74505E-06	**Aa**	3.78094E-06	**Aa**	3.28626E-06	**Aa**	3.17553E-06	**Aa**
Glutaric acid	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	9.68973E-07	**Aa**	9.06244E-07	**Aa**
Glycerate	1.28407E-06	**Ab**	7.74268E-07	**Ac**	3.16007E-06	**Ba**	4.16026E-06	**Aa**	1.54389E-06	**Ab**	2.11151E-06	**Ab**
Glyceric acid-3-phosphate	4.04299E-06	**Ab**	2.37279E-06	**Ac**	1.17311E-05	**Aa**	1.32341E-05	**Aa**	4.33042E-06	**Ab**	6.30442E-06	**Ab**
Glycolic acid	3.5204E-06	**Ab**	2.94113E-06	**Ab**	4.83526E-06	**Ba**	6.64877E-06	**Aa**	3.47107E-06	**Ab**	3.28607E-06	**Ab**
Hexacosanoic acid methyl ester, n-	6.94187E-07	**Aa**	6.56747E-07	**Aa**	3.45612E-07	**Ab**	5.10962E-07	**Ab**	2.37404E-07	**Ac**	2.37691E-07	**Ac**
Hexadecanoic acid methyl ester, n-	6.9595E-06	**Aa**	7.54169E-06	**Aa**	3.40055E-06	**Bb**	6.59649E-06	**Aa**	1.89947E-06	**Ac**	2.05614E-06	**Ab**
Hexanoic acid, 2-ethyl-	7.18977E-07	**Aa**	6.81053E-07	**Aa**	4.19371E-07	**Bb**	7.11831E-07	**Aa**	1.86879E-07	**Ac**	2.16134E-07	**Ab**
Lactate	1.18948E-05	**Ba**	1.57375E-05	**Aa**	1.28819E-05	**Ba**	1.95555E-05	**Aa**	6.29269E-06	**Bb**	7.72497E-06	**Ab**
Malate	2.57185E-05	**Ab**	1.97898E-05	**Ab**	4.09215E-05	**Ba**	5.55113E-05	**Aa**	2.58677E-05	**Ab**	2.76852E-05	**Ab**
Malonic acid	7.76886E-08	**Bc**	8.04057E-08	**Ac**	2.94473E-07	**Ba**	3.68461E-07	**Aa**	1.23311E-07	**Bb**	2.12787E-07	**Ab**
Nonadecanoic acid methyl ester	2.85495E-08	**Aa**	2.91996E-08	**Aa**	1.19476E-08	**Bb**	2.05978E-08	**Ab**	6.0222E-09	**Bc**	1.00799E-08	**Ac**
Octadecanoic acid	3.76216E-06	**Aa**	4.53206E-06	**Aa**	2.76312E-06	**Aa**	4.73062E-06	**Aa**	1.3671E-06	**Ab**	1.53258E-06	**Ab**
Palmitic acid	2.32406E-05	**Ab**	2.3743E-05	**Ab**	5.28728E-05	**Aa**	7.89529E-05	**Aa**	2.94674E-05	**Ab**	3.97193E-05	**Ab**
Pyruvate	3.3322E-05	**Aa**	2.81311E-05	**Ab**	2.34107E-05	**Ba**	6.18614E-05	**Aa**	1.61612E-05	**Aa**	2.02082E-05	**Ab**
Ribonic acid	5.3245E-08	**Ab**	4.84009E-08	**Ac**	1.24858E-07	**Ba**	1.79805E-07	**Aa**	7.6503E-08	**Ab**	1.00709E-07	**Ab**
Sinapic acid	1.0426E-07	**Aa**	2.19898E-07	**Aa**	4.38472E-08	**Aa**	1.837E-07	**Aa**	1.58118E-07	**Aa**	3.23272E-08	**Aa**
Succinate	1.15252E-05	**Ab**	1.01796E-05	**Ab**	2.78142E-05	**Ba**	4.18524E-05	**Aa**	1.55235E-05	**Ab**	1.70808E-05	**Ab**
Tetracosanoic acid methyl ester	1.69487E-06	**Aa**	1.6159E-06	**Aa**	8.24083E-07	**Ab**	1.27428E-06	**Ab**	4.7444E-07	**Ac**	5.27046E-07	**Ac**

SI Table 13. *(continuation)* Relative abundance of primary metabolites of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Organic acid	**0h_Control**		**0h_5µM rap**		**24h_Control**		**24h_5µM rap**		**48h_Control**		**48h_5µM rap**	
Tetradecanoic acid methyl ester	3.13809E-08	**Bb**	3.80085E-08	**Ab**	4.15597E-08	**Ba**	6.55502E-08	**Aa**	2.02236E-08	**Bb**	3.18439E-08	**Ab**
Threonic acid	3.21615E-06	**Ac**	3.94434E-06	**Ac**	7.87618E-06	**Ba**	1.3115E-05	**Aa**	5.41505E-06	**Ab**	5.94493E-06	**Ab**
2-Oxoglutaric acid	5.02766E-06	**Ac**	3.96526E-06	**Ac**	4.98767E-05	**Ba**	7.96033E-05	**Aa**	3.66671E-05	**Ab**	4.45116E-05	**Ab**
4-hydroxy-benzoic acid	-1.17141E-06	**Ab**	-1.11703E-06	**Ab**	-4.5882E-07	**Aab**	-5.50514E-07	**Aab**	3.47465E-07	**Aa**	1.79279E-07	**Aa**
4-Hydroxycinnamic acid	3.85762E-08	**Aa**	5.44068E-08	**Aa**	1.1609E-08	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
Sugar	**0h_Control**		**0h_5µM rap**		**24h_Control**		**24h_5µM rap**		**48h_Control**		**48h_5µM rap**	
Altrose	3.69845E-06	**Ac**	2.91484E-06	**Ac**	1.74515E-05	**Aa**	1.58119E-05	**Aa**	6.04928E-06	**Ab**	8.17977E-06	**Ab**
Arabinose	5.52232E-06	**Aa**	3.92381E-06	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
Fructose	2.79325E-07	**Ab**	1.95883E-07	**Aa**	9.08977E-06	**Aa**	7.67589E-07	**Ba**	2.54872E-07	**Ab**	3.48E-07	**Aa**
Fructose-6-Phosphate	6.25372E-08	**Ab**	4.14602E-08	**Ab**	2.59382E-07	**Aa**	2.00401E-07	**Aa**	5.84848E-08	**Ab**	9.81091E-08	**Ab**
Fucose	4.35137E-06	**Aa**	3.03677E-06	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
Galactose, 3,6-anhydro-	3.08751E-07	**Aa**	NA	**Ba**	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Aa**
Glucose	NA	**Ab**	NA	**Aa**	3.86476E-06	**Aa**	NA	**Ba**	NA	**Ab**	NA	**Aa**
Glucose-6-Phosphate	6.36146E-06	**Ab**	3.17553E-06	**Ab**	3.03723E-05	**Aa**	2.29238E-05	**Aa**	6.19411E-06	**Ab**	1.03583E-05	**Ab**
Isomaltose	NA	**Ab**	NA	**Aa**	1.47805E-08	**Aa**	NA	**Ba**	NAN	**Ab**	NA	**Aa**
Mannose	1.23418E-06	**Aa**	1.11197E-06	**Ac**	NA	**Bb**	3.83202E-06	**Aa**	1.50891E-06	**Ba**	1.87461E-06	**Ab**
Maltose	1.98547E-07	**Aa**	2.43468E-06	**Aa**	1.0821E-06	**Aa**	1.28142E-06	**Aa**	4.081E-07	**Aa**	4.80489E-07	**Aa**
Melibiose	7.15696E-07	**Ab**	6.80857E-07	**Ab**	1.16995E-06	**Ba**	1.72922E-06	**Aa**	6.16311E-07	**Ab**	7.05068E-07	**Ab**
Raffinose	NA	**Ab**	NA	**Aa**	2.03304E-09	**Aa**	NA	**Ba**	1.90354E-09	**Aa**	NA	**Ba**
Sucrose	1.72199E-07	**Ab**	1.41549E-07	**Aa**	5.73769E-06	**Aa**	1.18097E-07	**Ba**	7.48147E-08	**Ab**	6.82539E-07	**Aa**
Trehalose	7.43306E-06	**Ac**	9.89392E-06	**Ac**	5.769E-05	**Aa**	7.69857E-05	**Aa**	2.37108E-05	**Ab**	2.72702E-05	**Ab**
Xylose	5.52232E-06	**Ab**	3.92381E-06	**Ab**	1.03911E-05	**Aa**	1.15429E-05	**Aa**	6.33401E-06	**Ab**	7.51277E-06	**Ab**
1,6-Anhydro-beta-D-glucose	4.3575E-07	**Bb**	4.23903E-07	**Ab**	1.05499E-06	**Ba**	1.97579E-06	**Aa**	3.38045E-07	**Bb**	4.73476E-07	**Ab**
Arabitol	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	6.33188E-06	**Aa**	7.28698E-06	**Aa**
Erythritol	NA	**Bc**	0.00023555	**Aa**	0.000112852	**Ba**	0.000193356	**Ab**	5.49118E-05	**Ab**	6.90536E-05	**Ac**
Glycerol	8.10192E-07	**Ab**	7.84235E-07	**Ab**	1.40109E-06	**Aa**	1.67191E-06	**Aa**	1.33655E-06	**Aa**	1.53508E-06	**Aa**
Glycerol 3-P	7.81244E-05	**Aab**	7.4024E-05	**Ab**	9.0128E-05	**Ba**	0.00014376	**Aa**	5.72E-05	**Ab**	6.6308E-05	**Ab**
Mannitol	7.54687E-05	**Ab**	8.07398E-05	**Ab**	0.000143469	**Ba**	0.000254741	**Aa**	5.62093E-05	**Ab**	5.70438E-05	**Ab**

SI Table 13. *(continuation)* Relative abundance of primary metabolites of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Others	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Putrescine	0.000886803	**Aa**	0.000896921	**Aa**	0.000444353	**Bb**	0.000814717	**Aa**	0.000211891	**Ac**	0.000186882	**Ab**
Spermidine	5.52658E-05	**Aa**	3.67532E-05	**Aa**	2.12441E-05	**Ab**	2.98966E-05	**Ab**	1.3258E-05	**Ab**	1.70134E-05	**Ab**
Adenine	2.1516E-05	**Aa**	1.8166E-05	**Ac**	2.78758E-05	**Ba**	4.22594E-05	**Aa**	2.42036E-05	**Aa**	2.90363E-05	**Ab**
Adenosine-5-Monophosphate	3.23885E-08	**Aa**	2.65329E-08	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
Guanidine	1.09813E-05	**Aa**	1.28521E-05	**Aa**	3.62989E-05	**Aa**	7.54908E-06	**Aa**	2.82556E-06	**Aa**	6.1047E-06	**Aa**
Uracil	2.41139E-05	**Ab**	2.91132E-05	**Ac**	6.07426E-05	**Ba**	9.80172E-05	**Aa**	4.60741E-05	**Aa**	4.85151E-05	**Ab**
4-Aminobutanoate	1.64778E-05	**Ab**	1.74099E-05	**Ac**	5.74121E-05	**Ba**	0.000118077	**Aa**	5.34703E-05	**Aa**	4.87387E-05	**Ab**
Galactinol	NA	**Aa**	NA	**Ab**	NA	**Ba**	6.27147E-07	**Aa**	NA	**Aa**	NA	**Ab**
Isopropyl-beta-D-thiogalactoside	6.89921E-07	**Bc**	8.30306E-07	**Ac**	3.45266E-06	**Ba**	4.79215E-06	**Aa**	2.34418E-06	**Bb**	2.541E-06	**Ab**
Myo-Inositol	9.28223E-06	**Ac**	9.76764E-06	**Ac**	5.91547E-05	**Ba**	9.98736E-05	**Aa**	2.71936E-05	**Ab**	2.63811E-05	**Ab**
Phosphoric acid	0.000129431	**Aa**	0.00013262	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**

SI Table 14. Relative abundance of primary metabolites of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Alanine	0.000246295	**Aa**	0.000234334	**Aa**	9.07276E-05	**Bb**	0.000134983	**Ab**	NA	**Bc**	6.33128E-05	**Ac**
Asparagine	NA	**Aa**	NA	**Ab**	1.5724E-07	**Ba**	2.6267E-06	**Aa**	7.51047E-08	**Aa**	4.30303E-07	**Ab**
Aspartate	2.56611E-05	**Aa**	2.44641E-05	**Ab**	2.56673E-05	**Ba**	5.03328E-05	**Aa**	1.51881E-05	**Bb**	2.42023E-05	**Ab**
b-Alanine	8.36131E-07	**Bb**	8.07151E-07	**Ab**	7.15075E-07	**Bb**	1.86281E-06	**Ab**	1.8273E-06	**Ba**	3.30373E-06	**Aa**
Butanoic acid, 2-amino-	2.21191E-06	**Aa**	1.63297E-06	**Ab**	3.0164E-06	**Ba**	3.95564E-06	**Aa**	2.60738E-06	**Ba**	4.69767E-06	**Aa**
Citrulline	3.95712E-08	**Aa**	5.34027E-08	**Ac**	1.27845E-07	**Ba**	1.15501E-06	**Aa**	2.01395E-08	**Ba**	2.61245E-07	**Ab**
Cysteine	1.74045E-08	**Aa**	1.40267E-08	**Aa**	7.17153E-09	**Ab**	9.8235E-09	**Ab**	6.43649E-09	**Ab**	9.76229E-09	**Ab**
Cysteinyl-glycine	1.67376E-07	**Bb**	1.65601E-07	**Ab**	2.38324E-07	**Bab**	3.67581E-07	**Aab**	2.932E-07	**Ba**	5.59834E-07	**Aa**
Glutamate	9.74416E-05	**Aa**	9.62866E-05	**Aa**	3.3717E-05	**Bb**	4.49974E-05	**Ab**	1.30186E-05	**Bc**	2.12031E-05	**Ac**
Glutamine	1.33331E-07	**Aa**	1.36034E-07	**Ac**	1.31993E-07	**Ba**	4.50035E-06	**Aa**	6.535E-08	**Ba**	9.95E-07	**Ab**

SI Table 14. *(continuation)* Relative abundance of primary metabolites of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Glycine	2.25832E-06	**Aa**	2.64137E-06	**Aa**	4.71681E-06	**Aa**	9.20016E-06	**Aa**	2.21143E-06	**Aa**	1.10995E-05	**Aa**
Histidine	1.84674E-06	**Aa**	1.8556E-06	**Ac**	1.5528E-06	**Ba**	5.12326E-06	**Aa**	1.02152E-06	**Bb**	2.66789E-06	**Ab**
Hydroxyproline	2.36996E-08	**Bb**	2.44002E-08	**Ab**	2.89019E-08	**Ba**	3.94134E-08	**Aa**	3.02603E-08	**Ba**	4.01323E-08	**Aa**
Isoleucine	9.813E-06	**Aa**	7.72135E-06	**Bc**	9.64759E-06	**Ba**	1.39155E-05	**Ab**	1.01571E-05	**Ba**	1.64125E-05	**Aa**
Leucine	8.24074E-06	**Ab**	6.81239E-06	**Ab**	8.77252E-06	**Aab**	9.90598E-06	**Aab**	9.95363E-06	**Aa**	1.1612E-05	**Aa**
Methionine	2.35003E-06	**Aa**	2.36498E-06	**Ac**	2.33875E-06	**Ba**	3.66611E-06	**Ab**	2.7722E-06	**Ba**	4.66942E-06	**Aa**
O-acetyl-serine	NA	**Ac**	NA	**Ac**	8.19872E-07	**Ba**	1.22147E-06	**Aa**	3.45613E-07	**Bb**	5.77605E-07	**Ab**
Ornithine	2.66213E-05	**Aa**	2.38553E-05	**Aa**	1.42089E-05	**Bb**	1.85785E-05	**Ab**	3.03066E-06	**Bc**	8.70972E-06	**Ac**
Phenylalanine	2.31385E-06	**Ab**	2.09734E-06	**Ab**	2.48383E-06	**Ab**	1.96103E-06	**Ab**	3.3404E-06	**Aa**	3.28184E-06	**Aa**
Proline	0.000115008	**Aa**	0.000108512	**Aa**	5.43304E-05	**Bb**	0.00011777	**Aa**	2.08132E-05	**Bc**	5.4423E-05	**Ab**
Pyroglutamic acid	8.18933E-05	**Aa**	8.2087E-05	**Ab**	7.66115E-05	**Ba**	0.000131303	**Aa**	3.88864E-05	**Bb**	6.45299E-05	**Ac**
Serine	1.01234E-05	**Aa**	9.83551E-06	**Ab**	1.09222E-05	**Ba**	1.89904E-05	**Aa**	9.54036E-06	**Ba**	1.59119E-05	**Aa**
Threonine	5.97028E-06	**Aa**	5.28611E-06	**Ac**	7.21496E-06	**Ba**	1.45648E-05	**Aa**	5.97579E-06	**Ba**	1.14016E-05	**Ab**
Tryptophan	1.60027E-06	**Ab**	1.48322E-06	**Bb**	1.84337E-06	**Ab**	1.31724E-06	**Bb**	2.04588E-06	**Aa**	1.91749E-06	**Ba**
Tyrosine	7.58829E-06	**Ab**	6.62593E-06	**Ab**	1.01081E-05	**Aa**	8.43276E-06	**Ab**	9.24E-06	**Bab**	1.27038E-05	**Aa**
Valine	2.08105E-05	**Aa**	1.68888E-05	**Bb**	2.06177E-05	**Ba**	2.88779E-05	**Aa**	1.57811E-05	**Bb**	2.42002E-05	**Aa**
Organic acid	**0h_Control**		**0h_5µM rap**		**24h_Control**		**24h_5µM rap**		**48h_Control**		**48h_5µM rap**	
Ascorbic acid	2.87075E-07	**Ab**	1.37281E-07	**Ab**	6.10596E-07	**Ab**	7.95115E-07	**Ab**	1.41027E-06	**Aa**	1.69581E-06	**Aa**
Benzoate	7.23882E-07	**Aa**	7.01512E-07	**Aa**	3.68273E-07	**Ab**	3.3329E-07	**Ab**	1.4446E-07	**Ab**	3.01151E-07	**Ab**
Capric acid	1.2873E-08	**Ba**	1.33751E-08	**Aa**	1.27994E-08	**Ba**	1.55214E-08	**Aa**	1.39424E-08	**Ba**	2.16271E-08	**Aa**
Citramalate	4.98925E-08	**Bb**	6.23961E-08	**Ab**	6.71628E-08	**Ba**	1.04416E-07	**Aa**	3.72665E-08	**Bb**	5.4643E-08	**Ab**
Citrate	7.96618E-06	**Aa**	7.74572E-06	**Aa**	7.64668E-06	**Aa**	5.78787E-06	**Bb**	4.24681E-06	**Bb**	7.86681E-06	**Aa**
Dehydroascorbate	1.34733E-06	**Ab**	1.48802E-06	**Ab**	2.94412E-06	**Ba**	4.14201E-06	**Aa**	3.20027E-06	**Ba**	4.22292E-06	**Aa**
Docosanoic acid	3.01679E-09	**Ab**	1.73812E-09	**Ab**	1.21579E-08	**Aa**	1.2176E-08	**Aa**	7.35833E-09	**Aab**	7.60667E-09	**Aab**
Eicosanoic acid methyl ester	3.90365E-07	**Aa**	3.7049E-07	**Aa**	1.33029E-07	**Ab**	1.95565E-07	**Ab**	5.55123E-08	**Ac**	1.01977E-07	**Ac**
Fumarate	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	1.42934E-06	**Aa**	3.21738E-06	**Aa**
Galacturonic acid	3.25651E-08	**Aa**	2.4168E-08	**Aa**	1.2581E-08	**Ab**	1.50897E-08	**Ab**	1.00606E-08	**Ab**	1.27855E-08	**Ab**
Gluconate	5.63438E-07	**Aa**	4.49E-07	**Aa**	3.70673E-07	**Aab**	4.00729E-07	**Aab**	2.56323E-07	**Ab**	2.71473E-07	**Ab**
Glycerate	1.69103E-07	**Ac**	1.42861E-07	**Ac**	3.52679E-07	**Ab**	2.99015E-07	**Ab**	4.43036E-07	**Aa**	5.03019E-07	**Aa**

