GABRIEL WILLIAM DIAS FERREIRA

HARVEST RESIDUES MANAGEMENT AND SILVICULTURAL OPERATIONS IMPACT ON SOIL PHYSICAL AND ORGANIC MATTER QUALITY OF EUCALYPT PLANTATIONS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de *Doctor Scientiae.*

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APROVADA: 26 de janeiro de 2017.

Carlos Alberto Silva

Leônidas Carrijo Azevedo Melo

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(Coorientador)

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(Orientadora)

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BIOGRAFIA

GABRIEL WILLIAM DIAS FERREIRA, filho de Mozart Martins Ferreira e Maria José Dias Ferreira, nasceu em Lavras – Minas Gerais, em 3 de junho de 1987.

Em março de 2005, iniciou a graduação em Engenharia Florestal na Universidade Federal de Lavras. Foi bolsista de iniciação científica do Laboratório de Estudos e Projetos em Manejo Florestal entre março/2007 e fevereiro/2010. Graduou-se em julho de 2010, obtendo o título de Engenheiro Florestal.

Em agosto de 2010, iniciou o mestrado em Solos e Nutrição de Plantas na Universidade Federal de Viçosa, concluindo-o em 26 de junho de 2012. Em novembro do mesmo ano, iniciou o doutorado também em Solos e Nutrição de Plantas pela mesma instituição. Realizou parte do seu doutoramento na University of New Hampshire/USA, entre outubro/2015 e setembro/2016. Submeteu-se à defese de tese em 26 de janeiro de 2017.

SUMÁRIO

ABSTRACT

FERREIRA, Gabriel William Dias, D.Sc., Universidade Federal de Viçosa, January, 2017. **Harvest residues management and silvicultural operations impact on soil physical and organic matter quality of eucalypt plantations**. Adviser: Emanuelle Mercês Barros Soares. Co-adviser: Ivo Ribeiro da Silva.

Brazil is among the world's largest timber producers and *Eucalyptus* planted forests are responsible for a significant portion of wood products supply. Furthermore, these forests play an important role on mitigation of increasing atmospheric greenhouse gases. The period between harvesting and new rotation establishment, *i.e.* its first two or three years, concentrates the major part of forest mechanical operations that may define achievable productivity while causing harmful impacts on soil physical and organic properties. This thesis is divided in three chapters that evaluated and tried to provide a better understanding of these operations impact on eucalypt forest dynamics and soil properties. The first chapter approached how the management of eucalypt harvest residues (HR) could alter its decomposition pattern and the dynamics of two soil organic matter (SOM) fractions (Particulate Organic Matter – POM, and Associated to Minerals – MAOM). The experiment was set up in an area where natural grasslands of Pampa Biome in the Rio Grande do Sul State, Brazil, have been recently converted to eucalypt forests. Removal of all residues (-R), only bark removal (-B) and maintenance of all residues (+B), all of them combined with external 200 kg ha^{-1} of N addition (+N) or not (-N), were simulated in PVC micro-plots in a 3x2 full factorial with 4 replications. A 10 % ^{15}N-enriched solution was used as N source to track the role of mineral-N in the process. Whole micro-plots were sampled at 0, 3, 6, 12 and 36 mo. after experiment establishment and taken to the lab for further analysis. All sampling times were used to build decomposition curve, while C, $\delta^{13}C$, N and ^{15}N content associated with both SOM fractions were determined on 36 mo. samples in 0 -1 and 1 -5 cm soil layers. In these same layers, SOM fractions from 12 and 36 mo. samples were characterized with Pyrolysis associated with Gas Chromatography/Mass Spectrometry (Py-GC/MS) to obtain a better understanding of SOM formation pathway under different HR management. Almost 80 % of HR have been decomposed until 3 yr. Bark ($p<0.05$) and mineral-N ($p=0.06$) presence slowed down decomposition. Overall, changes in C and N content and $\delta^{13}C$ due to residues presence were more expressive in 0-1 cm layer and POM fraction. +B tended to increase HR contribution, but its effect was more distinguished in -N treatments. Mineral-N fraction was always higher when HR were present, particularly in +B treatments, but its