SI Table 14. *(continuation)* Relative abundance of primary metabolites of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Organic acid	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Glyceric acid-3-phosphate	7.57055E-07	**Aa**	7.22208E-07	**Aa**	7.73247E-07	**Aa**	8.39075E-07	**Aa**	7.15894E-07	**Aa**	6.15435E-07	**Aa**
Glycolic acid	3.92769E-07	**Ab**	3.87804E-07	**Aa**	5.21601E-07	**Aa**	3.91395E-07	**Ba**	2.77868E-07	**Ab**	3.31672E-07	**Aa**
Hexacosanoic acid methyl ester, n-	8.1146E-08	**Aa**	9.22869E-08	**Aa**	3.6356E-08	**Ab**	4.95886E-08	**Ab**	1.37138E-08	**Ac**	1.90806E-08	**Ac**
Hexadecanoic acid methyl ester, n-	7.48711E-10	**Ba**	4.475E-09	**Aa**	1.53521E-09	**Aa**	9.09831E-10	**Ab**	3.80531E-10	**Aa**	NA	**Ab**
Hexanoic acid, 2-ethyl-	8.62379E-08	**Aa**	9.31645E-08	**Aa**	3.45848E-08	**Ab**	4.58753E-08	**Ab**	1.45547E-08	**Ac**	2.29506E-08	**Ac**
Lactate	3.38973E-06	**Aa**	3.25081E-06	**Aa**	2.14363E-06	**Ab**	2.64079E-06	**Ab**	1.0618E-06	**Ac**	1.37479E-06	**Ac**
Malate	6.28513E-06	**Aa**	4.35484E-06	**Aa**	4.62032E-06	**Aab**	5.34363E-06	**Aab**	3.64242E-06	**Ab**	3.87791E-06	**Ab**
Malonic acid	7.37844E-09	**Aa**	7.9994E-09	**Ab**	1.17179E-08	**Aa**	1.22202E-08	**Aab**	9.52753E-09	**Ba**	1.56919E-08	**Aa**
Nicotinate	NA	**Ac**	NA	**Ac**	1.56856E-05	**Ba**	2.25093E-05	**Aa**	5.1069E-06	**Bb**	1.01887E-05	**Ab**
Nonadecanoic acid methyl ester	3.97496E-09	**Aa**	3.86506E-09	**Aa**	1.27937E-09	**Ab**	1.45169E-09	**Ab**	4.87216E-10	**Ac**	7.58459E-10	**Ac**
Octadecanoic acid	8.57345E-07	**Aa**	3.63351E-07	**Aa**	8.57033E-08	**Ab**	1.68197E-07	**Ab**	3.79267E-08	**Ab**	1.18339E-07	**Ab**
Palmitic acid	1.11258E-05	**Aa**	6.77778E-06	**Aa**	3.4639E-06	**Ab**	4.33795E-06	**Ab**	1.92647E-06	**Ab**	2.85753E-06	**Ab**
Pyruvate	6.64923E-06	**Ab**	4.68979E-06	**Ac**	8.28796E-06	**Ab**	8.18236E-06	**Ab**	1.60174E-05	**Ba**	2.45112E-05	**Aa**
Ribonic acid	1.86863E-08	**Ac**	1.59376E-08	**Ac**	2.57329E-08	**Ab**	3.02602E-08	**Ab**	3.31584E-08	**Aa**	3.72715E-08	**Aa**
Sinapic acid	8.10898E-09	**Aa**	4.8043E-09	**Aa**	NA	**Ab**	NA	**Ab**	-4.55733E-10	**Ab**	1.69983E-09	**Ab**
Succinate	1.94161E-06	**Ac**	1.58299E-06	**Ac**	4.44701E-06	**Aa**	4.63663E-06	**Aa**	2.59862E-06	**Ab**	3.54556E-06	**Ab**
Tetracosanoic acid methyl ester	2.04567E-07	**Aa**	1.83876E-07	**Aa**	8.26998E-08	**Ab**	1.03075E-07	**Ab**	2.95238E-08	**Ac**	4.71103E-08	**Ac**
Tetradecanoic acid methyl ester	6.77847E-09	**Aa**	4.24541E-09	**Aa**	2.76823E-09	**Ab**	3.24415E-09	**Ab**	1.88641E-09	**Ab**	2.64718E-09	**Ab**
Threonic acid	1.11041E-06	**Aa**	1.06749E-06	**Aa**	1.09886E-06	**Aa**	1.16106E-06	**Aa**	5.01271E-07	**Ab**	5.36881E-07	**Ab**
2-Oxoglutaric acid	1.15479E-06	**Aa**	9.01881E-07	**Ab**	1.65449E-06	**Aa**	1.34817E-06	**Ab**	1.50128E-06	**Ba**	2.31944E-06	**Aa**
4-hydroxy-benzoic acid	-1.82437E-07	**Bb**	1.29281E-07	**Aa**	1.71937E-08	**Aa**	2.91812E-08	**Aab**	1.6818E-08	**Aa**	-6.70379E-09	**Ab**
4-Hydroxycinnamic acid	3.19501E-09	**Aa**	2.53542E-09	**Aa**	5.9636E-10	**Ab**	6.77986E-10	**Ab**	4.37846E-10	**Ab**	2.41063E-09	**Ab**

SI Table 14. *(continuation)* Relative abundance of primary metabolites of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Sugar	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Altrose	4.27695E-07	**Aa**	3.56446E-07	**Aa**	4.22012E-07	**Aa**	4.13957E-07	**Aa**	1.8019E-07	**Ab**	2.8223E-07	**Ab**
Fructose	5.62248E-08	**Aa**	4.60573E-08	**Aa**	4.35943E-08	**Aa**	7.04147E-08	**Aa**	3.3785E-08	**Aa**	3.61255E-08	**Aa**
Fructose-6-Phosphate	3.72283E-08	**Aa**	4.85037E-08	**Aa**	4.2742E-08	**Aa**	4.37231E-08	**Aa**	1.96393E-08	**Ab**	2.57208E-08	**Ab**
Fucose	3.93182E-07	**Aa**	3.33681E-07	**Ab**	3.72135E-07	**Ba**	6.86453E-07	**Aa**	3.17515E-07	**Ba**	5.80338E-07	**Aa**
Galactose, 3,6-anhydro-	NA	**Aa**	NA	**Ac**	3.93824E-08	**Ba**	9.68212E-07	**Aa**	4.22232E-08	**Ba**	1.76593E-07	**Ab**
Glucose	NA	**Aa**	NA	**Ab**	NA	**Ba**	5.61373E-07	**Aa**	NA	**Aa**	NA	**Ab**
Glucose-6-Phosphate	3.11984E-06	**Ab**	3.87125E-06	**Ab**	4.68763E-06	**Aa**	5.0011E-06	**Aa**	2.0033E-06	**Ac**	2.65041E-06	**Ac**
Isomaltose	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Aa**	3.3432E-09	**Aa**	NA	**Ba**
Mannose	1.22714E-07	**Aa**	1.3372E-07	**Aa**	1.37332E-07	**Aa**	1.61298E-07	**Aa**	1.17687E-07	**Aa**	1.19242E-07	**Aa**
Maltose	2.8108E-08	**Ba**	3.69485E-08	**Aa**	3.07128E-08	**Ba**	3.41219E-08	**Aa**	NA	**Bb**	2.32564E-08	**Ab**
Melibiose	4.78329E-08	**Ab**	5.14463E-08	**Ab**	7.61172E-08	**Aa**	7.58228E-08	**Aa**	NA	**Ac**	NA	**Ac**
Raffinose	1.07097E-09	**Aa**	NA	**Ba**	NA	**Ab**	4.52936E-10	**Aa**	3.94305E-10	**Aab**	3.7818E-10	**Aa**
Sucrose	2.15021E-06	**Aa**	2.00171E-06	**Aa**	6.53461E-07	**Bb**	9.85857E-07	**Ab**	2.66932E-07	**Ac**	NA	**Bc**
Trehalose	1.82468E-07	**Ac**	2.19018E-07	**Ac**	9.857E-07	**Aa**	1.29028E-06	**Aa**	4.8974E-07	**Ab**	3.9597E-07	**Ab**
Xylose	1.3777E-06	**Aa**	1.6047E-06	**Aa**	7.64558E-07	**Ab**	NA	**Bb**	NA	**Ac**	NA	**Ab**
1,6-Anhydro-beta-D-glucose	7.27594E-08	**Aa**	6.15342E-08	**Ab**	6.68076E-08	**Ba**	1.05984E-07	**Aa**	1.09E-07	**Aa**	8.86E-08	**Aab**
Arabitol	NA	**Ab**	NA	**Ab**	7.08158E-07	**Aa**	9.59933E-07	**Aa**	6.78933E-07	**Ba**	1.27962E-06	**Aa**
Erythritol	3.06038E-05	**Aa**	2.67946E-05	**Ba**	8.96333E-06	**Bb**	1.24759E-05	**Ab**	3.66436E-06	**Bc**	6.26266E-06	**Ac**
Glycerol	3.49367E-07	**Aa**	2.92669E-07	**Ba**	2.59499E-07	**Ab**	2.84192E-07	**Aa**	1.69722E-07	**Ac**	1.97039E-07	**Ab**
Glycerol 3-P	2.05627E-05	**Aa**	1.79128E-05	**Ba**	9.32275E-06	**Bb**	1.259E-05	**Ab**	4.19109E-06	**Bc**	6.67083E-06	**Ac**
Mannitol	1.81542E-07	**Ab**	1.36972E-07	**Ab**	2.86528E-07	**Aa**	4.61905E-07	**Aa**	3.48493E-07	**Aa**	3.9112E-07	**Aa**
Others	**0h_Control**		**0h_5µM rap**		**24h_Control**		**24h_5µM rap**		**48h_Control**		**48h_5µM rap**	
Putrescine	1.37366E-05	**Aa**	1.22231E-05	**Aa**	6.60017E-06	**Bb**	1.2571E-05	**Aa**	3.03562E-06	**Bb**	7.25253E-06	**Ab**
Spermidine	1.68908E-05	**Aa**	1.4134E-05	**Aa**	4.64733E-06	**Ab**	4.78855E-06	**Ab**	1.6661E-06	**Ab**	3.39491E-06	**Ab**
Adenine	2.53247E-06	**Ba**	2.78568E-06	**Aa**	1.79745E-06	**Bb**	2.08919E-06	**Ab**	1.75698E-06	**Bab**	2.97539E-06	**Aab**
Adenosine-5-Monophosphate	1.13779E-08	**Aa**	NA	**Ba**	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Aa**
Guanidine	3.29671E-06	**Aa**	1.57243E-06	**Aa**	4.47788E-07	**Aa**	1.7189E-06	**Aa**	7.12291E-07	**Aa**	6.69291E-06	**Aa**
Uracil	3.80566E-06	**Aa**	2.83091E-06	**Bab**	2.76841E-06	**Ab**	3.12968E-06	**Aa**	1.43149E-06	**Bc**	2.30873E-06	**Ab**
4-Aminobutanoate	1.9124E-06	**Aa**	1.75031E-06	**Ab**	2.73413E-06	**Ba**	5.12937E-06	**Aa**	2.02047E-06	**Ba**	4.86825E-06	**Aa**

SI Table 14. *(continuation)* Relative abundance of primary metabolites of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Others	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Isopropyl-beta-D-thiogalactoside	NA	**Ab**	NA	**Ac**	4.11166E-07	**Aa**	3.81693E-07	**Ab**	4.32391E-07	**Ba**	5.59514E-07	**Aa**
Myo-Inositol	1.29254E-06	**Ab**	1.39692E-06	**Ab**	2.6649E-06	**Aa**	2.22016E-06	**Aa**	1.95566E-06	**Aa**	2.24098E-06	**Aa**
Phosphoric acid	1.78206E-05	**Aa**	1.75532E-05	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**

SI Table 15. Relative abundance of primary metabolites of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Alanine	0.000309687	**Aa**	0.00033118	**Aa**	9.22756E-05	**Ab**	NA	**Bb**	NA	**Ac**	NA	**Ab**
Asparagine	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Aa**	3.7484E-08	**Aa**	NA	**Ba**
Aspartate	6.49463E-05	**Aa**	6.93182E-05	**Ab**	3.63988E-05	**Bb**	0.000100314	**Aa**	NA	**Bc**	2.25273E-05	**Ac**
b-Alanine	1.933E-06	**Aa**	1.77693E-06	**Ab**	2.95691E-06	**Ba**	7.97662E-06	**Aa**	3.47137E-06	**Aa**	5.67878E-06	**Aa**
Butanoic acid, 2-amino-	4.88998E-06	**Aa**	5.20099E-06	**Ab**	4.92191E-06	**Ba**	1.33295E-05	**Aa**	6.09202E-06	**Aa**	5.30458E-06	**Ab**
Citrulline	1.57766E-07	**Aa**	1.53721E-07	**Ab**	1.83054E-07	**Ba**	2.36865E-06	**Aa**	NA	**Aa**	9.61995E-08	**Ab**
Cysteine	2.73787E-08	**Ba**	3.68985E-08	**Aa**	9.49971E-09	**Bb**	2.0493E-08	**Ab**	1.38478E-08	**Ab**	1.28287E-08	**Ac**
Cysteinyl-glycine	1.85049E-07	**Aa**	2.1376E-07	**Ab**	1.68742E-07	**Ba**	5.05745E-07	**Aa**	2.11177E-07	**Aa**	2.30935E-07	**Ab**
Glutamate	0.000133153	**Aa**	0.000129875	**Aa**	3.13936E-05	**Bb**	8.45646E-05	**Ab**	1.29011E-05	**Ac**	1.92124E-05	**Ac**
Glutamine	4.36109E-07	**Aa**	4.86261E-07	**Ab**	2.92068E-07	**Bab**	2.48876E-06	**Aa**	6.69059E-08	**Ab**	2.30018E-07	**Ab**
Glycine	6.98454E-06	**Aa**	1.09928E-05	**Aa**	1.02714E-05	**Aa**	4.92601E-05	**Aa**	6.53912E-06	**Aa**	7.60675E-06	**Aa**
Histidine	4.29389E-06	**Aa**	4.74924E-06	**Ab**	3.20792E-06	**Ba**	1.20314E-05	**Aa**	2.51519E-06	**Aa**	2.65889E-06	**Ab**
Hydroxyproline	5.99372E-08	**Ba**	7.69925E-08	**Ab**	5.70328E-08	**Bab**	1.25754E-07	**Aa**	4.17651E-08	**Ab**	4.83455E-08	**Ac**
Isoleucine	2.18311E-05	**Aa**	2.3105E-05	**Ab**	1.5903E-05	**Bb**	4.20441E-05	**Aa**	1.3906E-05	**Bb**	1.92942E-05	**Ab**
Leucine	2.05944E-05	**Aa**	2.22422E-05	**Ab**	1.5215E-05	**Bb**	2.97849E-05	**Aa**	1.40966E-05	**Ab**	1.43092E-05	**Ac**
Methionine	6.94925E-06	**Aa**	7.37125E-06	**Ab**	5.62503E-06	**Bab**	1.31582E-05	**Aa**	4.37863E-06	**Ab**	4.02841E-06	**Ac**
O-acetyl-serine	NA	**Ba**	2.59788E-06	**Aa**	8.34099E-07	**Ba**	2.33029E-06	**Aa**	3.61408E-07	**Aa**	5.41738E-07	**Ab**
Ornithine	5.19978E-05	**Aa**	6.46139E-05	**Aa**	2.32966E-05	**Bb**	6.9817E-05	**Aa**	2.0151E-06	**Ac**	4.07599E-06	**Ab**

SI Table 15. *(continuation)* Relative abundance of primary metabolites of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Amino acid	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Phenylalanine	5.19467E-06	**Aa**	5.83463E-06	**Ab**	4.82574E-06	**Ba**	8.64584E-06	**Aa**	5.90019E-06	**Aa**	4.46952E-06	**Bc**
Proline	0.000138051	**Aa**	0.000153782	**Ab**	6.15234E-05	**Bb**	0.000242314	**Aa**	1.73815E-05	**Ac**	3.88427E-05	**Ac**
Pyroglutamic acid	0.000191757	**Aa**	0.000213088	**Ab**	9.3421E-05	**Bb**	0.000251752	**Aa**	3.85486E-05	**Ac**	5.77801E-05	**Ac**
Serine	3.50846E-05	**Ba**	4.30843E-05	**Ab**	1.83153E-05	**Bb**	5.50441E-05	**Aa**	1.19811E-05	**Ab**	1.80295E-05	**Ac**
Threonine	1.70696E-05	**Ba**	1.97981E-05	**Ab**	1.17861E-05	**Bb**	3.58517E-05	**Aa**	8.09308E-06	**Bc**	1.19475E-05	**Ac**
Tryptophan	3.27922E-06	**Ba**	4.46471E-06	**Aa**	3.90776E-06	**Ba**	5.2594E-06	**Aa**	3.91869E-06	**Aa**	2.83772E-06	**Bb**
Tyrosine	1.65234E-05	**Ab**	1.99974E-05	**Ab**	2.78484E-05	**Ba**	3.72267E-05	**Aa**	1.2759E-05	**Ab**	1.49955E-05	**Ac**
Valine	4.77258E-05	**Aa**	4.7392E-05	**Ab**	3.25321E-05	**Bb**	8.70613E-05	**Aa**	1.79913E-05	**Ac**	2.49538E-05	**Ac**
Organic acid	**0h_Control**		**0h_5µM rap**		**24h_Control**		**24h_5µM rap**		**48h_Control**		**48h_5µM rap**	
Ascorbic acid	2.63339E-07	**Ab**	1.95304E-07	**Ab**	8.92962E-07	**Ab**	1.55086E-06	**Ab**	1.75408E-06	**Aa**	3.37612E-06	**Aa**
Benzoate	8.12027E-07	**Ba**	1.07103E-06	**Aa**	4.1478E-07	**Bb**	7.24E-07	**Ab**	1.96667E-07	**Bc**	1.88995E-07	**Ac**
Capric acid	NA	**Bb**	1.79554E-08	**Ab**	1.85981E-08	**Ba**	3.75816E-08	**Aa**	7.59777E-09	**Ab**	7.98083E-09	**Ac**
Citramalate	5.78772E-08	**Ba**	9.40286E-08	**Ab**	8.96136E-08	**Ba**	2.50579E-07	**Aa**	NA	**Ab**	NA	**Ac**
Citrate	7.00233E-06	**Aa**	7.59741E-06	**Ab**	8.29975E-06	**Ba**	2.12247E-05	**Aa**	6.00364E-06	**Aa**	7.32957E-06	**Ab**
Docosanoic acid	7.64595E-09	**Bab**	8.62017E-09	**Aab**	8.45314E-09	**Ba**	1.64246E-08	**Aa**	2.4586E-09	**Bb**	5.3739E-09	**Ab**
Eicosanoic acid methyl ester	4.8875E-07	**Aa**	4.33521E-07	**Aa**	1.456E-07	**Bb**	4.01E-07	**Aa**	6.09602E-08	**Ab**	1.02329E-07	**Ab**
Fumarate	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	1.28033E-06	**Aa**	1.50016E-06	**Aa**
Galacturonic acid	2.14003E-08	**Aa**	1.09032E-08	**Bb**	1.62527E-08	**Bab**	2.76286E-08	**Aa**	6.22009E-09	**Ab**	1.18871E-08	**Ab**
Gluconate	4.36142E-07	**Aa**	3.93808E-07	**Aab**	3.27058E-07	**Ba**	4.76253E-07	**Aa**	1.87845E-07	**Bb**	3.05849E-07	**Ab**
Glutaric acid	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	1.43828E-07	**Ba**	1.75524E-07	**Aa**
Glycerate	2.1702E-07	**Bb**	3.62982E-07	**Ab**	3.97388E-07	**Ba**	8.80551E-07	**Aa**	3.3312E-07	**Aab**	2.97127E-07	**Ab**
Glyceric acid-3-phosphate	9.68E-07	**Ba**	1.70815E-06	**Aa**	5.06747E-07	**Ba**	1.47E-06	**Aa**	4.28917E-07	**Aa**	1.71567E-07	**Ab**
Glycolic acid	5.92298E-07	**Ba**	8.07126E-07	**Aa**	5.90042E-07	**Ba**	8.10736E-07	**Aa**	3.82419E-07	**Ab**	3.00479E-07	**Ab**
Hexacosanoic acid methyl ester, n-	1.34332E-07	**Aa**	1.21046E-07	**Aa**	4.0884E-08	**Ab**	7.13371E-08	**Ab**	1.32515E-08	**Ac**	2.08799E-08	**Ac**
Hexadecanoic acid methyl ester, n-	NA	**Bb**	4.24974E-09	**Aa**	9.62895E-10	**Aa**	-8.14088E-10	**Bc**	3.55495E-10	**Aab**	3.12633E-10	**Ab**
Hexanoic acid, 2-ethyl-	1.20853E-07	**Aa**	1.30677E-07	**Aa**	3.61755E-08	**Bb**	9.10449E-08	**Ab**	1.42149E-08	**Ac**	1.87261E-08	**Ac**
Lactate	4.95637E-06	**Aa**	5.38107E-06	**Ab**	2.17439E-06	**Bb**	6.75666E-06	**Aa**	8.66344E-07	**Ac**	6.92473E-07	**Ac**
Malate	1.04585E-05	**Ba**	1.09996E-05	**Aa**	5.1505E-06	**Bb**	9.9693E-06	**Ab**	3.89288E-06	**Bc**	5.28318E-06	**Ac**
Malonic acid	4.0808E-08	**Ba**	6.04498E-08	**Ab**	3.57721E-08	**Ba**	8.14386E-08	**Aa**	6.11231E-09	**Ab**	9.81221E-09	**Ac**