contribution to SOM fractions was always smaller than 4 %. Py-GC/MS products revealed a direct role of HR on POM formation, but their effect on MAOM seems to be indirect by altering microbial composition and its products. In summary, we showed how HR management drives SOM fractions chemistry and we conclude that a sustainable management of HR can enhance soil C accrual. In the second chapter was evaluated how two different harvest systems would change soil physical properties, soil C content and soil CO_2 efflux, and initial tree growth under two different silvicultural system. To this end, two adjacent stands located in Estrela do Sul/MG were selected. Each stand was harvested and logged with a different system (Feller + Forwarder – F+F; and Feller + Skidder – F+S) and soil physical properties [Soil Bulk Density (*Ds*), Micro (*Mi*), Macro (*Ma*) and Total Porosity (*TP*), and Penetration Resistance (*PR*)], SOM properties [Labile-C, C associated with Particulate (C-POM) and Mineral (C-MAOM) fractions] soil CO_2 efflux and stump mortality rate, were assessed after harvesting operations and compared with reference (before harvesting). Afterwards, each stand was divided in coppice and replanting, and we followed soil CO_2 efflux after planting and one year after planting, when trees height (*H*) and SOM properties were also measured in both areas and system to evaluate how areas would recover from harvesting impacts. All soil variables were assessed at planting and inter-planting row positions. Both systems affected soil density and soil pore configuration, but in different ways. F+F concentrates traffic at inter-planting row position, and therefore causes a slightly higher compaction in this region, while F+S does not follow traffic routes and impacts planting-row similarly, resulting in higher stump mortality. Harvesting operations increased *Ds*, *Mi* and *PR* and reduced *Ma* and *TP* at all layers. Soil CO_2 efflux was not affected by harvesting operations. Differences in soil CO_2 were found after planting, when it was higher in F+S system, that also presented higher labile-C and C-POM. One year after planting, mortality rate was still higher under coppice in F+S system, that also presented lower trees. In turn, trees were higher in replanting in this area. Soil respiration behaved similarly, *i.e.*, higher in F+F under coppice and lower under replanting. Overall, after one year coppice system presented higher Labile-C, C-POM and C-MAOM. We concluded that each harvest system affects row and inter-row differently, creating different soil functional zones inside same area, that might be enhanced by the next silvicultural system chosen, and should be observed when assessing ecosystem services and site condition. Lastly, the third chapter evaluated the effect of land use change from natural grasslands of Pampa Biome to eucalypt plantations, as well as N fertilization effects on initial eucalypt growth, fine-root biomass (*FRB*) and its spatial distribution, and C, $\delta^{13}C$ and N content associated

with SOM fractions (POM and MAOM). 4 N-levels were tested (24, 36, 48 e 108 kg ha^{-1} of N) on initial tree growth (until 2 yr.). Afterwards, representative trees were chosen to evaluate *FRB* until 40 cm depth, and soil samples also until 40 cm depth were collected for SOM evaluation. Positive effect of N on tree growth (diameter and height) was seen initially after fertilization, *i.e.*, 1.5 yr., whereas at 2 yr. N effects were seen only at trees height. The *FRB* fitted regression showed increase of FRB until 56 kg ha^{-1} of N, and after that level a decrease in *FRB* was observed, and the highest N level used resulted in the lowest *FRB*. Both horizontal and vertical anisotropy in fine-root distribution were observed, and besides differences among N levels, we could not see a clear relation between N fertilization and fine-root spatial distribution. Overall, land use change to eucalypt plantations increased soil C content, particularly in top-soil layers. 36 kg ha^{-1} resulted in higher C-POM in 0 -10, while 48 kg ha^{-1} resulted in higher C-MAOM in this layer. C and N dynamics were tightly correlated, especially in MAOM fraction. C-POM was positively correlated with *FRB*. Tillage had a strong control on soil C and N stocks, enhancing C deposition and turnover at row (ridge) region, most likely for favoring roots development in this region. Therefore, it is shown that N fertilization may alter initial tree growth, but its effects don't seem to last longer. Nevertheless, N effects can be reflected on fine-root biomass and distribution and C and N of SOM fractions. We hope that our findings could guide the adoption of proper management practices in eucalypt plantations in Brazil, enhancing the productivity and sustainability of these forests.