SI Table 15. *(continuation)* Relative abundance of primary metabolites of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Organic acid	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
Nonadecanoic acid methyl ester	4.567E-09	**Aa**	4.1059E-09	**Aa**	9.5557E-10	**Bb**	3.04204E-09	**Ab**	NA	**Ab**	5.40349E-10	**Ac**
Octadecanoic acid	8.20233E-07	**Aa**	6.32127E-07	**Aa**	2.93355E-07	**Bb**	8.64E-07	**Aa**	6.16869E-08	**Ab**	8.95444E-08	**Ab**
Palmitic acid	6.78687E-06	**Ba**	9.7005E-06	**Aa**	5.81159E-06	**Ba**	1.14534E-05	**Aa**	1.73014E-06	**Bb**	1.65218E-06	**Ab**
Pyruvate	9.24459E-06	**Ab**	1.39417E-05	**Ab**	2.14174E-05	**Ba**	4.33273E-05	**Aa**	2.12913E-05	**Aa**	1.97494E-05	**Ab**
Ribonic acid	2.42238E-08	**Ab**	2.69701E-08	**Ac**	2.87286E-08	**Bb**	5.70829E-08	**Aa**	4.5557E-08	**Aa**	4.37941E-08	**Ab**
Sinapic acid	1.13195E-08	**Aa**	5.04393E-09	**Aa**	6.42325E-09	**Aa**	2.78475E-08	**Aa**	4.53369E-09	**Aa**	3.57142E-09	**Aa**
Succinate	4.3276E-06	**Aab**	5.16616E-06	**Ab**	6.30077E-06	**Ba**	1.50923E-05	**Aa**	3.31794E-06	**Ab**	3.73712E-06	**Ab**
Tetracosanoic acid methyl ester	2.9726E-07	**Aa**	2.7756E-07	**Aa**	7.87604E-08	**Bb**	1.83488E-07	**Ab**	2.95864E-08	**Ab**	5.04017E-08	**Ac**
Tetradecanoic acid methyl ester	4.60495E-09	**Aa**	5.10391E-09	**Ab**	3.56596E-09	**Ba**	9.36909E-09	**Aa**	1.74475E-09	**Aa**	2.83669E-09	**Ab**
Threonic acid	1.43964E-06	**Ba**	1.79243E-06	**Aa**	8.84816E-07	**Bb**	2.06712E-06	**Aa**	4.30892E-07	**Ac**	5.10946E-07	**Ab**
2-Oxoglutaric acid	2.50065E-06	**Aa**	3.22144E-06	**Aa**	2.51249E-06	**Aa**	4.10803E-06	**Aa**	8.97909E-07	**Ab**	5.91972E-07	**Ab**
4-hydroxy-benzoic acid	-2.01039E-07	**Ab**	-1.3203E-07	**Ab**	2.58095E-08	**Aa**	-5.94373E-08	**Aa**	2.35537E-08	**Aa**	1.24905E-08	**Aa**
4-Hydroxycinnamic acid	6.34224E-07	**Aa**	3.44136E-09	**Aa**	NA	**Aa**	4.10022E-09	**Aa**	NA	**Aa**	NA	**Aa**
Sugar	**0h_Control**		**0h_5µM rap**		**24h_Control**		**24h_5µM rap**		**48h_Control**		**48h_5µM rap**	
Altrose	1.11845E-06	**Aa**	6.78676E-07	**Aa**	4.23147E-07	**Aa**	1.32085E-06	**Aa**	5.26069E-08	**Ab**	7.08154E-08	**Ab**
Fructose	1.63917E-07	**Aa**	2.32984E-06	**Aa**	1.09929E-07	**Aa**	1.5928E-06	**Aa**	2.054E-08	**Aa**	1.71569E-08	**Aa**
Fructose-6-Phosphate	5.48019E-08	**Aa**	7.13968E-08	**Ab**	5.04585E-08	**Ba**	1.57824E-07	**Aa**	2.78993E-09	**Aa**	3.44674E-09	**Ac**
Fucose	4.33798E-07	**Ba**	5.97539E-07	**Ab**	NA	**Bc**	1.62371E-06	**Aa**	2.46798E-07	**Ab**	3.08055E-07	**Ac**
Galactose, 3,6-anhydro-	NA	**Aa**	NA	**Ab**	NA	**Ba**	5.89532E-07	**Aa**	NA	**Aa**	NA	**Ab**
Glucose	NA	**Aa**	NA	**Ab**	NA	**Ba**	6.52468E-07	**Aa**	NA	**Aa**	NA	**Ab**
Glucose-6-Phosphate	5.02361E-06	**Aa**	8.5488E-06	**Ab**	4.44697E-06	**Bab**	1.49712E-05	**Aa**	3.15049E-07	**Ab**	3.83281E-07	**Ac**
Mannose	2.69712E-07	**Aa**	2.12288E-06	**Aa**	3.0248E-07	**Aa**	1.93849E-06	**Aa**	7.26277E-08	**Aa**	1.06581E-07	**Aa**
Maltose	5.72525E-08	**Bb**	1.13608E-07	**Ab**	1.09933E-07	**Ba**	1.84868E-07	**Aa**	NA	**Bc**	NA	**Ac**
Melibiose	1.60621E-07	**Aa**	1.6474E-07	**Aa**	9.9948E-08	**Bb**	1.64256E-07	**Aa**	5.26581E-08	**Ac**	6.87417E-08	**Ab**
Raffinose	NA	**Ba**	1.3615E-09	**Aa**	NA	**Aa**	NA	**Ab**	2.9385E-10	**Aa**	2.27157E-10	**Ab**
Sucrose	3.14537E-06	**Aa**	2.60005E-06	**Ba**	NA	**Bb**	1.88853E-06	**Ab**	2.60611E-07	**Ab**	4.40413E-07	**Ac**
Trehalose	4.99671E-06	**Ab**	6.98695E-06	**Ab**	8.83469E-06	**Ba**	1.6438E-05	**Aa**	1.22406E-07	**Ac**	1.60892E-07	**Ac**
Xylose	1.34516E-06	**Aa**	1.50471E-06	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
1,6-Anhydro-beta-D-glucose	1.79287E-07	**Aa**	1.63756E-07	**Ab**	1.82932E-07	**Ba**	5.95141E-07	**Aa**	6.33323E-08	**Aa**	5.35103E-08	**Ab**

SI Table 15. *(continuation)* Relative abundance of primary metabolites of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Sugar	**0h_Control**		**0h_5µM rap**		**24h_Control**		**24h_5µM rap**		**48h_Control**		**48h_5µM rap**	
Arabitol	NA	**Ab**	NA	**Ab**	1.3065E-06	**Ba**	3.081E-06	**Aa**	3.79979E-07	**Ab**	3.77418E-07	**Ab**
Erythritol	3.87312E-05	**Aa**	3.49911E-05	**Ba**	9.01801E-06	**Bb**	2.44804E-05	**Ab**	3.84981E-06	**Ac**	5.45673E-06	**Ac**
Glycerol	4.38974E-07	**Aa**	5.10274E-07	**Aa**	2.47818E-07	**Bb**	5.18624E-07	**Aa**	1.24221E-07	**Ac**	1.46753E-07	**Ab**
Glycerol 3-P	2.77834E-05	**Aa**	2.73157E-05	**Aa**	9.95499E-06	**Bb**	2.50757E-05	**Aa**	4.07541E-06	**Ac**	6.22728E-06	**Ab**
Mannitol	4.68086E-07	**Aa**	3.2155E-06	**Aa**	1.14694E-06	**Aa**	4.27033E-06	**Aa**	1.17751E-07	**Aa**	2.11245E-07	**Aa**
Others	**0h_Control**		**0h_5µM rap**		**24h_Control**		**24h_5µM rap**		**48h_Control**		**48h_5µM rap**	
Putrescine	1.56056E-05	**Ba**	2.34738E-05	**Aa**	NA	**Ab**	NA	**b**	2.11068E-06	**Ab**	4.15839E-06	**Ab**
Spermidine	1.0822E-05	**Ba**	1.66562E-05	**Aa**	4.84477E-06	**Bb**	7.72447E-06	**Ab**	1.36719E-06	**Bc**	1.15896E-06	**Ac**
Adenine	3.7775E-06	**Ba**	4.99199E-06	**Ab**	3.32E-06	**Ba**	6.78372E-06	**Aa**	1.76566E-06	**Ab**	2.188E-06	**Ac**
Guanidine	9.99804E-07	**Aa**	9.349E-06	**Aa**	1.01488E-06	**Aa**	1.04536E-05	**Aa**	8.17507E-07	**Aa**	4.79864E-07	**Aa**
Uracil	3.08936E-06	**Aa**	2.90288E-06	**Ab**	3.40562E-06	**Ba**	6.48899E-06	**Aa**	1.75506E-06	**Ab**	2.30336E-06	**Ab**
4-Aminobutanoate	7.15635E-06	**Aa**	6.34141E-06	**Ab**	6.59812E-06	**Ba**	1.712E-05	**Aa**	3.06179E-06	**Ba**	7.3134E-06	**Ab**
Galactinol	NA	**Ab**	NA	**Ab**	2.03213E-07	**Ba**	4.26472E-07	**Aa**	NA	**Ab**	NA	**Ab**
Isopropyl-beta-D-thiogalactoside	3.53017E-07	**Aab**	4.32069E-07	**Ab**	5.1476E-07	**Ba**	9.36596E-07	**Aa**	2.91045E-07	**Ab**	3.86775E-07	**Ab**
Myo-Inositol	1.14623E-06	**Aa**	1.13469E-06	**Ac**	1.9659E-06	**Ba**	7.02265E-06	**Aa**	1.92778E-06	**Aa**	2.3569E-06	**Ab**
Phosphoric acid	2.1735E-05	**Aa**	2.16649E-05	**Aa**	5.4106E-06	**Ab**	NA	**Bb**	NA	**Ac**	NA	**Ab**

SI Table 16. Relative abundance of lipids of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
DGDG 32:0	116629.7213	**Bc**	204649.0991	**Ac**	308916.5594	**Bb**	495289.9671	**Ab**	637651.9658	**Ba**	795010.3593	**Aa**
DGDG 32:1	46410.75159	**Bc**	80618.59898	**Ac**	216505.5559	**Bb**	324040.4776	**Ab**	394992.4327	**Ba**	513690.8027	**Aa**
DGDG 32:2	15866.12385	**Ab**	27643.01974	**Ab**	30695.54513	**Ab**	48107.99968	**Ab**	78434.39973	**Aa**	90361.28872	**Aa**
DGDG 32:3	81986.46535	**Bb**	152010.5606	**Ab**	134939.6409	**Bb**	229927.8687	**Ab**	363576.1989	**Ba**	441848.1642	**Aa**
DGDG 32:3.1	262562.7226	**Bc**	470430.1241	**Ac**	1066107.576	**Bb**	1703973.312	**Ab**	2179838.988	**Ba**	2788907.664	**Aa**
DGDG 34:0	44897.72905	**Bb**	77781.8091	**Ab**	214885.4998	**Ba**	303079.6908	**Aa**	287626.5332	**Ba**	354297.3683	**Aa**
DGDG 34:1	3190589.803	**Bc**	5593287.724	**Ac**	11844746.98	**Bb**	17749551.58	**Ab**	17013906.02	**Ba**	21700766.96	**Aa**
DGDG 34:2	3153791.206	**Bb**	5766949.575	**Ab**	14572637.89	**Ba**	22053328.1	**Aa**	19062806.43	**Ba**	24286314.86	**Aa**
DGDG 34:3	6078840.99	**Bc**	10997334.76	**Ac**	31795848.61	**Bb**	55637536.46	**Ab**	48461704.35	**Ba**	65476761.16	**Aa**
DGDG 34:4	1319303.203	**Bc**	2632236.029	**Ac**	6632756.924	**Bb**	9393201.35	**Ab**	8875395.122	**Ba**	11640032.56	**Aa**
DGDG 34:5	2274571.497	**Ab**	4169689.409	**Ac**	10107290.3	**Ba**	15042885.51	**Ab**	NA	**Bb**	22117770.65	**Aa**
DGDG 34:6	8789206.064	**Bc**	15313637.25	**Ac**	29318557.35	**Bb**	45953074.03	**Ab**	50161996.26	**Ba**	73213807.04	**Aa**
DGDG 36:2	13176.39143	**Ba**	24326.97777	**Ab**	NA	**Bb**	68016.06742	**Aa**	NA	**Ab**	NA	**Ac**
DGDG 36:5	65575.23385	**Ac**	118616.4271	**Ac**	288423.0864	**Ab**	268551.2256	**Ab**	444924.0309	**Aa**	456139.5811	**Aa**
DGDG 36:6	853419.6205	**Ab**	1558012.123	**Ab**	1774816.842	**Ab**	2025317.185	**Ab**	3151580.355	**Aa**	3104878.782	**Aa**
MGDG 34:1	NA	**Bb**	NA	**Ab**	449131.8036	**Ba**	831965.1498	**Aa**	750963.5324	**Ba**	985153.454	**Aa**
MGDG 34:3	42945.88039	**Bb**	82146.84049	**Ab**	445404.3382	**Ba**	821694.4417	**Aa**	551343.1027	**Ba**	714087.3847	**Aa**
MGDG 34:4	87852.78298	**Aa**	153888.9425	**Ab**	NA	**Ba**	1228938.583	**Aa**	NA	**Ba**	1090985.509	**Aa**
MGDG 34:4.1	16808.70138	**Bb**	34131.46666	**Ab**	29726.84552	**Bab**	75758.3589	**Aab**	65511.66708	**Ba**	63675.01308	**Aa**
MGDG 34:5	464627.9552	**Ba**	931613.9335	**Aa**	NA	**Ab**	NA	**Ab**	581975.0324	**Ba**	1170895.925	**Aa**
MGDG 34:6	412425.3256	**Bb**	760916.0633	**Ab**	1293286.893	**Ba**	2442457.479	**Aa**	1841964.678	**Ba**	2529602.557	**Aa**
MGDG 34:7	1348151.823	**Bb**	2478085.103	**Ab**	2035893.978	**Bab**	4389142.06	**Aab**	3527109.614	**Ba**	4708344.579	**Aa**
MGDG 34:7.1	1000371.353	**Bb**	1760160.854	**Ab**	1285369.642	**Bab**	2528462.165	**Aab**	1996585.524	**Ba**	2756547.752	**Aa**
MGDG 36:5.1	37115.52296	**Bb**	58262.62438	**Ab**	44546.69028	**Bab**	84441.13913	**Aab**	69104.47595	**Ba**	95177.01865	**Aa**
MGDG 36:6	87636.90696	**Aab**	175968.9172	**Aab**	97912.93513	**Ab**	121127.5767	**Ab**	248482.1303	**Aa**	186108.3855	**Aa**
SQDG 32:0	4193324.453	**Bb**	7885060.093	**Ac**	7845175.643	**Ba**	13272660.16	**Ab**	NA	**Bc**	16522096.33	**Aa**
SQDG 32:1	139389.6591	**Ab**	227160.2472	**Aa**	179367.3697	**Ab**	240662.6639	**Aa**	355649.4537	**Aa**	NA	**Bb**
SQDG 32:2	26910.22833	**Bb**	53667.14863	**Aa**	NA	**Ac**	NA	**Ab**	45614.97465	**Aa**	54838.36825	**Aa**
SQDG 32:3	32230.98025	**Ab**	NA	**Bb**	46773.89003	**Aa**	50834.1836	**Aa**	NA	**Ac**	NA	**Ab**

SI Table 16. *(continuation)* Relative abundance of lipids of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
SQDG 34:1	NA	**Ab**	NA	**Ab**	1651133.804	**Ba**	2402725.469	**Aa**	NA	**Ab**	NA	**Ab**
SQDG 34:2	295077.1093	**Ab**	574343.5906	**Ab**	2613813.321	**Aa**	3662149.712	**Aa**	2858107.19	**Aa**	3060136.632	**Aa**
SQDG 34:3	3424754.785	**Bc**	6222441.976	**Ac**	6484369.57	**Bb**	9624875.386	**Ab**	11373250.34	**Ba**	11717507.12	**Aa**
SQDG 34:4	40530.81287	**Ab**	81846.20915	**Ab**	75224.48647	**Ab**	90086.28094	**Ab**	130625.1889	**Aa**	128980.5617	**Aa**
SQDG 34:4.1	9247.308606	**Ab**	18019.67455	**Ab**	13453.11756	**Ab**	18633.55797	**Ab**	33604.96664	**Aa**	32952.00715	**Aa**
SQDG 34:6	89477.11182	**Ab**	168711.6213	**Ab**	170094.7012	**Ab**	176630.8258	**Ab**	270099.0356	**Aa**	255515.5446	**Aa**
DAG 34:2	38107.24515	**Ac**	74090.4557	**Ab**	737333.7723	**Ab**	1216126.545	**Aa**	1448083.486	**Aa**	NA	**Bb**
DAG 34:3	222175.5041	**Ac**	478733.814	**Ac**	1313191.662	**Ab**	1924845.829	**Ab**	3036738.334	**Aa**	3127221.693	**Aa**
DAG 34:4	11284.20406	**Ab**	NA	**Ab**	84513.66509	**Ab**	NA	**Bb**	181745.1353	**Aa**	240568.8433	**Aa**
DAG 34:5	18170.30627	**Ac**	35786.73607	**Ac**	74068.32883	**Ab**	124490.5344	**Ab**	188780.3844	**Aa**	242381.026	**Aa**
DAG 34:6	70087.56111	**Ab**	147612.1719	**Ab**	98947.08093	**Ab**	158671.9139	**Ab**	345716.8815	**Aa**	337969.8262	**Aa**
DAG 36:1	2283.286688	**Ab**	4548.702188	**Ab**	30492.70359	**Aa**	NA	**Bb**	33174.14408	**Ba**	58291.02974	**Aa**
DAG 36:3	4830.669509	**Ab**	9262.997053	**Ab**	151704.6252	**Aa**	208114.2283	**Aa**	161416.8583	**Aa**	207469.1668	**Aa**
DAG 36:4	NA	**Bb**	NA	**Ab**	266253.8485	**Ba**	442995.2919	**Aa**	304791.2821	**Ba**	436839.1095	**Aa**
DAG 36:5	9915.951989	**Ab**	21225.80126	**Ab**	226197.8569	**Aa**	296901.5172	**Aa**	267201.1088	**Aa**	327346.3928	**Aa**
DAG 36:6	19235.13712	**Ac**	42146.88405	**Ac**	143054.5264	**Ab**	199731.2207	**Ab**	288047.1231	**Aa**	337990.7869	**Aa**
LysoPC 16:0	4543.713525	**Ab**	17652.01094	**Ac**	97134.74961	**Ba**	223325.631	**Aa**	119733.4725	**Aa**	133761.9836	**Ab**
LysoPC 16:0.1	7751.617028	**Ab**	13820.52303	**Ab**	33967.62487	**Ab**	49266.37378	**Ab**	117804.822	**Aa**	126987.076	**Aa**
LysoPC 18:1	57971.79437	**Ab**	114692.9386	**Ab**	211420.2087	**Ab**	318002.9247	**Ab**	874098.3817	**Aa**	1150442.963	**Aa**
LysoPC 18:1.1	803017.2668	**Ab**	2169530.216	**Ab**	2144526.86	**Ab**	4400787.712	**Ab**	8450019.061	**Aa**	10704580.46	**Aa**
LysoPC 18:2	16212.67077	**Ac**	31975.02826	**Ac**	177113.8243	**Ab**	291569.9306	**Ab**	578595.6056	**Aa**	498706.6462	**Aa**
LysoPC 18:2.1	14977.84701	**Ab**	27551.69837	**Ab**	126707.7606	**Aa**	118557.8691	**Aa**	79693.90431	**Aa**	117410.5484	**Aa**
LysoPC 18:3	2373.546423	**Ac**	4496.46395	**Ac**	26490.09646	**Ab**	37640.81389	**Ab**	63625.56477	**Aa**	57801.58796	**Aa**
LysoPC 18:3.1	3980.533971	**Ab**	8256.57771	**Ab**	41398.04853	**Aa**	34841.97812	**Aa**	25415.57088	**Aa**	33436.89265	**Aa**
PC 32:3	13961.99097	**Aa**	19229.18697	**Aa**	27286.77613	**Aa**	35822.4009	**Aa**	NA	**Ba**	45382.11422	**Aa**
PC 34:2	NA	**Ab**	NA	**Ab**	1095658.351	**Aa**	1280767.096	**Aa**	NA	**Ab**	NA	**Ab**
PC 34:3	78616.48399	**Ab**	171683.8973	**Ab**	464228.3996	**Aa**	526474.0196	**Aa**	176561.8662	**Ab**	241940.7915	**Ab**
PC 34:5	10106.56085	**Aa**	13041.42234	**Aa**	23857.92243	**Aa**	28320.22941	**Aa**	NA	**Ba**	35462.45731	**Aa**
PC 34:6	26518.65071	**Ab**	41658.66989	**Ab**	87210.96482	**Aab**	115128.2072	**Aab**	117391.9384	**Aa**	184674.9282	**Aa**

SI Table 16. *(continuation)* Relative abundance of lipids of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
PC 36:1	308465.3196	**Bb**	635889.0777	**Ab**	387110.0789	**Bb**	515607.2529	**Ab**	1617344.213	**Ba**	2104829.644	**Aa**
PC 36:2	11914276.96	**Bb**	21547792.27	**Ab**	14699407.95	**Bb**	22289157.63	**Ab**	61795264.93	**Ba**	81143758.86	**Aa**
PC 36:3	69151.73611	**Ab**	129840.5968	**Ab**	319275.5876	**Ab**	410951.1794	**Ab**	927117.2529	**Aa**	1434142.248	**Aa**
PC 36:3.1	2498631.886	**Ab**	4487725.793	**Ab**	3126206.602	**Ab**	5226486.724	**Ab**	15945326.31	**Aa**	20782215.5	**Aa**
PC 36:5	312966.7301	**Ab**	668258.269	**Ab**	2507133.794	**Aa**	3019133.599	**Aa**	1266871.213	**Ab**	1785546.078	**Ab**
PC 36:6	69136.90323	**Ab**	140027.9781	**Ab**	367328.5083	**Aa**	423214.2442	**Aa**	255437.0812	**Aa**	311160.1262	**Aa**
PC 38:2	39931.0282	**Bb**	69931.71921	**Ab**	33118.06747	**Bb**	48881.56875	**Ab**	110216.4699	**Ba**	161424.336	**Aa**
PC 38:3	2221.762645	**Ab**	4521.458636	**Ab**	25445.12161	**Aa**	30061.31017	**Aa**	14334.34833	**Aa**	20511.25924	**Aa**
PC 38:3.1	15046.31577	**Bb**	28513.53729	**Ab**	18841.11935	**Bb**	31640.86504	**Ab**	76566.61732	**Ba**	96489.24764	**Aa**
PE 32:1	36265.32419	**Bb**	78227.3439	**Ab**	381024.3581	**Ba**	550225.5146	**Aa**	316856.5905	**Ba**	446103.1427	**Aa**
PE 34:2	794464.9136	**Bb**	1465706.827	**Ab**	3943852.835	**Ba**	5705283.107	**Aa**	4263839.393	**Ba**	5842135.384	**Aa**
PE 34:3	31562.79944	**Ab**	57764.14609	**Ab**	266466.5392	**Aa**	350020.5288	**Aa**	343224.0035	**Aa**	426200.745	**Aa**
PE 34:4	9199.471529	**Ab**	16770.57023	**Ab**	37073.46067	**Ba**	47979.72993	**Aa**	26443.06141	**Aa**	NA	**Bc**
PE 34:5	6266.747826	**Ab**	11392.29967	**Ab**	19692.94786	**Aa**	21711.56871	**Aa**	15065.65401	**Aa**	18858.93153	**Aa**
PE 34:6	2715.247575	**Ab**	4055.98632	**Ab**	6884.336865	**Ab**	6305.862464	**Ab**	10657.04499	**Aa**	14080.69236	**Aa**
PE 36:2	6151354.354	**Bb**	10865828.66	**Ab**	8082372.746	**Bb**	11924238.98	**Ab**	13486357.64	**Ba**	18616005.29	**Aa**
PE 36:3	1359945.931	**Bb**	2401179.828	**Ab**	2641940.064	**Ba**	4324848.861	**Aa**	3369137.692	**Ba**	4583695.417	**Aa**
PE 36:4	83901.10036	**Bb**	167812.9142	**Ab**	334584.5211	**Ba**	471898.3638	**Aa**	165161.7833	**Bb**	242097.737	**Ab**
PE 36:5	38632.37095	**Bb**	84602.17599	**Ab**	104245.5903	**Ba**	127063.1743	**Aa**	58849.78332	**Bb**	76557.29852	**Ab**
PE 36:6	10530.42612	**Ac**	21175.91546	**Ac**	60373.78783	**Ab**	72765.99256	**Ab**	85647.82822	**Aa**	114294.3149	**Aa**
PE 38:2	1149694.31	**Bb**	2071422.042	**Ab**	1435269.104	**Bb**	2147144.466	**Ab**	5079314.698	**Ba**	6368339.006	**Aa**
PE 38:3	278134.4976	**Bb**	517805.4209	**Ab**	450577.249	**Bb**	785658.095	**Ab**	1062380.561	**Ba**	1395844.959	**Aa**
PE 40:2	277479.2555	**Bb**	510704.8517	**Ab**	299097.7968	**Bb**	464414.0715	**Ab**	1579538.406	**Ba**	2281960.326	**Aa**
PE 40:3	5869.212202	**Ab**	9498.67403	**Ab**	13603.69606	**Ab**	NA	**Ab**	61715.41462	**Aa**	77012.01214	**Aa**
PG 34:2	93736.8566	**Bc**	180905.8371	**Ac**	309373.1289	**Bb**	410532.4403	**Ab**	415081.9349	**Ba**	568437.4063	**Aa**
PG 34:3	19701.48057	**Bb**	43974.48813	**Ab**	77149.76687	**Ba**	104255.3461	**Aa**	67401.76803	**Ba**	93211.44995	**Aa**
PG 34:4	1098574.617	**Ab**	NA	**Bc**	1568600.059	**Ab**	2070856.449	**Ab**	2710991.812	**Aa**	3090725.997	**Aa**
PG 36:2	919380.5704	**Bb**	1866913	**Ab**	1381201.88	**Bb**	1874703.657	**Ab**	2807927.923	**Ba**	3448445.443	**Aa**
PG 36:3	30098.53693	**Ac**	NA	**Ac**	56493.56862	**Ab**	63220.19642	**Ab**	143575.0095	**Aa**	152151.8597	**Aa**

SI Table 16. *(continuation)* Relative abundance of lipids of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 42:0	9908.984945	**Bb**	24249.55578	**Ab**	26959.15432	**Ba**	48363.58674	**Aa**	25875.6782	**Ba**	38142.37712	**Aa**
TAG 42:1	3690.784371	**Ab**	NA	**Ac**	53624.22457	**Ba**	126166.1697	**Aa**	41065.07438	**Aa**	58887.70144	**Ab**
TAG 44:0	36543.24304	**Ab**	86052.58001	**Ac**	249542.2294	**Ba**	622528.1718	**Aa**	211368.6038	**Ba**	339354.3588	**Ab**
TAG 44:1	18424.63968	**Ab**	41155.11179	**Ab**	283736.9014	**Ba**	860055.0514	**Aa**	102767.7594	**Ab**	155852.7766	**Ab**
TAG 46:0	69015.88361	**Bb**	154929.7366	**Ab**	314551.6579	**Ba**	634243.3514	**Aa**	466793.8062	**Ba**	702773.3498	**Aa**
TAG 46:1	95931.91946	**Ab**	227654.5615	**Ac**	5680889.699	**Ba**	17050581.23	**Aa**	3303225.111	**Ba**	6158192.344	**Ab**
TAG 46:2	25711.85333	**Ab**	54098.08088	**Ac**	3551520.947	**Ba**	10457346.39	**Aa**	1720633.264	**Aab**	3290000.008	**Ab**
TAG 46:2.1	13081.17956	**Ac**	29140.3847	**Ac**	112295.6081	**Ba**	339305.1518	**Aa**	41228.67315	**Bb**	89677.64495	**Ab**
TAG 48:0	125747.6195	**Ab**	277445.4699	**Ab**	2969639.332	**Ba**	6790457.457	**Aa**	4605247.06	**Ba**	7652768.475	**Aa**
TAG 48:1	203360.0682	**Ab**	478097.4541	**Ac**	7167008.627	**Ba**	18101789.97	**Aa**	6203581.673	**Ba**	11106734	**Ab**
TAG 48:2	39809.32386	**Ab**	80474.42081	**Ab**	4102967.057	**Ba**	10032257.05	**Aa**	4553204.323	**Ba**	8005787.927	**Aa**
TAG 48:4.1	4336.440579	**Ab**	8338.352425	**Ac**	556708.5287	**Ba**	1349654.742	**Aa**	454571.4286	**Ba**	784007.4245	**Ab**
TAG 48:5	633.8263475	**Aa**	882.9098179	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
TAG 50:0	76818.1254	**Ab**	152680.8506	**Ab**	623556.29	**Ba**	1221946.003	**Aa**	626663.6339	**Ba**	983314.6248	**Aa**
TAG 50:1	677092.4257	**Ab**	1551239.106	**Ab**	52249888.45	**Ba**	123333101.9	**Aa**	69181386.06	**Ba**	123675286.6	**Aa**
TAG 50:2	348201.7887	**Ab**	727082.9119	**Ab**	46337500.32	**Ba**	110790018.6	**Aa**	59438443.74	**Ba**	109638999.3	**Aa**
TAG 50:3	546467.1826	**Ab**	916858.9656	**Ab**	36784808	**Ba**	93102878.42	**Aa**	61633924.68	**Ba**	98318947.63	**Aa**
TAG 50:4	104702.6461	**Ab**	202841.0041	**Ab**	12702072.13	**Bab**	30589121.32	**Aa**	21065514.37	**Ba**	39217710.7	**Aa**
TAG 50:5	39103.46935	**Ab**	73095.38357	**Ab**	5496202.169	**Bab**	15064702.16	**Aa**	10096283.05	**Ba**	19082911.68	**Aa**
TAG 50:6	61273.45343	**Bc**	101376.4112	**Ac**	3003869.376	**Bb**	8172133.354	**Ab**	6764473.381	**Ba**	12046455.12	**Aa**
TAG 50:7	1823.997914	**Bc**	1640.524603	**Ac**	205780.9769	**Bb**	550463.5243	**Ab**	442261.6631	**Ba**	783699.6753	**Aa**
TAG 50:8	1269.178153	**Bb**	2559.953693	**Ab**	157300.3878	**Ba**	415202.9541	**Aa**	318740.5925	**Ba**	549547.148	**Aa**
TAG 50:9	2550.43235	**Bc**	4654.714356	**Ac**	125186.3451	**Bb**	283642.063	**Ab**	368453.6896	**Ba**	533999.0441	**Aa**
TAG 52:0	56724.80021	**Bb**	112966.5279	**Ab**	160979.3879	**Ba**	309764.8847	**Aa**	153583.4459	**Ba**	239548.2574	**Aa**
TAG 52:1	321637.3892	**Ac**	736002.7265	**Ac**	16781569.62	**Ba**	38990400.06	**Aa**	9401527.372	**Bb**	16752708.98	**Ab**
TAG 52:2	703213.5109	**Ab**	1484814.909	**Ac**	55629960.74	**Ba**	131378797.3	**Aa**	43771131.16	**Ba**	80424623.9	**Ab**
TAG 52:3	316324.1614	**Ab**	655339.7993	**Ac**	52301518.75	**Ba**	112855740.3	**Aa**	44483606.85	**Ba**	83827541.44	**Ab**
TAG 52:3.1	23038.52062	**Ab**	45562.8967	**Ab**	3183192.205	**Ba**	7945932.377	**Aa**	3909739.916	**Ba**	6948958.751	**Aa**
TAG 52:4	357749.3066	**Ab**	730954.1849	**Ab**	52107009.81	**Ba**	122381929	**Aa**	58411914.26	**Ba**	106312840.8	**Aa**

SI Table 16. *(continuation)* Relative abundance of lipids of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 52:4.1	141460.569	**Ab**	265835.3505	**Ab**	20120228.4	**Ba**	43755836.78	**Aa**	33166213.18	**Ba**	53147918.41	**Aa**
TAG 52:5	110701.9146	**Ab**	228750.0317	**Ab**	25702577.93	**Ba**	63709541.81	**Aa**	32194909.34	**Ba**	59273611.2	**Aa**
TAG 52:5.1	153054.5436	**Bb**	305083.2887	**Ab**	22456809.63	**Ba**	47792451.29	**Aa**	34948443.25	**Ba**	61240353.55	**Aa**
TAG 52:6	147562.4771	**Ab**	270018.6803	**Ab**	13421434.46	**Bab**	36629426.93	**Aa**	24663761.41	**Aa**	34398006.9	**Aa**
TAG 52:6.1	219608.8307	**Bc**	411470.3933	**Ac**	18095705.64	**Bb**	38923904.2	**Ab**	33022012.02	**Ba**	53329267.82	**Aa**
TAG 52:7	21183.50722	**Ab**	40898.31084	**Ab**	2910890.988	**Bab**	7614762.362	**Aa**	4449933.86	**Ba**	7895891.285	**Aa**
TAG 52:8	9829.602835	**Ab**	18804.9894	**Ab**	1052678.264	**Bab**	2905686.189	**Aa**	1740872.28	**Ba**	3133935.767	**Aa**
TAG 52:9	15950.16675	**Bc**	29556.74755	**Ac**	380209.7972	**Bb**	943571.3247	**Ab**	927893.2366	**Ba**	1373565.848	**Aa**
TAG 54:0	20621.86453	**Bb**	38053.97303	**Ab**	55827.71848	**Ba**	96310.3047	**Aa**	56993.51108	**Ba**	74741.49886	**Aa**
TAG 54:1	89510.69138	**Ab**	171737.7075	**Ac**	2368518.641	**Ba**	4224434.754	**Aa**	871618.5681	**Ab**	1475993.533	**Ab**
TAG 54:2	217303.8865	**Ac**	422430.532	**Ac**	11461890.08	**Ba**	21333729.18	**Aa**	5634760.317	**Bb**	11056445.77	**Ab**
TAG 54:3	373636.9251	**Ab**	697301.3555	**Ac**	17877705.98	**Ba**	30845204.96	**Aa**	6961807.223	**Bb**	13668550.09	**Ab**
TAG 54:4	230920.534	**Ab**	457845.5934	**Ac**	21024068.08	**Ba**	34736726.57	**Aa**	8442512.113	**Bb**	16053051.84	**Ab**
TAG 54:5	120314.7533	**Ac**	247807.419	**Ac**	20984191.18	**Ba**	32537247.76	**Aa**	7601130.193	**Ab**	11576596.19	**Ab**
TAG 54:6	61988.00293	**Bc**	139305.4079	**Ac**	13072443.51	**Ba**	20022098.78	**Aa**	5562172.535	**Bb**	9261266.27	**Ab**
TAG 54:7	33587.92276	**Bc**	82633.91618	**Ac**	7710577.041	**Ba**	12918764.45	**Aa**	4902926.894	**Bb**	7201516.096	**Ab**
TAG 54:8	9326.819325	**Bb**	22761.44721	**Ab**	2443231.582	**Ba**	4488597.953	**Aa**	2062165.962	**Ba**	2946025.016	**Aa**
TAG 54:9	8119.436773	**Bb**	18678.48015	**Ab**	754438.2055	**Ba**	1560709.638	**Aa**	1139205.666	**Ba**	1389020.352	**Aa**
TAG 56:0	13787.60875	**Bb**	25872.42233	**Ab**	37885.36006	**Ba**	64766.25722	**Aa**	37195.42461	**Ba**	50190.99485	**Aa**
TAG 56:1	13274.36843	**Ab**	24865.36745	**Ac**	422282.2287	**Ba**	683173.319	**Aa**	137886.6054	**Bb**	256229.9028	**Ab**
TAG 56:2	27834.30287	**Ab**	55435.99038	**Ac**	996563.3315	**Ba**	1636357.615	**Aa**	355842.2505	**Bb**	721416.0984	**Ab**
TAG 56:3	15425.1847	**Ab**	30837.61362	**Ab**	1469465.418	**Aa**	1712918.913	**Aa**	460673.6149	**Ab**	NA	**Ab**
TAG 56:4	14836.30826	**Ab**	28217.86927	**Ab**	1793399.774	**Aa**	1725476.727	**Aa**	460161.8451	**Ab**	853470.6748	**Ab**
TAG 56:5	8552.761815	**Ab**	16131.48562	**Ab**	2461012.861	**Aa**	2177066.576	**Aa**	716595.216	**Ab**	1004826.704	**Ab**
TAG 56:5.1	NA	**Ab**	NA	**Ab**	253215.6343	**Ba**	527249.6268	**Aa**	264096.5576	**Ba**	512781.8779	**Aa**
TAG 56:6	4751.814365	**Ab**	9970.788236	**Ab**	1986132.76	**Aa**	1638795.124	**Aa**	540345.9088	**Ab**	886056.7987	**Ab**
TAG 56:7	4748.218354	**Ac**	8227.345663	**Ac**	1044159.378	**Aa**	1073147.934	**Aa**	389410.5127	**Ab**	571229.9262	**Ab**
TAG 58:0	6552.792256	**Bb**	12078.64433	**Ab**	24015.20078	**Ba**	44962.88776	**Aa**	27788.9543	**Ba**	42526.40345	**Aa**
TAG 58:1	12814.12316	**Ab**	23611.77909	**Ac**	424131.8732	**Ba**	739190.1095	**Aa**	157135.7519	**Ab**	265688.7932	**Ab**