RESUMO

FERREIRA, Gabriel William Dias, D.Sc., Universidade Federal de Viçosa, janeiro de 2017. **Impacto do manejo dos resíduos da colheita e de operações silviculturais sobre a qualidade física e da matéria orgânica do solo em plantações de eucalipto**. Orientador: Emanuelle Mercês Barros Soares. Coorientador: Ivo Ribeiro da Silva.

O Brasil é um dos maiores produtores mundiais de madeira e plantações de eucalipto são responsáveis por boa parte do suprimento dos produtos madeireiros no país. Além disso, essas plantações desempenham papel importante na mitigação do aumento dos gases de efeito estufa na atmosfera. No período compreendido entre a colheita de um ciclo e o estabelecimento de uma nova rotação, ou seja, os seus primeiros dois ou três anos, se concentra a maior parte das operações florestais, que podem definir o sucesso do povoamento e ao mesmo tempo causar impactos negativos em propriedades físicas e orgânicas do solo. Esta tese, dividida em três capítulos, teve como objetivo avaliar o impacto dessas operações sobre a dinâmica das florestas de eucalipto e propriedades do solo. No primeiro capítulo foi abordado como o manejo dos resíduos da colheita do eucalipto pode alterar a sua decomposição e a dinâmica de duas frações (Matéria Orgânica Particulada – MOP e Associada aos Minerais – MAM) da matéria orgânica do solo (MOS). O experimento foi conduzido em área onde pastagens naturais do Pampa Gaúcho foram recentemente convertidas em plantios de eucalipto. Foram simuladas a remoção total dos resíduos (-R), a remoção apenas da casca (-B) e a manutenção total dos resíduos (+B), combinados (+N) ou não (-N) com a adição de 200 kg ha^{-1} de N mineral, em um fatorial 3x2 completo com 4 repetições. Uma solução enriquecida em 10 % com ^{15}N foi utilizada como fonte de N para traçar o N mineral em todo o processo. Aos 0, 3, 6, 12 e 36 meses após a instalação amostras dos resíduos e do solo foram coletadas e levadas ao laboratório. Dados de todos os tempos foram utilizados para construir a curva de decomposição dos resíduos, enquanto que os teores de C, $\delta^{13}C$, N e $\delta^{15}N$ associados às duas frações da MOS foram determinados no último tempo nas camadas de 0 -1 e 1 -5 cm do solo. Nessas mesmas camadas, as frações da MOS dos solos de 12 e 36 meses foram submetidas à pirolise associada com cromatografia gasosa e espectrometria de massa (Pi-CG/EM) para caracterização química e tentativa de melhor entendimento sobre os processos de formação da MOS sob os diferentes manejos de resíduos. Ao final dos três anos, cerca de 80 % de todo o resíduo tinha sido decomposto. A presença da casca ($p<0,05$) e do N-mineral ($p=0,06$) diminuíram a taxa de decomposição do resíduo. O efeito dos resíduos sobre o C e N das frações da MOS foram mais expressivos na fração