SI Table 16. *(continuation)* Relative abundance of lipids of *C. reinhardtii* CC503 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 58:2	22009.06122	**Ab**	44233.72692	**Ab**	590777.18	**Ba**	950934.0382	**Aa**	NA	**Bb**	185923.5623	**Ab**
TAG 58:3.1	10147.32527	**Ab**	20878.83849	**Ab**	716246.2591	**Aa**	718614.6004	**Aa**	121230.5339	**Ab**	190386.1037	**Ab**
TAG 58:5	605.4304678	**Ab**	957.0264156	**Ab**	NA	**Ab**	NA	**Ab**	22448.97895	**Ba**	42332.73892	**Aa**
TAG 58:6	843.6633655	**Ab**	1446.655774	**Ac**	54828.89968	**Ba**	90295.63816	**Aa**	65265.27648	**Aa**	60274.31572	**Ab**
TAG 60:0	1697.616024	**Bb**	3426.036411	**Ab**	4498.951044	**Ba**	8757.368185	**Aa**	5810.278533	**Ba**	8230.862471	**Aa**
TAG 60:1	8873.460961	**Ab**	16530.25957	**Ab**	299744.8445	**Ba**	668532.5873	**Aa**	294840.98	**Ba**	563898.4195	**Aa**
TAG 60:2	13346.91325	**Ab**	27130.35577	**Aa**	397890.9531	**Aa**	NA	**Ba**	NA	**Ab**	NA	**Aa**
TAG 60:4	8717.891168	**Ab**	20535.97817	**Ab**	287437.4828	**Ba**	452526.2446	**Aa**	41130.87562	**Ab**	74754.5981	**Ab**
TAG 60:5	3867.320422	**Ba**	8482.778344	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
TAG 60:5.1	680.928666	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	33745.89466	**Ba**	64462.24524	**Aa**
TAG 60:6	62320.17137	**Ab**	112552.6137	**Ab**	521262.0413	**Ab**	905001.5399	**Ab**	1972834.101	**Aa**	2650841.9	**Aa**
FA 16:0	400625.8585	**Ab**	755495.5616	**Ab**	2272363.155	**Ab**	3604707.353	**Ab**	9019936.882	**Aa**	9385814.504	**Aa**
FA 16:2	14242.95921	**Ab**	25279.44978	**Ab**	62573.81431	**Ab**	76756.78255	**Ab**	299417.3611	**Aa**	312623.7337	**Aa**
FA 16:3	14047.29493	**Ab**	22946.24655	**Ab**	117583.9712	**Ab**	141559.1052	**Ab**	576469.1025	**Aa**	592468.9203	**Aa**
FA 18:0	415253.1757	**Ac**	828547.8485	**Ac**	1344401.636	**Ab**	2336619.543	**Ab**	3299828.602	**Aa**	3826420.131	**Aa**
FA 18:1	NA	**Ac**	NA	**Ac**	10400995.12	**Ab**	17754146.76	**Ab**	28187525.13	**Aa**	34801451.13	**Aa**
FA 18:2	477853.087	**Ac**	881461.2602	**Ac**	7584363.125	**Ab**	11777934.54	**Ab**	17291082.66	**Aa**	19353362.97	**Aa**
FA 18:3	8490642.948	**Ab**	15102461.2	**Ab**	31080965.09	**Ab**	50087753.54	**Ab**	86993451.3	**Aa**	86923010.7	**Aa**
FA 20:0	9327.772871	**Bb**	19002.62966	**Ab**	32548.00855	**Ba**	59857.32941	**Aa**	53383.71355	**Ba**	58880.90041	**Aa**
FA 20:1	25048.61341	**Ac**	42381.59119	**Ac**	152323.0385	**Ab**	205697.6365	**Ab**	315229.2428	**Aa**	315960.7923	**Aa**
FA 20:3	9812.992612	**Ac**	17915.03472	**Ac**	32130.54046	**Ab**	50345.15101	**Ab**	81692.16692	**Aa**	82451.71993	**Aa**
FA 22:0	5261.321787	**Bc**	9840.617702	**Ac**	31341.92357	**Bb**	43135.10229	**Ab**	45548.21629	**Ba**	55376.93857	**Aa**
FA 22:1	2179.134596	**Ac**	3882.855405	**Ac**	5988.301788	**Ab**	10880.06318	**Ab**	12104.28696	**Aa**	14740.08863	**Aa**
FA 24:0	13527.28666	**Bc**	23447.39667	**Ac**	66482.62622	**Bb**	113872.3065	**Ab**	132875.1132	**Ba**	167926.9384	**Aa**
FA 24:1	800.5878357	**Ab**	1310.714868	**Ab**	3059.56916	**Aa**	4129.3648	**Aa**	4895.995559	**Aa**	3747.50665	**Aa**
FA 26:0	15460.6199	**Ab**	27530.48131	**Ab**	149792.1848	**Ab**	268187.2213	**Ab**	674178.3773	**Aa**	835611.6644	**Aa**
FA 28:0	3080.343168	**Ab**	6068.314153	**Ab**	10255.53384	**Ab**	20190.10814	**Ab**	52506.31878	**Aa**	56862.86634	**Aa**
FA 30:0	1247.89056	**Ac**	2011.768685	**Ac**	2283.587886	**Ab**	4717.446497	**Ab**	6294.399519	**Aa**	7288.269737	**Aa**

SI Table 17. Relative abundance of lipids of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
DGDG 32:0	46028.55655	**Ab**	66039.00307	**Aa**	44511.25764	**Ab**	52203.88655	**Aa**	200999.5898	**Aa**	72100.95902	**Ba**
DGDG 32:1	13670.42597	**Ab**	19798.45036	**Aa**	14956.81401	**Ab**	24924.11817	**Aa**	79118.80526	**Aa**	36240.06954	**Ba**
DGDG 32:2	37919.70683	**Ab**	56016.14001	**Aa**	65399.3837	**Ab**	55614.23995	**Aa**	301638.4371	**Aa**	79512.5058	**Ba**
DGDG 32:3	183631.1578	**Ab**	264093.1695	**Aa**	309118.0724	**Ab**	323416.3307	**Aa**	1167609.88	**Aa**	552849.7776	**Ba**
DGDG 32:3.1	78119.41938	**Ab**	109122.7356	**Ab**	111603.8803	**Bb**	205240.7619	**Ab**	584598.6903	**Aa**	333439.7523	**Ba**
DGDG 34:0	21824.78391	**Ab**	31692.67218	**Aa**	18189.96562	**Ab**	17706.1369	**Aa**	99317.91604	**Aa**	34891.67024	**Ba**
DGDG 34:1	1570285.43	**Ab**	2390968.762	**Aa**	1573781.737	**Ab**	2090834.074	**Aa**	6781000.187	**Aa**	2811627.571	**Ba**
DGDG 34:2	2847339.309	**Ab**	4254362.847	**Aa**	2437538.541	**Ab**	2820113.66	**Aa**	8522342.308	**Aa**	2954488.345	**Ba**
DGDG 34:3	2689765.644	**Ab**	3952048.339	**Aa**	2733399.159	**Ab**	4262172.283	**Aa**	9735922.32	**Aa**	5167660.178	**Ba**
DGDG 34:4	2230608.377	**Ab**	3246144.096	**Aa**	2496623.985	**Ab**	2278876.904	**Aa**	11218965.38	**Aa**	2717815.766	**Ba**
DGDG 34:5	7395039.096	**Ab**	10150453.31	**Aa**	8558394.057	**Ab**	10568330.75	**Aa**	38673302	**Aa**	13122302.1	**Ba**
DGDG 34:6	18382217.75	**Ab**	26029207.54	**Ab**	27282378.82	**Ab**	40543972.44	**Aab**	126484572.9	**Aa**	62764920.6	**Ba**
DGDG 36:2	10225.32727	**Ba**	18306.90858	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
DGDG 36:4	144608.7499	**Ab**	210116.0281	**Aa**	199153.9118	**Ab**	287042.9791	**Aa**	646227.8719	**Aa**	343603.6242	**Ba**
DGDG 36:5	511090.1514	**Ab**	742933.0646	**Ab**	675030.9695	**Bb**	1110556.613	**Aab**	2166668.957	**Aa**	1437968.979	**Ba**
DGDG 36:6	1340840.988	**Ab**	1940185.742	**Ab**	1580603.004	**Ab**	2786691.786	**Ab**	6594847.964	**Aa**	5298811.72	**Aa**
MGDG 34:1	89529.74876	**Ab**	128103.3249	**Ab**	144156.7069	**Ab**	200969.1348	**Ab**	875838.4996	**Aa**	524577.8602	**Ba**
MGDG 34:2	NA	**Ac**	NA	**Ac**	153331.5324	**Ab**	141419.355	**Ab**	700317.4251	**Aa**	365246.6088	**Ba**
MGDG 34:3	473715.8731	**Ab**	720830.3989	**Aa**	459653.8832	**Ab**	757893.8824	**Aa**	1814017.683	**Aa**	1033420.558	**Ba**
MGDG 34:4	1073634.625	**Ab**	1538037.087	**Aa**	812235.7945	**Ab**	1276045.062	**Aa**	2410565.604	**Aa**	1429418.27	**Ba**
MGDG 34:4.1	5529.839754	**Bb**	13193.06424	**Aa**	NA	**Ab**	NA	**Ab**	22306.07859	**Aa**	NA	**Bb**
MGDG 34:5	11169.78676	**Ab**	14627.84866	**Ab**	18674.07742	**Ab**	31517.88234	**Ab**	142776.4951	**Aa**	63703.75871	**Ba**
MGDG 34:6	1563105.412	**Ab**	2222283.18	**Aa**	1156589.583	**Ab**	1876880.311	**Aa**	2952377.349	**Aa**	2108643.092	**Aa**
MGDG 34:7	301248.7743	**Ab**	343075.8049	**Ab**	384117.9595	**Ab**	422024.1484	**Ab**	2175195.229	**Aa**	1500540.844	**Aa**
MGDG 36:5	223356.736	**Ab**	273533.7256	**Ab**	148535.3658	**Ab**	374760.6226	**Ab**	486473.1115	**Aa**	395054.6653	**Aa**
MGDG 36:5.1	46679.45423	**Aa**	NA	**Bb**	29904.49818	**Ba**	47109.75352	**Aa**	NA	**Bb**	60295.99091	**Aa**
MGDG 36:6	322498.725	**Bb**	465703.2653	**Ab**	278095.3963	**Bb**	589652.4152	**Ab**	844721.467	**Ba**	871561.8956	**Aa**

SI Table 17. *(continuation)* Relative abundance of lipids of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
SQDG 32:0	1375540.086	**Ab**	2147591.419	**Aa**	1671934.186	**Ab**	2237803.794	**Aa**	6688967.318	**Aa**	3494054.054	**Ba**
SQDG 32:1	21953.99463	**Aa**	33214.58439	**Aa**	25023.16736	**Aa**	26995.41225	**Aa**	NA	**Ab**	NA	**Ab**
SQDG 32:2	52129.00585	**Ab**	66416.66596	**Ab**	86295.52591	**Ab**	92098.40078	**Aab**	471969.7077	**Aa**	170298.436	**Ba**
SQDG 32:3	139690.4536	**Ab**	203838.6936	**Ab**	262882.6101	**Ab**	358058.5484	**Ab**	1494310.013	**Aa**	822826.5452	**Ba**
SQDG 34:1	546911.1191	**Ab**	867013.647	**Aa**	735254.0031	**Ab**	803471.3983	**Aa**	2403302.954	**Aa**	1124241.447	**Ba**
SQDG 34:2	2977566.397	**Ab**	4437582.645	**Aa**	3095418.019	**Ab**	2411963.887	**Aa**	11672951.21	**Aa**	3524202.991	**Ba**
SQDG 34:3	3306887.081	**Ab**	5495838.449	**Aa**	3870336.123	**Ab**	5671947.03	**Aa**	16060869.24	**Aa**	8453820.614	**Ba**
SQDG 34:4	23541.28396	**Ab**	30645.47775	**Aa**	21535.95546	**Ab**	19704.61829	**Aa**	98482.50406	**Aa**	42499.18628	**Ba**
SQDG 34:4.1	NA	**Ab**	NA	**Ac**	NA	**Bb**	16535.87086	**Ab**	55601.59196	**Aa**	36335.10255	**Ba**
SQDG 34:6	141427.3372	**Aa**	NA	**Bb**	NA	**Ab**	NA	**Ab**	NA	**Bb**	833885.7764	**Aa**
DAG 34:1	46787.94959	**Ac**	45537.7434	**Ac**	83193.43621	**Ab**	180696.3415	**Ab**	220747.8678	**Aa**	204519.3289	**Aa**
DAG 34:2	83931.33748	**Ab**	93727.69659	**Ab**	95247.29349	**Ab**	138984.7377	**Aab**	441860.2143	**Aa**	214695.674	**Ba**
DAG 34:3	55504.00387	**Ab**	64770.45932	**Ab**	67955.69508	**Ab**	124266.1206	**Aab**	328435.0654	**Aa**	182590.0106	**Ba**
DAG 34:4	59552.84673	**Ab**	58887.44263	**Aa**	94247.12846	**Ab**	117855.4806	**Aa**	487728.4102	**Aa**	128450.8724	**Ba**
DAG 34:5	261826.8195	**Ab**	243823.564	**Aa**	357197.184	**Ab**	473833.4781	**Aa**	1672063.911	**Aa**	525262.8083	**Ba**
DAG 34:6	771112.3987	**Ab**	689994.134	**Ab**	1176279.508	**Ab**	1973720.668	**Aab**	5702205.747	**Aa**	2543753.316	**Ba**
DAG 36:1	995.721439	**Ab**	NA	**Bb**	1434.996118	**Bb**	2948.160957	**Aa**	3603.634772	**Aa**	3935.24296	**Aa**
DAG 36:3	43634.11303	**Ab**	46421.30138	**Ab**	65225.66415	**Ab**	157461.4808	**Ab**	288723.2382	**Aa**	268091.5034	**Aa**
DAG 36:4	NA	**Ab**	62921.14593	**Ab**	74195.15394	**Ab**	211945.1796	**Ab**	443833.123	**Aa**	437320.7332	**Aa**
DAG 36:5	152874.665	**Ab**	168907.0394	**Ab**	212098.8282	**Ab**	454175.3817	**Aab**	1126908.133	**Aa**	670415.3545	**Ba**
DAG 36:6	113569.2261	**Ab**	125954.9152	**Ab**	174278.7481	**Bb**	393540.712	**Aa**	919565.3409	**Aa**	620315.7479	**Ba**
LysoPC 16:0	51261.87453	**Ab**	72192.91179	**Aa**	348563.1913	**Ab**	136300.7621	**Aa**	3312390.834	**Aa**	489681.4161	**Ba**
LysoPC 16:0.1	9813.006747	**Ab**	13260.56906	**Ab**	17771.67083	**Ab**	21278.59845	**Ab**	113912.9751	**Aa**	56929.46415	**Ba**
LysoPC 16:2	9283.004921	**Ab**	20421.00564	**Aa**	12761.51832	**Ab**	6137.065561	**Aa**	119149.7151	**Aa**	22929.17924	**Ba**
LysoPC 16:3	74287.43121	**Ab**	133253.7226	**Ab**	84640.93294	**Ab**	98051.80121	**Ab**	596239.5614	**Aa**	266500.0217	**Ba**
LysoPC 18:1	195004.744	**Ab**	307650.0118	**Ab**	244521.3893	**Ab**	233724.8238	**Ab**	2145349.444	**Aa**	811354.0596	**Ba**
LysoPC 18:1.1	1516421.599	**Ab**	2154330.573	**Ab**	1809466.721	**Ab**	2520286.048	**Ab**	7947829.035	**Aa**	5388794.539	**Ba**
LysoPC 18:2	66309.25042	**Ab**	105694.9764	**Aa**	213823.566	**Ab**	93446.09096	**Aa**	2009741.686	**Aa**	323180.6252	**Ba**
LysoPC 18:2.1	173287.8787	**Ab**	297010.5749	**Ab**	200474.1448	**Ab**	233196.872	**Ab**	935565.7933	**Aa**	551719.8682	**Ba**

SI Table 17. *(continuation)* Relative abundance of lipids of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
LysoPC 18:3	87361.93507	**Ab**	123684.5259	**Aa**	551727.7131	**Ab**	194835.4547	**Aa**	4895722.456	**Aa**	968976.6506	**Ba**
LysoPC 18:3.1	167904.2636	**Ab**	284288.9615	**Ab**	157247.9973	**Ab**	201735.2513	**Ab**	723656.0436	**Aa**	450622.8635	**Ba**
PC 32:0	NA	**Ab**	NA	**Ab**	NA	**Bb**	6392.153173	**Aab**	25602.03432	**Aa**	12429.24502	**Ba**
PC 32:1	19245.55687	**Ab**	27234.95921	**Ab**	32909.49757	**Ab**	50596.46322	**Ab**	276220.481	**Aa**	159081.7083	**Ba**
PC 32:1.1	55290.39102	**Ab**	75627.06688	**Ab**	94945.59301	**Ab**	90266.90802	**Aab**	640458.1527	**Aa**	196695.2118	**Ba**
PC 32:2	1184360.731	**Ab**	1595925.107	**Aa**	2091792.192	**Ab**	1946017.225	**Aa**	14255257.74	**Aa**	3484659.001	**Ba**
PC 32:3	4280607.041	**Ab**	5610758.648	**Ab**	7093565.356	**Ab**	8278671.497	**Ab**	42493692.54	**Aa**	18156387.8	**Ba**
PC 32:4	54213.98039	**Ab**	80855.52512	**Ab**	149047.4037	**Ab**	90310.82947	**Ab**	1671571.536	**Aa**	343142.3219	**Ba**
PC 34:2	2610926.95	**Ab**	3590923.423	**Ab**	4402206.539	**Ab**	6299965.74	**Aab**	21114113.86	**Aa**	9820887.314	**Ba**
PC 34:3	2163910.301	**Ab**	2936050.14	**Ab**	3405796.506	**Ab**	4980844.644	**Ab**	23387722.48	**Aa**	10449467.87	**Ba**
PC 34:4	1094964.048	**Ab**	1561405.615	**Ab**	2119151.519	**Ab**	2186325.443	**Aab**	20553324.69	**Aa**	5525836.807	**Ba**
PC 34:5	2618799.409	**Ab**	3479741.714	**Ab**	5205591.77	**Ab**	6135611.185	**Aab**	51993568.15	**Aa**	16477653.06	**Ba**
PC 34:6	3582068.87	**Ab**	4998718.013	**Ab**	11003734.86	**Ab**	14209277.06	**Ab**	102682770.5	**Aa**	42770198.57	**Ba**
PC 36:1	153870.1249	**Ab**	198653.8945	**Ab**	271224.147	**Ab**	396491.5601	**Aab**	2019320.077	**Aa**	937095.4856	**Ba**
PC 36:2	5375584.263	**Ab**	6723550.439	**Ab**	8331483.397	**Ab**	13565126.02	**Ab**	63545892.07	**Aa**	38841648.15	**Ba**
PC 36:3	505797.7096	**Ab**	703342.6418	**Ab**	796342.5623	**Ab**	1256540.486	**Ab**	4070107.539	**Aa**	2287481.917	**Ba**
PC 36:3.1	471228.6031	**Ab**	601347.7762	**Ab**	937648.3172	**Ab**	1456487.627	**Ab**	9030539.605	**Aa**	5159650.122	**Ba**
PC 36:5	7435728.521	**Ab**	10365147.48	**Ab**	10777753.09	**Ab**	15168817.88	**Ab**	53857238.36	**Aa**	27027823.14	**Ba**
PC 36:6	7175748.603	**Ab**	9784371.757	**Ab**	10298722.83	**Ab**	15494633.58	**Ab**	57979052.14	**Aa**	32506057.28	**Ba**
PC 38:2	13117.4768	**Ab**	17955.94315	**Ab**	19498.58679	**Ab**	30512.77543	**Ab**	148133.3743	**Aa**	97214.92393	**Aa**
PC 38:3	27701.50839	**Ab**	35794.05185	**Ab**	42451.8539	**Ab**	42505.91266	**Ab**	198829.8344	**Aa**	102108.5745	**Ba**
PC 38:3.1	3287.102094	**Ab**	3307.217491	**Ab**	4563.131325	**Ab**	7446.779544	**Ab**	40915.46158	**Aa**	19470.13914	**Ba**

SI Table 17. *(continuation)* Relative abundance of lipids of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
PE 32:1	18958.91666	**Ac**	31537.37436	**Ab**	171505.0762	**Ab**	272280.5076	**Aa**	539314.3349	**Aa**	303695.3983	**Ba**
PE 34:2	NA	**Ac**	NA	**Ab**	1823331.442	**Ab**	2790461.817	**Aa**	6600761.538	**Aa**	3440096.189	**Ba**
PE 34:3	440537.5538	**Ab**	697330.8294	**Aa**	368886.193	**Bb**	667941.4808	**Aab**	887565.7789	**Aa**	379184.0088	**Bb**
PE 34:4	200700.1124	**Ab**	299068.38	**Aa**	166862.1242	**Ab**	250968.408	**Aa**	619261.621	**Aa**	218435.3518	**Ba**
PE 34:5	449587.79	**Ab**	691969.779	**Aa**	432990.296	**Ab**	614598.0748	**Aa**	1670383.171	**Aa**	553414.9048	**Ba**
PE 34:6	383922.2046	**Ab**	590538.6441	**Ab**	556731.1125	**Ab**	841926.4009	**Aab**	2986903.107	**Aa**	1321853.183	**Ba**
PE 36:2	4214077.187	**Ab**	6383316.193	**Aa**	4585614.518	**Bb**	7861246.315	**Aa**	7724433.364	**Aa**	5007949.864	**Ba**
PE 36:3	551196.879	**Ab**	800882.0178	**Ab**	738073.0154	**Bab**	1218079.558	**Aa**	1015770.582	**Aa**	532504.6274	**Bb**
PE 36:4	768220.3334	**Ab**	1159559.486	**Aa**	553647.044	**Bb**	1002028.138	**Aab**	1626496.595	**Aa**	630066.9879	**Bb**
PE 36:5	1637513.126	**Ab**	2582526.741	**Aa**	1846574.789	**Ab**	2861770.824	**Aa**	5523778.743	**Aa**	2442817.076	**Ba**
PE 36:6	870438.1919	**Ab**	1281086.736	**Aa**	1136998.113	**Ab**	1859483.42	**Aa**	4005385.435	**Aa**	1840800.883	**Ba**
PE 38:2	585405.9439	**Ab**	727042.0526	**Ab**	835153.7531	**Ab**	1428127.157	**Ab**	2725983.863	**Aa**	1903812.31	**Aa**
PE 38:3	79977.15885	**Ab**	98243.15212	**Aa**	110143.8644	**Ab**	185987.8711	**Aa**	312185.7949	**Aa**	174410.3032	**Ba**
PE 40:2	57073.65399	**Ab**	75552.37699	**Ab**	86304.05559	**Ab**	136667.6532	**Ab**	794295.7052	**Aa**	548536.9735	**Aa**
PE 40:3	37475.15962	**Ab**	54520.98362	**Ab**	48142.74461	**Ab**	62799.04458	**Ab**	225383.1796	**Aa**	110846.6308	**Ba**
PE 40:4	49085.43419	**Ab**	68754.63423	**Ab**	71263.23576	**Ab**	94623.83427	**Ab**	409085.7012	**Aa**	184943.7895	**Ba**
PG 34:2	639229.9857	**Ab**	1052456.733	**Aa**	727333.8388	**Ab**	725028.7502	**Aa**	2329765.694	**Aa**	892416.5384	**Ba**
PG 34:3	746187.5627	**Ab**	1143757.854	**Aa**	821998.8773	**Ab**	897635.6741	**Aa**	3003793.576	**Aa**	1303208.814	**Ba**
PG 34:4	579213.4912	**Ab**	916168.1176	**Ab**	932400.4528	**Ab**	1036807.41	**Aab**	4090421.407	**Aa**	1935258.939	**Ba**
PG 36:2	1172612.949	**Ab**	1688877.273	**Aa**	1435129.866	**Ab**	NA	**Bb**	3493395.61	**Aa**	2368120.134	**Ba**
PG 36:3	32466.95011	**Ab**	48110.99146	**Ab**	53840.84778	**Ab**	NA	**Bc**	107501.2979	**Aa**	109193.9006	**Aa**
TAG 42:0	9379.464433	**Ab**	13222.8044	**Aa**	7697.855283	**Bb**	19234.76628	**Aa**	21907.39758	**Aa**	16644.53212	**Aa**
TAG 42:1	7320.874838	**Aa**	11312.99661	**Ab**	16888.44774	**Ba**	74088.68352	**Aa**	15180.07846	**Aa**	NA	**Ab**
TAG 42:2	1257.851124	**Ab**	1522.247359	**Aa**	NA	**Ac**	NA	**Ab**	4460.05462	**Aa**	NA	**Bb**
TAG 44:0	36522.68646	**Aa**	50130.04783	**Aa**	20713.05267	**Ba**	51916.00085	**Aa**	41384.60722	**Aa**	32536.63925	**Aa**
TAG 44:1	22534.95057	**Ab**	34432.30466	**Ab**	83125.44173	**Ba**	267984.0093	**Aa**	44192.92173	**Aab**	36498.45678	**Ab**
TAG 46:0	59731.09835	**Ab**	84144.26414	**Aa**	45808.84143	**Bb**	95257.56584	**Aa**	103045.8158	**Aa**	82665.15648	**Aa**
TAG 46:1	441326.6265	**Aa**	638455.8052	**Ab**	606308.5779	**Ba**	2389885.842	**Aa**	145954.851	**Aa**	191738.45	**Ab**
TAG 46:2	163229.4939	**Aa**	238644.9463	**Ab**	129343.4448	**Ba**	351160.8443	**Aa**	110037.5228	**Aa**	76633.99473	**Ac**

SI Table 17. *(continuation)* Relative abundance of lipids of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 46:2.1	NA	**Ab**	NA	**Ac**	NA	**Bb**	109873.451	**Aa**	41973.00209	**Aa**	33004.48949	**Ab**
TAG 46:4	NA	**Aa**	NA	**Ab**	8451.928093	**Ba**	45926.63156	**Aa**	NA	**Aa**	NA	**Ab**
TAG 48:0	192118.4512	**Ba**	277788.4676	**Aa**	122541.8235	**Ba**	293163.8398	**Aa**	196148.9883	**Ba**	223878.6187	**Aa**
TAG 48:1	386820.4123	**Ab**	587233.5049	**Ab**	1578246.162	**Ba**	4735263.649	**Aa**	470394.7972	**Ab**	693634.4958	**Ab**
TAG 48:2	561840.9091	**Aa**	821458.6061	**Ab**	870257.6349	**Ba**	3947540.629	**Aa**	549169.7142	**Aa**	1220722.409	**Ab**
TAG 48:3	918937.8422	**Aa**	1272132.241	**Ab**	558204.8408	**Ba**	4974080.111	**Aa**	1017951.213	**Ba**	2628124.188	**Ab**
TAG 48:4	62009.2549	**Aa**	78733.20128	**Ac**	64724.32868	**Ba**	715554.7177	**Aa**	199355.4942	**Ba**	446430.5286	**Ab**
TAG 48:4.1	68372.24106	**Ba**	99028.24905	**Aa**	43701.53968	**Aa**	NA	**Bb**	NA	**Ab**	NA	**Ab**
TAG 48:5	70538.70794	**Aa**	89646.11973	**Ac**	49651.71753	**Ba**	561108.5724	**Aa**	177154.1244	**Aa**	305251.4281	**Ab**
TAG 48:6	41747.81961	**Aa**	52049.33925	**Ab**	33412.90229	**Ba**	398428.2478	**Aa**	134834.0154	**Ba**	277866.5265	**Aa**
TAG 48:7	5002.892684	**Ab**	5411.20381	**Ab**	3759.686614	**Bb**	31898.10077	**Aa**	31195.82244	**Aa**	29795.66393	**Aa**
TAG 48:8	6201.738854	**Ab**	6280.434508	**Ab**	4061.609538	**Bb**	30477.6193	**Aa**	32082.54316	**Aa**	28002.29558	**Aa**
TAG 48:9	3741.944805	**Ab**	2912.875522	**Ab**	2445.565096	**Bb**	21698.71644	**Aa**	23682.18709	**Aa**	27310.28214	**Aa**
TAG 50:0	100664.161	**Aab**	144457.448	**Aa**	79714.12632	**Bb**	179949.5312	**Aa**	149260.179	**Aa**	131633.1017	**Aa**
TAG 50:1	7834039.444	**Aa**	10820726.95	**Ac**	8873938.21	**Ba**	42316856.39	**Aa**	8372953.839	**Ba**	22179423.15	**Ab**
TAG 50:2	4648315.355	**Aa**	6208832.742	**Ab**	4728611.534	**Ba**	17790976.13	**Aa**	4943224.194	**Aa**	8140923.681	**Ab**
TAG 50:3	1194062.8	**Aa**	1702215.848	**Ac**	1187252.223	**Ba**	9500029.064	**Aa**	2247126.89	**Ba**	5392554.534	**Ab**
TAG 50:4	1136143.889	**Aa**	1587352.459	**Ac**	1077027.124	**Ba**	13208978.63	**Aa**	2500517.363	**Ba**	8406888.901	**Ab**
TAG 50:5	784048.6376	**Aa**	1041970.564	**Ac**	577855.2365	**Ba**	6722208.344	**Aa**	1866234.995	**Ba**	3615050.905	**Ab**
TAG 50:6	730368.1461	**Aa**	996411.5381	**Ac**	544325.9576	**Ba**	7585504.36	**Aa**	1716281.912	**Ba**	4800528.106	**Ab**
TAG 50:7	64983.53681	**Ab**	80836.45293	**Ab**	62446.96556	**Bb**	676392.8132	**Aa**	339321.9669	**Aa**	493166.7734	**Aa**
TAG 50:8	78165.93635	**Ab**	89648.00126	**Ab**	79038.88643	**Bb**	468932.5051	**Aa**	392374.9969	**Aa**	365117.3908	**Aa**
TAG 50:9	81231.63681	**Ab**	85545.62573	**Ab**	85165.27621	**Bb**	444895.7493	**Aa**	373755.8513	**Aa**	467789.6706	**Aa**
TAG 52:0	72627.31885	**Ab**	102566.2924	**Aa**	58549.36192	**Bb**	123073.4833	**Aa**	136678.0895	**Aa**	111161.6165	**Aa**
TAG 52:1	1414789.377	**Aa**	2037473.117	**Ab**	2852858.075	**Ba**	11528126.15	**Aa**	1133806.592	**Ba**	3372990.54	**Ab**
TAG 52:2	7937682.594	**Aa**	11339626.65	**Ac**	16022316.53	**Ba**	68522389.21	**Aa**	14515437.05	**Ba**	44409669.45	**Ab**
TAG 52:3	4658695.511	**Aa**	6544621.064	**Ac**	6289924.89	**Ba**	36033735.96	**Aa**	10186230.49	**Ba**	23032928.27	**Ab**
TAG 52:4	4032681.324	**Aa**	5698481.205	**Ac**	3988583.325	**Ba**	39105411.67	**Aa**	8372129.016	**Ba**	25872166.29	**Ab**
TAG 52:4.1	8477.936595	**Ba**	12850.90115	**Aa**	NA	**Ba**	46201.21698	**Aa**	16657.85962	**Ba**	45959.32055	**Aa**

SI Table 17. *(continuation)* Relative abundance of lipids of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 52:5	1742351.5	**Aa**	2546036.645	**Ac**	1433453.356	**Ba**	10536952.23	**Aa**	3976072.552	**Aa**	6581460.382	**Ab**
TAG 52:5.1	9294.28745	**Aa**	13180.44327	**Ab**	15968.13006	**Ba**	120846.134	**Aa**	9990.88134	**Ba**	57525.46162	**Ab**
TAG 52:6	NA	**Aa**	NA	**Ac**	489025.8157	**Ba**	7166093.651	**Aa**	1782085.492	**Ba**	4577168.655	**Ab**
TAG 52:6.1	5263.48224	**Ab**	6447.849645	**Ab**	5010.808138	**Bb**	20466.63413	**Aa**	19501.87607	**Aa**	16478.88634	**Aa**
TAG 52:7	517628.2271	**Aa**	668843.9277	**Ab**	487086.3454	**Ba**	6915215.551	**Aa**	1816229.177	**Ba**	4793453.73	**Aa**
TAG 52:8	377057.6607	**Ab**	489940.6063	**Ab**	631174.0649	**Bb**	1995342.458	**Aa**	2542044.706	**Aa**	1624933.926	**Ba**
TAG 52:9	521224.0549	**Ab**	697906.1421	**Ab**	1183756.408	**Ab**	2303414.34	**Aa**	4157337.101	**Aa**	2821701.036	**Ba**
TAG 54:0	31363.58301	**Ab**	45142.59268	**Aa**	25754.22888	**Bb**	45619.03408	**Aa**	61725.56393	**Aa**	47193.66955	**Aa**
TAG 54:1	136922.7985	**Aa**	200616.502	**Ac**	113391.6277	**Ba**	690536.8281	**Aa**	180330.998	**Ba**	425179.7057	**Ab**
TAG 54:2	1036128.555	**Aa**	1546341.856	**Ac**	1324690.906	**Ba**	6743819.416	**Aa**	1174424.86	**Ba**	3748320.261	**Ab**
TAG 54:3	2470681.278	**Aa**	3663054.437	**Ab**	3670802.955	**Ba**	28418203.81	**Aa**	5931074.205	**Ba**	23233226.9	**Aa**
TAG 54:4	1368501.817	**Aa**	2081745.988	**Ab**	1965658.2	**Ba**	20953057.48	**Aa**	3745316.822	**Ba**	14530607.52	**Aa**
TAG 54:5	1405584.094	**Aa**	2090160.939	**Ab**	2271061.43	**Ba**	28602807.57	**Aa**	5090000.719	**Ba**	21453458.19	**Aa**
TAG 54:6	586189.6748	**Aa**	853955.1873	**Ac**	898802.4836	**Ba**	11141334.2	**Aa**	2789504.151	**Ba**	7533053.981	**Ab**
TAG 54:7	325928.3162	**Aa**	494501.581	**Ab**	484487.5047	**Ba**	6813926.926	**Aa**	1995608.827	**Ba**	5054643.131	**Aa**
TAG 54:8	174105.7104	**Ab**	280771.1069	**Ab**	262214.7182	**Bb**	1558428.68	**Aa**	1269122.418	**Aa**	1376576.977	**Aa**
TAG 54:9	72867.35962	**Ab**	115313.2816	**Ab**	123186.9837	**Bb**	431692.6744	**Aa**	605573.6376	**Aa**	500500.4693	**Aa**
TAG 56:0	23421.93712	**Ab**	33986.11605	**Aa**	17821.45379	**Bb**	31360.31673	**Aa**	46922.5938	**Aa**	35824.39231	**Aa**
TAG 56:1	21421.94038	**Aa**	29872.95552	**Ac**	18933.65479	**Ba**	132422.5944	**Aa**	35352.71611	**Ba**	90955.89156	**Ab**
TAG 56:2	71933.70446	**Aa**	106601.1322	**Ac**	89689.64445	**Ba**	557646.2559	**Aa**	103973.643	**Ba**	310441.8279	**Ab**
TAG 56:3	159406.8721	**Aa**	238235.2762	**Ab**	263678.2771	**Ba**	1718073.803	**Aa**	284647.4967	**Ba**	1228828.018	**Aa**
TAG 56:4	111637.5579	**Aa**	168519.399	**Ac**	154826.4491	**Ba**	930851.2552	**Aa**	228678.8911	**Ba**	597808.9525	**Ab**
TAG 56:5	70143.87344	**Aa**	100877.0589	**Ab**	129256.8633	**Ba**	1129424.298	**Aa**	186987.3289	**Ba**	795521.6476	**Aa**
TAG 56:6	32658.73292	**Aa**	46179.08045	**Ab**	49947.72217	**Ba**	348968.6615	**Aa**	112074.9219	**Ba**	240749.1567	**Aa**
TAG 56:7	11029.53797	**Aa**	15463.77485	**Ab**	14984.02725	**Ba**	161196.0815	**Aa**	41230.3841	**Ba**	118592.6178	**Aa**
TAG 58:0	10036.39657	**Ab**	13942.33037	**Aa**	7385.483166	**Bb**	13786.44842	**Aa**	19673.96151	**Aa**	14662.69958	**Aa**
TAG 58:1	20657.70723	**Aa**	31128.67477	**Ab**	17399.9397	**Ba**	112770.4629	**Aa**	35065.72582	**Ba**	85100.38522	**Aa**
TAG 58:2	24047.62577	**Aa**	34498.28555	**Ac**	48194.43287	**Ba**	214590.6999	**Aa**	42226.57942	**Ba**	110353.1847	**Ab**
TAG 58:3	NA	**Ab**	NA	**Ac**	104697.1174	**Ba**	237693.6556	**Aa**	32687.09776	**Ab**	53697.94408	**Ab**

SI Table 17. *(continuation)* Relative abundance of lipids of *Chlamydomonas* sp. BR020 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 58:3.1	19943.3692	**Ab**	29467.20817	**Ab**	NA	**Ac**	NA	**Ac**	47993.94303	**Aa**	82704.3185	**Aa**
TAG 58:4	NA	**Ab**	NA	**Aa**	35732.62674	**Aa**	NA	**Ba**	34161.39289	**Aa**	NA	**Ba**
TAG 58:5	4003.143866	**Aa**	5605.441295	**Ab**	4823.243193	**Ba**	26993.89555	**Aa**	10565.8578	**Ba**	19232.33129	**Aa**
TAG 58:6	4970.048375	**Aa**	7078.831028	**Ab**	4502.740881	**Ba**	41402.54281	**Aa**	9696.501197	**Ba**	33282.29897	**Aa**
TAG 60:0	2689.854833	**Ab**	3924.586622	**Aa**	1984.40789	**Ab**	3468.421044	**Aa**	5288.805662	**Aa**	3587.224404	**Aa**
TAG 60:1	15915.28496	**Aa**	20703.23921	**Ab**	10675.62888	**Ba**	65920.27028	**Aa**	25056.97478	**Ba**	61594.81937	**Aa**
TAG 60:2	21637.74459	**Aa**	32050.27559	**Ac**	26157.60009	**Ba**	143989.9516	**Aa**	37555.83666	**Ba**	99950.63958	**Ab**
TAG 60:3	12585.35233	**Ab**	19180.24353	**Ac**	78982.24467	**Ba**	225202.2998	**Aa**	47426.56116	**Aab**	84589.42133	**Ab**
TAG 60:4	7448.499139	**Aa**	10704.8994	**Ac**	32664.65309	**Ba**	102795.1571	**Aa**	29566.74757	**Ba**	63786.57914	**Ab**
TAG 60:5	7269.402822	**Aa**	12076.09249	**Ab**	7889.988301	**Ba**	31249.90894	**Aa**	12394.65483	**Aa**	21156.09161	**Aab**
TAG 60:5.1	NA	**Ab**	NA	**Ab**	NA	**Bb**	17397.49062	**Aa**	13389.5604	**Aa**	19422.51284	**Aa**
TAG 60:6	2817.551198	**Aa**	4775.553491	**Ab**	3321.509933	**Ba**	28064.25731	**Aa**	9055.186995	**Ba**	28068.63183	**Aa**
FA 16:0	517360.9737	**Ab**	683305.4251	**Ab**	713342.5496	**Ab**	847982.8126	**Ab**	3083215.588	**Aa**	1996201.831	**Ba**
FA 16:2	162114.642	**Ab**	192469.2774	**Ab**	541036.6135	**Ab**	492493.9907	**Ab**	6601405.97	**Aa**	1623896.924	**Ba**
FA 16:3	332464.9559	**Ab**	380280.1386	**Ab**	1136403.449	**Ab**	1595229.791	**Ab**	11796995.95	**Aa**	4122491.277	**Ba**
FA 18:0	834515.6569	**Ab**	1090206.71	**Aa**	777583.4235	**Ab**	1173654.352	**Aa**	2377843.699	**Aa**	1720923.248	**Ba**
FA 18:1	8682238.352	**Ab**	10189635.06	**Ab**	10462149.13	**Ab**	12736701.36	**Ab**	43596604.08	**Aa**	31111381.98	**Ba**
FA 18:2	1081733.815	**Ab**	1506563.629	**Ab**	4000585.783	**Ab**	3225223.414	**Ab**	36537470.49	**Aa**	11428654.05	**Ba**
FA 18:3	3278094.403	**Ab**	4421514.632	**Ab**	10934367.39	**Ab**	13687227.1	**Ab**	94035324.39	**Aa**	48822965.06	**Ba**
FA 20:0	13338.9385	**Ab**	17781.40864	**Ab**	13022.24283	**Ab**	19536.70101	**Ab**	35165.02693	**Aa**	30414.45325	**Aa**
FA 20:1	30267.83017	**Ab**	39739.03131	**Ab**	37986.18918	**Ab**	46487.72758	**Ab**	164237.5835	**Aa**	115758.9296	**Ba**
FA 20:3	4647.337901	**Ab**	7079.766025	**Ab**	7744.966517	**Ab**	8242.893189	**Ab**	40814.8256	**Aa**	27947.5139	**Ba**
FA 22:0	13828.03452	**Ab**	19754.84663	**Ab**	11056.77059	**Ab**	17125.98056	**Ab**	31946.83797	**Aa**	26737.2832	**Aa**
FA 22:1	2076.577502	**Ab**	4749.441448	**Aa**	3711.168276	**Ab**	5045.541157	**Aa**	17514.76372	**Aa**	8416.018607	**Ba**
FA 24:0	26212.45394	**Ab**	39071.85878	**Ab**	30745.07031	**Ab**	38524.17898	**Ab**	124773.884	**Aa**	72978.71939	**Ba**
FA 24:1	909.7376008	**Ab**	1248.596309	**Ab**	1091.600741	**Ab**	1798.641432	**Ab**	7280.754553	**Aa**	3134.543053	**Ba**
FA 26:0	14216.36294	**Ab**	19558.03386	**Aab**	13489.39316	**Ab**	16707.63149	**Ab**	47819.61768	**Aa**	29505.1145	**Ba**
FA 28:0	4513.992976	**Ab**	6282.080303	**Aa**	3661.011042	**Ab**	5899.005031	**Aa**	12966.58692	**Aa**	8031.855683	**Ba**
FA 30:0	1705.360776	**Bb**	2779.051945	**Aa**	1397.888273	**Ab**	1942.589788	**Aa**	3610.68023	**Aa**	2464.626775	**Ba**