particulada e camada de 0 -1 cm. Em geral, a presença dos resíduos aumentou os teores de C e N, e diminuiu os valores de $\delta^{13}C$, indicando contribuição dos resíduos para as frações da MOS. A contribuição dos resíduos é maior na presença da casca, especialmente nos tratamentos -N. A contribuição do N mineral para as frações da MOS foi inferior a 4 %, mas foi sempre maior quando os resíduos estavam presentes. Os resultados da Pi-CG/EM indicaram contribuição direta dos resíduos para a fração MOP, enquanto que a influencia na fração MAM parece ser indireta pela alteração da comunidade microbiana e seus produtos. Concluindo, foi mostrado como o manejo dos resíduos altera a composição da MOS e que a manutenção dos resíduos na área acarreta em aumento nos teores de C da MOS. No segundo capítulo, foi avaliado como dois sistemas diferentes de colheita de eucalipto alterariam propriedades físicas do solo, os teores de C e a emissão de CO_2 pelo solo, bem como o crescimento inicial das árvores após a adoção de dois sistemas silviculturais distintos. Para isso, dois talhões adjacentes localizados em Estrela do Sul/MG foram selecionados. Cada talhão foi colhido com um sistema diferente (*Feller* e *Forwarder* - F+F, ou *Feller* e *Skidder* - F+S) e propriedades físicas [Densidade do Solo (*Ds*), Micro (*Mi*), Macro (*Ma*), Porosidade Total (*PT*) e Resistência à Penetração (*RP*)], orgânicas (C lábil, e C associado às frações MOP e MAM), emissão de CO_2, e mortalidade das cepas foram avaliadas antes e depois da colheita. Posteriormente, cada área foi dividida em dois sistemas silviculturais: talhadia e alto-fuste (reforma), e a emissão de CO_2 pelo solo, frações de C do solo e a altura das árvores foram analisados após um ano para avaliar como cada área se recuperaria dos impactos da colheita. Todas as avaliações foram feitas nas linhas e entrelinhas de plantio. Ambos os sistemas de colheita afetaram negativamente as propriedades físicas do solo, mas de maneira diferente. Enquanto F+F concentra o impacto nas entrelinhas, o sistema F+S não segue linhas de trafego pré-estabelecidas, causando maior compactação nas linhas de plantio e consequentemente maior mortalidade de cepas. A colheita aumentou *Ds*, *Mi*, *RP* e reduziu *Ma* e *PT* em todas as posições, sistemas e profundidade. A emissão de CO_2 do solo não foi alterada pela colheita. Diferenças na emissão de CO_2 só foram encontradas após o plantio, quando foi mais elevada no F+S em ambos sistemas silviculturais, e C lábil e C-MOP também estavam mais elevados. Após um ano, a mortalidade ainda era maior no sistema F+S e altura menor no sistema de talhadia. Por outro lado, as árvores no F+S sob reforma eram mais altas. A respiração do solo apresentou padrão semelhante, ou seja, mais alta em área de talhadia no F+F e mais baixa nas áreas reformadas. C lábil, C-MOP e C-MAM foram significativamente mais altos nos sistemas de talhadia. Conclui-se que cada sistema de colheita afeta linha e entrelinha de plantio diferentemente, criando