SI Table 18. Relative abundance of lipids of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5µM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test (P<0.05). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5µM rap		24h_Control		24h_5µM rap		48h_Control		48h_5µM rap	
DGDG 32:0	33309.10611	**Ab**	45284.82892	**Aa**	50204.09006	**Ab**	36432.69552	**Aa**	123553.1942	**Aa**	43006.02036	**Ba**
DGDG 32:1	12154.68903	**Ab**	12503.86105	**Aa**	17528.75478	**Ab**	13116.04543	**Aa**	67362.71609	**Aa**	29868.85001	**Ba**
DGDG 32:2	25601.91407	**Ab**	35464.38054	**Aa**	51369.06973	**Ab**	21117.95417	**Ba**	161483.4241	**Aa**	52522.5256	**Ba**
DGDG 32:3	104791.2864	**Ab**	143415.3686	**Ab**	194556.6029	**Ab**	93292.43833	**Ab**	655496.2166	**Aa**	396598.8779	**Ba**
DGDG 32:3.1	65147.00701	**Ab**	91346.55698	**Ab**	100431.0129	**Ab**	143197.5123	**Aab**	254328.9482	**Aa**	189965.3177	**Ba**
DGDG 34:0	22088.24472	**Ab**	29185.17256	**Aa**	48044.64619	**Aab**	43853.8264	**Aa**	59448.29572	**Aa**	24716.00253	**Ba**
DGDG 34:1	1299846.951	**Ab**	1764856.025	**Aa**	1831224.027	**Ab**	1114974.534	**Aa**	4414748.019	**Aa**	1924015.456	**Ba**
DGDG 34:2	2614666.733	**Ab**	3431920.167	**Aa**	3208806.857	**Aab**	1739809.565	**Bb**	3971473.257	**Aa**	1775419.643	**Bb**
DGDG 34:3	2038362.67	**Ab**	2691220.952	**Aa**	2765472.507	**Ab**	2536780.225	**Aa**	4025995.817	**Aa**	2567957.689	**Ba**
DGDG 34:4	2982707.838	**Ab**	3708207.213	**Aa**	3260211.018	**Ab**	985546.6479	**Bb**	6735222.52	**Aa**	3504814.464	**Ba**
DGDG 34:5	7746123.234	**Ab**	9273897.041	**Ab**	11363592.47	**Ab**	7796035.599	**Bb**	20980682.34	**Aa**	13287931.54	**Ba**
DGDG 34:6	18253577.02	**Ac**	22191165.58	**Ac**	33973026.84	**Ab**	29037165.81	**Ab**	64355877.78	**Aa**	53728774.58	**Aa**
DGDG 36:2	17941.61444	**Aa**	NA	**Ba**	NA	**Ab**	NA	**Aa**	NA	**Ab**	NA	**Aa**
DGDG 36:4	193806.3095	**Aa**	247571.4947	**Aa**	210173.3783	**Aa**	91363.4206	**Bb**	287061.3081	**Aa**	200733.554	**Ba**
DGDG 36:5	526220.1862	**Ab**	661652.3284	**Aa**	608078.3102	**Ab**	310374.5259	**Bb**	1017515.601	**Aa**	803511.4043	**Aa**
DGDG 36:6	1111488.267	**Ab**	1404638.622	**Ab**	1243149.962	**Ab**	1073754.638	**Ab**	3071697.015	**Aa**	2115045.687	**Ba**
MGDG 34:1	44156.07938	**Ac**	62329.35944	**Aa**	321889.192	**Ab**	247345.9303	**Aa**	857736.7023	**Aa**	251910.9105	**Ba**
MGDG 34:2	NA	**Ac**	85433.4654	**Aa**	288551.9695	**Ab**	182831.3847	**Aa**	525111.1134	**Aa**	158720.457	**Ba**
MGDG 34:3	313752.2314	**Ab**	NA	**Ab**	660461.91	**Ab**	537393.7229	**Aa**	1211318.22	**Aa**	NA	**Bb**
MGDG 34:4	972744.3681	**Ab**	987563.1311	**Ab**	1002991.914	**Ab**	881200.7584	**Ab**	1648603.153	**Aa**	1212247.524	**Aa**
MGDG 34:4.1	NA	**Bb**	13082.71811	**Aa**	12184.92598	**Aa**	NA	**Bb**	15509.01487	**Aa**	NA	**Bb**
MGDG 34:5	8795.255502	**Ab**	13319.07044	**Ab**	15461.91066	**Bb**	41515.11268	**Aa**	45917.74116	**Aa**	38724.75835	**Aa**
MGDG 34:6	1448766.537	**Ba**	1368046.46	**Aa**	1309513.34	**Ba**	1946191.319	**Aa**	1639773.873	**Ba**	2295818.732	**Aa**
MGDG 34:7	240895.6753	**Ab**	375514.9857	**Ab**	566219.7608	**Ab**	628693.9014	**Ab**	1094646.369	**Aa**	1496602.558	**Aa**
MGDG 36:5	237479.9147	**Aab**	241438.2233	**Aab**	181658.8354	**Ab**	146091.9575	**Ab**	355533.9574	**Aa**	370107.0742	**Aa**
MGDG 36:5.1	41999.89973	**Ab**	36962.65021	**Ab**	30389.49834	**Ab**	46151.4366	**Ab**	55063.22402	**Aa**	59774.51355	**Aa**
MGDG 36:6	237203.7688	**Ab**	262707.9442	**Ab**	237613.4435	**Ab**	221443.0126	**Ab**	518284.7172	**Aa**	563193.093	**Aa**

SI Table 18. *(continuation)* Relative abundance of lipids of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
SQDG 32:0	1361316.709	**Ab**	1776788.29	**Ab**	1433648.696	**Ab**	1660571.281	**Ab**	3764112.653	**Aa**	2915133.574	**Ba**
SQDG 32:1	17003.25752	**Ba**	24992.62366	**Aa**	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**
SQDG 32:2	53458.93338	**Ab**	63345.78765	**Bb**	64842.0887	**Ab**	30337.78122	**Bb**	251018.1252	**Aa**	180243.5561	**Ba**
SQDG 32:3	125342.5337	**Ab**	151725.4138	**Ab**	181010.0781	**Ab**	123281.2718	**Ab**	848757.4976	**Aa**	833556.2286	**Aa**
SQDG 34:1	361769.2736	**Ab**	517987.484	**Ab**	555084.8924	**Ab**	285741.104	**Ab**	2115133.325	**Aa**	1226458.39	**Ba**
SQDG 34:2	2961766.596	**Bb**	3758764.437	**Aa**	2586728.87	**Ab**	1340785.504	**Bb**	6548582.385	**Aa**	3052573.916	**Ba**
SQDG 34:3	2842834.494	**Ab**	3522019.913	**Ab**	3186215.979	**Ab**	3597846.107	**Ab**	7405279.758	**Aa**	6751976.92	**Aa**
SQDG 34:4	28380.94599	**Ab**	28133.99062	**Ab**	28054.81769	**Ab**	13167.43306	**Ab**	121415.1443	**Aa**	63996.92835	**Ba**
SQDG 34:4.1	NA	**Ab**	NA	**Ac**	NA	**Bb**	8626.005834	**Ab**	20963.37911	**Ba**	35494.76328	**Aa**
SQDG 34:6	144039.7013	**Ac**	155677.5327	**Ab**	380919.0816	**Ab**	141473.9641	**Bb**	1635116.151	**Aa**	894158.2063	**Ba**
DAG 34:1	111119.7426	**Ab**	139690.9118	**Ab**	537432.3719	**Ba**	946157.8792	**Aa**	176529.2226	**Ab**	120563.2051	**Ab**
DAG 34:2	91225.69971	**Ac**	104545.6134	**Aab**	228800.2337	**Ab**	NA	**Bb**	358045.8947	**Aa**	118857.414	**Ba**
DAG 34:3	66149.75776	**Ab**	70374.13213	**Aa**	135315.8858	**Ab**	137708.2457	**Aa**	301799.4941	**Aa**	127018.9756	**Ba**
DAG 34:4	102866.5905	**Ab**	86453.84054	**Aa**	162855.0015	**Ab**	96505.20575	**Aa**	280896.8634	**Aa**	104108.887	**Ba**
DAG 34:5	395016.3278	**Ab**	304884.8756	**Bb**	432990.0152	**Ab**	297744.8391	**Bb**	915379.8429	**Aa**	503258.5041	**Ba**
DAG 34:6	920143.6262	**Ab**	692249.5621	**Ab**	1198605.517	**Ab**	1112938.114	**Ab**	2518472.081	**Aa**	1999995.009	**Aa**
DAG 36:1	2690.264144	**Ab**	2538.91261	**Ab**	7336.920769	**Aa**	8994.690166	**Aa**	2414.93752	**Ab**	2554.086173	**Ab**
DAG 36:3	62199.93611	**Ab**	83668.99568	**Ab**	191764.8741	**Aa**	242612.8912	**Aa**	246649.657	**Aab**	136829.5267	**Aab**
DAG 36:4	89332.68735	**Ab**	86889.15071	**Ab**	211179.7477	**Bab**	409986.4165	**Aa**	385691.55	**Aa**	229543.6567	**Aab**
DAG 36:5	252737.3455	**Ab**	224445.9355	**Aa**	346956.642	**Ab**	335873.8561	**Aa**	916711.4093	**Aa**	473829.7869	**Ba**
DAG 36:6	175229.4648	**Ab**	158006.8165	**Ab**	229499.4148	**Ab**	264525.8783	**Ab**	689547.5919	**Aa**	498917.2795	**Aa**
LysoPC 16:0	133594.1283	**Ab**	256129.6271	**Ab**	834777.5154	**Aa**	275931.2213	**Bb**	883156.8232	**Ba**	1737610.345	**Aa**
LysoPC 16:0.1	8420.661345	**Ac**	10238.56996	**Ac**	30160.77478	**Ab**	30488.50709	**Ab**	46237.88081	**Aa**	62490.15188	**Aa**
LysoPC 16:2	6743.75469	**Ab**	9877.466566	**Ab**	16230.62167	**Ab**	4319.701879	**Bb**	45943.7055	**Aa**	30351.12926	**Ba**
LysoPC 16:3	39452.40043	**Ab**	84570.80147	**Ab**	68971.37724	**Ab**	58304.5693	**Ab**	458182.9707	**Aa**	203227.3324	**Ba**
LysoPC 18:1	189620.6315	**Ab**	249361.8806	**Ab**	458596.8754	**Ab**	517520.1084	**Ab**	1122671.906	**Ba**	1846572.963	**Aa**
LysoPC 18:1.1	1607032.188	**Bc**	2605206.4	**Ac**	4055758.825	**Bb**	5376131.622	**Ab**	6502064.963	**Ba**	8533274.748	**Aa**
LysoPC 18:2	101019.055	**Ab**	118436.2503	**Ab**	425115.9688	**Aa**	107957.293	**Bb**	643302.9248	**Aa**	983158.1266	**Ba**
LysoPC 18:2.1	96597.50269	**Bb**	207016.5101	**Ab**	137930.9804	**Ab**	111048.9198	**Ab**	565420.1767	**Aa**	341812.9985	**Ba**

SI Table 18. *(continuation)* Relative abundance of lipids of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
LysoPC 18:3	167798.2967	**Ab**	200319.3986	**Ab**	1004637.755	**Aa**	254421.3248	**Bb**	1696224.304	**Ba**	3004443.794	**Aa**
LysoPC 18:3.1	74303.53976	**Bb**	163202.6807	**Ab**	93828.28916	**Ab**	90891.61756	**Ab**	471708.9935	**Aa**	266284.2871	**Ba**
PC 32:0	3754.566816	**Ac**	3488.30926	**Ab**	11411.27839	**Ab**	11762.36303	**Aa**	17649.36891	**Aa**	9424.136096	**Ba**
PC 32:1	28071.78213	**Ab**	38621.23817	**Ab**	60353.56966	**Ab**	58262.6072	**Ab**	223040.2117	**Aa**	143985.4304	**Ba**
PC 32:1.1	79044.92068	**Ab**	101649.8617	**Aa**	137997.0969	**Ab**	72715.3537	**Aa**	803727.5804	**Aa**	252399.7403	**Ba**
PC 32:2	1566730.962	**Ab**	1902727.785	**Ab**	3274915.57	**Ab**	1890454.691	**Ab**	9560587.74	**Aa**	3860671.843	**Ba**
PC 32:3	5289808.234	**Ab**	6554729.087	**Bb**	9741159.009	**Ab**	6938921.041	**Bb**	29909430.6	**Aa**	21379165.67	**Ba**
PC 32:4	79528.24241	**Ab**	116992.2204	**Aa**	222912.7749	**Ab**	59471.08021	**Ba**	1719317.542	**Aa**	NA	**Ba**
PC 34:2	3773537.996	**Ab**	4699665.999	**Aa**	6939444.294	**Ab**	4989540.715	**Aa**	12364600.94	**Aa**	6690936.03	**Ba**
PC 34:3	3154472.372	**Ab**	3786624.657	**Aa**	5571650.739	**Ab**	3932337.966	**Aa**	18545848.67	**Aa**	8201748.513	**Ba**
PC 34:4	1707384.075	**Ab**	2429806.483	**Aab**	3514350.287	**Ab**	1499777.606	**Ab**	20949919.3	**Aa**	6499383.686	**Ba**
PC 34:5	3735738.036	**Ab**	5048162.733	**Ab**	11008939.54	**Ab**	5049021.097	**Ab**	51533347.77	**Aa**	19163126.23	**Ba**
PC 34:6	5318404.051	**Ac**	6576824.027	**Ab**	21346226.2	**Ab**	13778139.22	**Ab**	94168987.92	**Aa**	50034293.23	**Ba**
PC 36:1	334239.0635	**Ac**	301711.8486	**Ac**	950825.819	**Ab**	1119994.005	**Ab**	2221043.252	**Aa**	1641908.34	**Ba**
PC 36:2	11663273.81	**Ac**	10308228.59	**Ac**	26703059.82	**Ab**	33310442.3	**Ab**	68759969.9	**Aa**	62578214.83	**Aa**
PC 36:3	847185.1576	**Ab**	1034691.813	**Ab**	1840313.55	**Ab**	1242265.734	**Ab**	3442016.271	**Aa**	2493907.669	**Aa**
PC 36:3.1	1388887.54	**Ac**	1323732.308	**Ac**	4998794.472	**Ab**	6596384.284	**Ab**	12830863.62	**Aa**	12439600.43	**Aa**
PC 36:5	8725357.154	**Ab**	11498649.84	**Ab**	15119362.77	**Ab**	10601094.12	**Ab**	43507314.99	**Aa**	25975426.53	**Ba**
PC 36:6	7798832.361	**Ab**	9767869.139	**Ab**	13815661.71	**Ab**	11638356.25	**Ab**	52996373.36	**Aa**	32980692.38	**Ba**
PC 38:2	46233.65512	**Bc**	38872.99742	**Ac**	93317.6843	**Bb**	145262.9031	**Ab**	177620.1022	**Ba**	249028.0291	**Aa**
PC 38:3	41537.36888	**Ab**	52625.98071	**Aa**	79633.52575	**Ab**	42438.87152	**Aa**	176893.0027	**Aa**	74527.76812	**Ba**
PC 38:3.1	7122.41998	**Ac**	6328.410038	**Ac**	22655.20531	**Ab**	27312.98851	**Ab**	43492.99989	**Aa**	40014.43945	**Aa**
PE 32:1	280983.3257	**Ac**	211236.826	**Ac**	995847.5512	**Aa**	1283753.679	**Aa**	559022.3103	**Ab**	515580.6384	**Ab**
PE 34:2	2488583.169	**Ac**	2265353.336	**Ac**	6945358.291	**Aa**	7639176.264	**Aa**	5734673.966	**Ab**	4590589.783	**Ab**
PE 34:3	447543.6599	**Aab**	556002.951	**Aa**	326218.3832	**Ab**	258411.0851	**Ab**	604723.3494	**Aa**	345999.3579	**Bab**
PE 34:4	241000.9375	**Ab**	262579.751	**Aa**	188135.0874	**Ab**	131192.5614	**Ab**	438791.3524	**Aa**	201295.2276	**Bab**
PE 34:5	557892.5914	**Ab**	642296.7946	**Aa**	581959.6359	**Ab**	374662.3997	**Aa**	1074261.597	**Aa**	478177.5361	**Ba**
PE 34:6	464611.2851	**Ab**	558064.0046	**Ab**	903620.2412	**Ab**	676256.7749	**Aab**	2397533.683	**Aa**	1150930.36	**Ba**
PE 36:2	4257288.461	**Ab**	3665722.781	**Ab**	5390140.834	**Aa**	7192369.333	**Aa**	5152539.171	**Aab**	4971550.216	**Aab**

SI Table 18. *(continuation)* Relative abundance of lipids of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
PE 36:3	415527.9384	**Ac**	375545.2127	**Ac**	1315736.445	**Aa**	1690980.281	**Aa**	987482.1962	**Ab**	717353.0685	**Ab**
PE 36:4	753791.1136	**Ab**	855323.5955	**Aa**	494534.4531	**Ab**	339279.7905	**Ab**	1172266.426	**Aa**	491553.0843	**Bab**
PE 36:5	1920080.032	**Ab**	2387108.825	**Aa**	1706954.903	**Ab**	1233212.314	**Ab**	3388615.67	**Aa**	1689862.775	**Bab**
PE 36:6	1007134.199	**Ab**	1300553.432	**Aa**	1169351.706	**Ab**	917403.2733	**Aa**	2442735.036	**Aa**	1420027.859	**Ba**
PE 38:2	964852.6333	**Ab**	822889.1468	**Ab**	2789930.338	**Aa**	3249087.677	**Aa**	2364860.443	**Aa**	2254307.191	**Aa**
PE 38:3	121412.2445	**Ac**	113098.6785	**Ac**	701690.8904	**Aa**	844603.3194	**Aa**	419966.821	**Ab**	332802.1958	**Ab**
PE 40:2	138299.8051	**Ac**	122552.0388	**Ac**	410229.4898	**Ab**	511398.2351	**Ab**	1399503.301	**Aa**	1297017.279	**Aa**
PE 40:3	48419.41081	**Ab**	59231.93972	**Ab**	78971.72226	**Ab**	50859.79731	**Ab**	318296.2759	**Aa**	195454.8233	**Ba**
PE 40:4	67255.35631	**Ab**	82414.3091	**Aab**	84993.25585	**Ab**	55920.57448	**Ab**	303728.8207	**Aa**	134730.1683	**Ba**
PG 34:2	633949.5062	**Ab**	769646.1404	**Aa**	958955.9103	**Ab**	633220.8548	**Ba**	1534818.695	**Aa**	688756.8866	**Ba**
PG 34:3	635606.2275	**Ab**	811335.6008	**Aa**	955245.5415	**Ab**	734590.3636	**Aa**	2275450.06	**Aa**	1143726.571	**Ba**
PG 34:4	435519.4557	**Ab**	554175.8219	**Ab**	534894.1072	**Ab**	331564.6913	**Ab**	2360067.259	**Aa**	1280353.138	**Ba**
PG 36:2	1514881.415	**Ac**	1455172.346	**Ac**	2555835.099	**Aa**	3196051.645	**Aa**	2075652.401	**Ab**	2417559.207	**Ab**
PG 36:3	36139.74044	**Ab**	34141.87011	**Ab**	138091.1516	**Aa**	162583.1472	**Aa**	130444.9023	**Aa**	140152.4836	**Aa**
TAG 42:0	21595.69319	**Aa**	31879.11466	**Ab**	27148.75967	**Ba**	49777.66495	**Aa**	16637.94066	**Aa**	15547.72027	**Ac**
TAG 42:1	31644.7949	**Aa**	32984.97337	**Ab**	115503.1328	**Ba**	400434.032	**Aa**	11083.16155	**Aa**	14568.13475	**Ab**
TAG 42:2	3253.104966	**Ab**	3528.729995	**Ab**	14341.27288	**Ba**	36242.4051	**Aa**	NA	**Ab**	NA	**Ab**
TAG 44:0	62181.6741	**Aab**	77624.18433	**Ab**	79764.32378	**Ba**	153784.9256	**Aa**	27337.4787	**Ab**	32454.03025	**Ac**
TAG 44:1	212694.0226	**Aab**	244415.3449	**Ab**	1036454.555	**Ba**	3128823.094	**Aa**	24287.34112	**Ab**	29762.68258	**Ab**
TAG 44:2	21195.96474	**Ab**	23910.41327	**Ab**	112136.4796	**Ba**	278134.5379	**Aa**	NA	**Ab**	NA	**Ab**
TAG 46:0	164070.7213	**Aab**	191180.6859	**Ab**	257205.6338	**Ba**	452768.1557	**Aa**	71142.14711	**Ab**	72865.69328	**Ab**
TAG 46:1	1811882.945	**Aab**	2069300.328	**Ab**	7464796.406	**Ba**	22746471.75	**Aa**	49278.97359	**Ab**	76275.87395	**Ab**
TAG 46:2	197593.7316	**Ab**	203800.2115	**Ab**	1238399.103	**Ba**	2759826.449	**Aa**	NA	**Ab**	NA	**Ab**
TAG 46:2.1	117163.7113	**Aab**	116744.6398	**Ab**	584323.2925	**Ba**	1848616.215	**Aa**	28029.51708	**Ab**	39316.23383	**Ab**
TAG 46:3	17555.31777	**Ab**	17088.22227	**Aa**	126657.6877	**Aa**	NA	**Bb**	NA	**Ac**	NA	**Ab**
TAG 46:4	6484.468649	**Ab**	NA	**Bc**	22642.52372	**Ba**	33814.47739	**Aa**	4154.045413	**Ab**	6399.860326	**Ab**
TAG 48:0	398720.7137	**Bb**	471256.8734	**Ab**	592589.4198	**Ba**	886408.2937	**Aa**	180987.7996	**Bc**	205483.8247	**Ac**

SI Table 18. *(continuation)* Relative abundance of lipids of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 48:1	7662756.941	**Aab**	8475639.407	**Ab**	24899790.1	**Ba**	64963204.55	**Aa**	265360.1614	**Ab**	377587.4195	**Ab**
TAG 48:2	1201770.421	**Ab**	1369360.719	**Ab**	8965938.003	**Ba**	19155165.77	**Aa**	329055.7485	**Ab**	523581.2888	**Ab**
TAG 48:3	607892.5686	**Aa**	758404.031	**Ab**	1632364.107	**Ba**	6425684.369	**Aa**	702622.8094	**Aa**	1081553.463	**Ab**
TAG 48:4	87016.00099	**Ab**	104307.4114	**Ab**	337689.6748	**Ba**	950870.964	**Aa**	182223.7339	**Aab**	215163.1443	**Ab**
TAG 48:5	82067.26799	**Aa**	96926.86479	**Ab**	235589.999	**Ba**	761789.3992	**Aa**	163657.7039	**Aa**	134902.6724	**Ab**
TAG 48:6	51197.54493	**Aa**	59984.47103	**Ab**	134955.9501	**Ba**	608208.2583	**Aa**	141544.0405	**Aa**	111790.1763	**Ab**
TAG 48:7	10559.22124	**Ab**	12448.62293	**Ab**	23669.40383	**Aab**	37795.82194	**Aa**	37752.1962	**Aa**	20564.82013	**Bab**
TAG 48:8	11472.59473	**Ab**	14824.23366	**Ab**	21766.39606	**Ab**	40034.61034	**Aa**	53233.80344	**Aa**	25112.69926	**Bab**
TAG 48:9	6601.451362	**Ab**	7465.152072	**Ab**	13660.87345	**Aab**	30811.18255	**Aab**	40003.52917	**Aa**	31929.81213	**Aa**
TAG 50:0	331918.6449	**Aa**	373352.807	**Ab**	370357.8282	**Ba**	625285.395	**Aa**	125755.7146	**Ab**	141979.224	**Ac**
TAG 50:1	23590536.44	**Aab**	27238841.39	**Ab**	52024844.28	**Ba**	118474665.5	**Aa**	6726429.452	**Ab**	12318277.17	**Ab**
TAG 50:2	9410207.158	**Ab**	10415759.13	**Ab**	43666975.72	**Ba**	89839075.89	**Aa**	2874945.43	**Ab**	3924783.126	**Ab**
TAG 50:3	1665297.813	**Ab**	2052945.923	**Ab**	9322235.23	**Ba**	14905734.1	**Aa**	1907378.648	**Ab**	2753577.483	**Ab**
TAG 50:4	1351740.759	**Aa**	1685696.592	**Ab**	4316464.477	**Ba**	15989625.76	**Aa**	2122878.06	**Aa**	3355432.148	**Ab**
TAG 50:5	846226.967	**Aa**	1077026.626	**Ab**	2742447.528	**Ba**	8445745.359	**Aa**	1497523.524	**Aa**	1347282.398	**Ab**
TAG 50:6	666032.3695	**Aa**	795430.2767	**Ab**	2298638.595	**Ba**	9426528.011	**Aa**	1414683.676	**Aa**	1311744.419	**Ab**
TAG 50:7	144295.2765	**Aa**	159233.9615	**Ab**	377428.0315	**Ba**	886707.3192	**Aa**	470547.7564	**Aa**	228954.0835	**Ab**
TAG 50:8	172954.1405	**Ab**	182396.2916	**Ab**	329917.5417	**Bab**	652352.7914	**Aa**	591407.841	**Aa**	216082.549	**Bb**
TAG 50:9	165771.4016	**Ab**	153287.9196	**Ab**	275582.5136	**Bb**	630954.4137	**Aa**	726195.4371	**Aa**	298772.3792	**Bb**
TAG 52:0	193567.775	**Aa**	216179.1774	**Ab**	211326.5309	**Ba**	346835.2839	**Aa**	133724.2799	**Aa**	124875.1245	**Ab**
TAG 52:1	16107202.3	**Aab**	17070322.29	**Ab**	24301799.93	**Ba**	69496472.57	**Aa**	796237.8383	**Ab**	2189931.57	**Ab**
TAG 52:2	29144762.14	**Ab**	32858908.59	**Ab**	92654779.13	**Ba**	172473039.2	**Aa**	11418996.28	**Ab**	22884121.7	**Ab**
TAG 52:3	8993630.915	**Ab**	10859205.7	**Ab**	45286803.97	**Ba**	84829707.69	**Aa**	6555672.634	**Ab**	10852732.74	**Ab**
TAG 52:4	3463618.63	**Ab**	4450938.305	**Ab**	11527890.27	**Ba**	38784233.58	**Aa**	5681257.521	**Aab**	8623543.815	**Ab**
TAG 52:4.1	8787.801512	**Ab**	9130.665493	**Aa**	NA	**Ac**	NA	**Ab**	14050.99779	**Aa**	2859.360648	**Ba**
TAG 52:5	1586335.665	**Ab**	2014741.56	**Ab**	3666098.64	**Ba**	11894180.54	**Aa**	2443961.8	**Aab**	2129627.563	**Ab**
TAG 52:5.1	2869.547419	**Ab**	2532.237557	**Ab**	28261.12153	**Ba**	165414.3653	**Aa**	4316.035156	**Ab**	7294.71143	**Ab**
TAG 52:6	862398.7402	**Aa**	976516.7978	**Ab**	2631604.293	**Ba**	8394464.879	**Aa**	1477141.794	**Aa**	1554562.234	**Ab**
TAG 52:6.1	NA	**Ab**	NA	**Ab**	14943.62958	**Ba**	33526.32712	**Aa**	3768.548697	**Ab**	3447.180594	**Ab**

SI Table 18. *(continuation)* Relative abundance of lipids of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 52:7	985728.5223	**Aa**	839005.9022	**Ab**	2191625.686	**Ba**	7615206.544	**Aa**	1479760.599	**Aa**	1388987.18	**Ab**
TAG 52:8	625927.2209	**Aa**	674827.2727	**Ab**	1103810.214	**Ba**	1894599.71	**Aa**	1270059.1	**Aa**	779609.5776	**Ab**
TAG 52:9	638648.328	**Ab**	655785.7285	**Ab**	1023650.756	**Bab**	1930174.244	**Aa**	1413524.28	**Aa**	1163127.223	**Ab**
TAG 54:0	38846.53682	**Aa**	38509.64321	**Aa**	42794.46636	**Aa**	63877.23155	**Aa**	41534.06785	**Aa**	47990.46506	**Aa**
TAG 54:1	408613.0947	**Aab**	465504.8111	**Ab**	716147.8036	**Ba**	2745864.032	**Aa**	117181.4075	**Ab**	307552.1498	**Ab**
TAG 54:2	2190878.1	**Ab**	2492003.973	**Ab**	6651213.206	**Ba**	17192522.17	**Aa**	718241.2353	**Ab**	2224887.471	**Ab**
TAG 54:3	3737489.253	**Ab**	4965491.18	**Ab**	15059765.52	**Ba**	28847203.63	**Aa**	5019713.789	**Ab**	11096866.18	**Ab**
TAG 54:4	1814136.12	**Aa**	2387363.944	**Ab**	5851904.795	**Ba**	20410101.59	**Aa**	2575904.906	**Aa**	5704830.752	**Ab**
TAG 54:5	1897796.172	**Aa**	2654090.937	**Ab**	6700669.295	**Ba**	26169799.53	**Aa**	3369940.642	**Aa**	6713521.278	**Ab**
TAG 54:6	831852.7546	**Aa**	1182246.112	**Ab**	2846101.459	**Ba**	10199612.06	**Aa**	1633400.23	**Aa**	2178833.44	**Ab**
TAG 54:7	537862.675	**Aa**	708875.76	**Ab**	1466395.393	**Ba**	5669977.261	**Aa**	1215631.37	**Aa**	1258839.056	**Ab**
TAG 54:8	330115.2055	**Aa**	439479.4958	**Ab**	656397.0513	**Ba**	1579997.816	**Aa**	781027.0052	**Aa**	546276.8898	**Ab**
TAG 54:9	127802.2641	**Ab**	149665.9945	**Ab**	188767.444	**Bab**	493973.6804	**Aa**	339272.7859	**Aa**	281019.8166	**Ab**
TAG 56:0	26298.44151	**Aa**	25050.65914	**Aa**	24428.10577	**Aa**	38868.54893	**Aa**	32158.39498	**Aa**	35055.42173	**Aa**
TAG 56:1	47149.21924	**Ab**	49683.52855	**Ab**	114288.7693	**Ba**	401918.4259	**Aa**	20807.25726	**Ab**	66512.31791	**Ab**
TAG 56:2	224761.7775	**Ab**	272621.9163	**Ab**	803629.8886	**Ba**	2508327.008	**Aa**	65416.74104	**Ab**	208493.7098	**Ab**
TAG 56:3	312061.3007	**Ab**	401662.2301	**Ab**	1120325.514	**Ba**	2270033.101	**Aa**	156339.63	**Ab**	459258.1181	**Ab**
TAG 56:4	160326.6187	**Ab**	207667.4936	**Ab**	446855.9911	**Ba**	914169.4313	**Aa**	105252.1285	**Ab**	244905.2005	**Ab**
TAG 56:5	108576.5636	**Aab**	148339.7053	**Ab**	346310.1726	**Ba**	1017390.619	**Aa**	72685.37198	**Ab**	187425.4863	**Ab**
TAG 56:6	45547.33872	**Ab**	64299.90692	**Ab**	140764.6706	**Ba**	303615.0814	**Aa**	46709.88577	**Ab**	61844.36997	**Ab**
TAG 56:7	15597.21965	**Aa**	21862.29805	**Ab**	46557.4873	**Ba**	112851.1954	**Aa**	19060.53325	**Aa**	16802.30976	**Ab**
TAG 58:0	10994.18474	**Ba**	10649.85618	**Aa**	10605.56921	**Ba**	18930.39333	**Aa**	12465.87391	**Ba**	15491.65295	**Aa**
TAG 58:1	36017.50803	**Ab**	40013.49531	**Ab**	86655.71778	**Ba**	297101.0869	**Aa**	27605.04985	**Ab**	53894.48404	**Ab**
TAG 58:2	165027.4401	**Aab**	181852.8229	**Ab**	704254.7922	**Ba**	2163923.378	**Aa**	26640.03818	**Ab**	72403.97886	**Ab**
TAG 58:3	167131.3543	**Ab**	172916.7268	**Ab**	1616447.333	**Ba**	2935472.978	**Aa**	24821.5379	**Ab**	NA	**Ab**
TAG 58:3.1	NA	**Ab**	NA	**Ab**	NA	**Ab**	NA	**Ab**	26083.73197	**Ba**	46356.17288	**Aa**
TAG 58:4	49064.54179	**Ab**	52609.11374	**Ab**	706344.9161	**Aa**	848499.7555	**Aa**	15784.19404	**Ab**	NA	**Ab**
TAG 58:5	8203.044892	**Ab**	7901.901281	**Ab**	37931.24261	**Ba**	86759.73992	**Aa**	16468.79306	**Ab**	10784.84462	**Ab**
TAG 58:6	7271.02021	**Ab**	7399.943124	**Ab**	31991.59275	**Ba**	151385.9244	**Aa**	15509.71516	**Aab**	13516.54181	**Ab**

SI Table 18. *(continuation)* Relative abundance of lipids of *Chlorella vulgaris* BR017 strain grown under two different concentrations of rapamycin (0 and 5μM) in three growth time (0h, 24h and 48h). Different lowercase letters refer to statistical difference between times within the same rapamycin concentration. Capital letter refer to statistical difference between rapamycin concentrations in same time according to the Tukey test ($P<0.05$). Metabolite not detected is represented by NA.

Lipids	0h_Control		0h_5μM rap		24h_Control		24h_5μM rap		48h_Control		48h_5μM rap	
TAG 60:0	3330.683312	**Ab**	2977.713601	**Ab**	5184.397348	**Aa**	5653.50148	**Aa**	3778.846639	**Ab**	4163.663576	**Ab**
TAG 60:1	19947.18621	**Aa**	23671.95997	**Ab**	50463.24144	**Ba**	154992.5795	**Aa**	20643.7941	**Aa**	40415.927	**Ab**
TAG 60:2	114187.8934	**Aab**	120639.2216	**Ab**	369173.1151	**Ba**	1135974.149	**Aa**	28194.79448	**Ab**	55621.15197	**Ab**
TAG 60:3	190849.8425	**Ab**	195319.8607	**Ab**	1297111.755	**Ba**	2941965.759	**Aa**	20069.96202	**Ab**	38258.53663	**Ab**
TAG 60:4	36454.18473	**Ab**	38436.57778	**Ab**	527080.0347	**Aa**	639294.0529	**Aa**	18607.37505	**Ab**	33591.28148	**Ab**
TAG 60:5	11676.84518	**Ab**	10987.53967	**Ab**	45048.38002	**Ba**	98279.21341	**Aa**	14853.12003	**Ab**	12892.25433	**Ab**
TAG 60:5.1	NA	**Ab**	NA	**Ab**	48514.91327	**Ba**	82386.51959	**Aa**	8580.216096	**Ab**	10907.00335	**Ab**
TAG 60:6	5986.21273	**Ab**	6672.742706	**Ab**	28058.7234	**Ba**	119396.5767	**Aa**	7366.238962	**Ab**	10876.12013	**Ab**
FA 16:0	454081.2202	**Ab**	642118.2684	**Ab**	1715127.862	**Aa**	2027802.714	**Aa**	2140396.696	**Aa**	1969654.046	**Aa**
FA 16:2	183024.0389	**Ab**	305270.9019	**Ab**	1216850.357	**Ab**	540204.7293	**Ab**	4388283.475	**Aa**	3238523.234	**Aa**
FA 16:3	275258.5359	**Ab**	436366.478	**Ab**	1923019.779	**Ab**	1620464.938	**Ab**	7812081.73	**Aa**	8332121.959	**Aa**
FA 18:0	637931.8324	**Ab**	835939.8527	**Ab**	1062208.81	**Aa**	1435338.58	**Aa**	1228945.948	**Aa**	1187920.22	**Aa**
FA 18:1	7973349.919	**Ab**	7899639.853	**Ab**	14700649.44	**Aa**	17340705.82	**Aa**	15696210.79	**Aa**	22279585.07	**Aa**
FA 18:2	1103098.138	**Ab**	1425306.569	**Ab**	7045891.283	**Ab**	3314767.514	**Ab**	21508729.08	**Aa**	17577943	**Aa**
FA 18:3	2362277.834	**Ab**	3124973.318	**Ab**	13349592.42	**Ab**	12458972.05	**Ab**	58449784.82	**Aa**	51330080.68	**Aa**
FA 20:0	14739.43295	**Ab**	15166.58268	**Ab**	35610.04782	**Aa**	45360.8524	**Aa**	24336.68025	**Ab**	23589.53837	**Ab**
FA 20:1	58378.36248	**Ab**	64910.9495	**Ab**	138263.0142	**Aa**	130567.4682	**Aa**	129573.0121	**Aa**	139453.0499	**Aa**
FA 20:3	5074.066827	**Ac**	5817.989642	**Bc**	11555.28716	**Ab**	7918.748455	**Bb**	22311.31331	**Aa**	16549.5463	**Ba**
FA 22:0	15641.61745	**Ab**	13939.01901	**Ab**	27342.64237	**Aa**	34411.09943	**Aa**	34084.32934	**Aa**	32833.73564	**Aa**
FA 22:1	3467.311938	**Ab**	4993.739918	**Ab**	14873.6684	**Aa**	12392.90113	**Aa**	9203.788421	**Aa**	10782.95852	**Aa**
FA 24:0	29508.15352	**Ac**	30763.46656	**Ac**	58864.84196	**Ab**	65897.09503	**Ab**	107109.6215	**Aa**	101843.0909	**Aa**
FA 24:1	2235.880002	**Ab**	2144.34259	**Ab**	10643.61547	**Aa**	11539.21584	**Aa**	4768.667917	**Ab**	2553.632589	**Ab**
FA 26:0	16118.30468	**Ab**	17302.97521	**Ab**	32323.0326	**Aa**	34948.52729	**Aa**	52085.91837	**Aa**	35287.00144	**Aa**
FA 28:0	4368.651476	**Ab**	5383.220836	**Aa**	5804.393811	**Ab**	6165.132789	**Aa**	10914.14594	**Aa**	7343.55538	**Ba**
FA 30:0	1815.495838	**Ab**	1848.003546	**Ab**	1769.797667	**Aab**	2300.517903	**Aab**	2899.340885	**Aa**	2646.255584	**Aa**