diferentes zonas no talhão, que podem ser exacerbadas com a adoção de diferentes sistemas silviculturais. Essas diferenças devem ser observadas quando são avaliados impactos sobre o solo. No terceiro capítulo foram avaliados o efeito da substituição de pastagens naturais do Pampa Gaúcho por plantios de eucalipto e o efeito da adubação nitrogenada sobre o crescimento inicial, a biomassa e distribuição espacial de raízes finas de eucalipto, e teores de C, $\delta^{13}C$ e N associados às frações MOP e MAM da MOS. Foram testadas 4 doses de N (24, 36, 48 e 108 kg ha^{-1} de N) e o crescimento inicial das árvores foi avaliado até a idade de dois anos. Posteriormente, foram selecionadas árvores representativas que tiveram a biomassa de raízes finas (BRF) avaliadas até a profundidade de 40 cm, ao mesmo tempo em que se realizaram as avaliações da MOS também até 40 cm. Efeitos positivos da adubação nitrogenada foram observados inicialmente sobre diâmetro e altura, mas aos dois anos apenas a altura foi positivamente influenciada. A regressão ajustada para a BRF indicou aumento da BRF até 56 kg ha^{-1} de N, sendo que após esse nível a BRF diminui e a maior dose de N testada resultou na menor BRF observada. Foi observada anisotropia na distribuição radicular horizontal e verticalmente e, apesar de diferenças entre as doses, não foi possível traçar uma relação clara entre as doses de N e a distribuição espacial de raízes finas. Em geral, a introdução de eucalipto elevou os teores de C do solo. Os efeitos foram maiores nas camadas superficiais. 36 kg ha^{-1} resultou nos maiores teores de C-MOP na camada 0 -10, enquanto que 48 kg ha^{-1} resultou nos maiores C-MAM nessa camada. As dinâmicas de C e N apresentaram-se fortemente correlacionadas, especialmente na fração MAM. Os teores de C-MOP correlacionaram-se positivamente com BRF. O sistema de preparo do solo influencia os teores de C e N no solo, aumentando-os na linha de plantio, provavelmente por favorecer o desenvolvimento de raízes nessa região. Assim, verificou-se que a adição de N pode afetar positivamente o crescimento inicial do eucalipto, mas os efeitos não parecem ser duradouros. Por outro lado, o efeito do N parece ser observado por mais tempo sobre a biomassa de raízes finas e C e N das frações da MOS. Espera-se que os resultados encontrados na presente tese possam ser utilizados para nortear as práticas de manejo adotadas em plantios de eucalipto no Brasil, trazendo maior produtividade e sustentabilidade à essas florestas.

I. GENERAL INTRODUCTION

It is estimated that timber world market will increase 40 % in the next 35 years. Considering current productivity levels of *Eucalyptus*, the most planted genus worldwide, this expected growth would require additional planting of 210 million of hectares (IBÁ, 2015). Brazil is among the world's largest wood producers and presents the seventh biggest forest planted area in the world (Colodette et al., 2014). Planted forests are responsible for 91 % of industrial wood production in the country. Estimates point out that about 7.5 million hectares of Brazilian territory are occupied by planted forests, being 72 % of those only by *Eucalyptus* genus (IBÁ, 2015). Although extensive, the area covered by planted forest covers only 0.9 % of national territory, which places the county in a highlighted position in future forecasts of the world wood chain supply. Moreover, the country already presents the world highest average productivity (~ 40 m^3 ha^{-1} yr^{-1} for *Eucalyptus* plantations) (IBÁ, 2015). The great pressure in international agreements to preserve native forests and mitigate increasing greenhouse gases concentration in the atmosphere and climate change further enhances the importance of these forest plantations.

Eucalypt forests are recognized as an efficient atmospheric CO_2 sink, and reforestation is considered as a sustainable practice by the *Clean Development Mechanism* of Kyoto Protocol and fomented by the Low Carbon Emission Agricultural Plan (Plano ABC) of the Brazilian National Government. The recent adoption of minimum tillage technique follows similar principles of no-till systems adopted in agriculture, that have resulted in significant increases in soil carbon (C) stock (Diekow et al., 2005; Lal, 2004), plays in favor of planted forests. However, these forests ability to long-term store C is still questionable, since they are constantly managed to obtain high productivity in increasingly shorter rotations, and an elevated C and nutrient export occurs at every harvesting (~ 7-year average rotation). An alternative would be to increase C transference to the soil, a larger and more stable pool (Lal, 2005, 2004), particularly by keeping non-or-less commercial components in the field after harvesting, as they could account for up to 40 % of above-ground biomass at harvesting (Gatto et al., 2010).

Nevertheless, reforestation effects on soil C are still not a consensus, and either increases or decreases on soil C stock have been observed due to several factors such as previous land use, edaphoclimatic conditions and management practices adopted (Cook et al., 2016; Li et al., 2012; Lima et al., 2006; Zinn et al., 2002). The lack of sensibility of total soil C concentration contributes to these divergences. Hence, the use of soil

organic matter (SOM) fractionation methods, isotopes and molecular techniques could bring new insights and provide a better understanding of SOM dynamics under eucalypt forests.

The period between harvesting and new rotation establishment, *i.e.* its first two or three years, concentrates the major part of forest mechanical operations and could define achievable productivity while may cause harmful impacts on soil physical, chemical and biological properties. We highlight harvesting and logging, harvest residues management, silvicultural system choice and fertilization as main defining operations. Studies addressing these operations effects on SOM are still scarce, making uncertain the real and potential role of eucalypt forests to mitigate climate change. These uncertainties are greater in new forest expansion areas, particularly in the Rio Grande do Sul State, Brazil, where expansion is occurring over natural grasslands of Pampa Biome, that is a fragile ecosystem that has a significant portion of its soil degraded due to inadequate management and excessive grazing (Pillar et al., 2012; Roesch et al., 2009).

This thesis is divided in three chapters aiming at filling some of these gaps, providing a better understanding on management practices effects on eucalypt forests and SOM dynamics. The first chapter uses a decomposition experiment to address harvest residues management effects on dynamics of C and N of SOM fractions. We selected a site in Rio Grande do Sul State where *Eucalyptus* afforestation over natural grassland was just happening and simulated harvest residues managements practices. Therefore, natural differences between the ^{13}C isotope signature of C_3 (new, eucalypt) and C_4 (old, grassland) plants could be used to quantify harvest residues contribution to SOM pools. We also took advantage of differences between SOM and harvest residues chemistry and used pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) to characterize changes in SOM overtime and elucidate SOM formation pathways under different harvest residues managements.

In the second chapter is studied how different harvest systems and afterwards different silvivultural systems would alter soil physical properties, soil CO_2 emission, C content in different SOM fractions and initial tree growth. We selected two adjacent loamy-soil sites in Minas Gerais State to compare these different systems, as loamy soils are more prone to physical damage. The two stands were followed over the course of one year from harvesting to fertilization operations. Lastly, the third chapter assesses the effect land use change from natural grassland of Pampa Biome to eucalypt forests by changes in SOM properties, and how nitrogen fertilization would affect initial tree growth, fine-root biomass and spatial distribution and SOM dynamics. Trees were

measured over the first two years, and early changes in SOM properties were evaluated by SOM fractionation in two pools with different turnover time and sensitivity to land use change, and C and N content, and the proportion of C-derived from eucalypt (C_3) using isotope techniques, were determined in each SOM pool.

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II. CHAPTER 1

Eucalyptus harvest residue management enhances SOM accrual and alters chemistry of SOM fractions

ABSTRACT

Eucalyptus forests have been considered a key low-carbon land use, efficiently sinking atmospheric CO_2. Its non-or-less-commercial components (Harvest residues – HR) can account for up to 40 % of the aboveground biomass at harvesting. Keeping HR in the field could enhance CO_2 sequestration, especially if they could be transformed into stable forms of soil organic carbon (SOC). We collected samples from an experiment set up in a new frontier of *Eucalyptus* forest expansion in Southern Brazil and used micro-plots of PVC inserted 10 cm into the soil to assess the effect of simulated management practices [removal of all residues (-R), only bark removal (-B) and maintenance of all residues (+B), all of them combined with external nitrogen addition (+N) or not (-N)] on decomposition rate, carbon (C) transfer and C stabilization. A 10 % ^{15}N-enriched solution was used as N source to track the role of mineral-N in the process. Whole micro-plots were sampled five times (0, 3, 6, 12 and 36 mo. after experiment establishment). At each time, residues were carefully separated from soil and soil samples were divided in 0 -1 and 1 -5 cm soil layers for analysis. We assess HR management effects on soil organic matter (SOM) fractions [Particulate organic matter (POM) and Mineral-associated organic matter (MAOM)] by changes in C and N content and changes in the natural abundance of δ^{13}C, as well as changes in soil fraction chemistry by Py-GC/MS. Almost 80 % of HR have been decomposed until 3 yr. Bark ($p<0.05$) and mineral-N ($p=0.06$) presence slowed down decomposition. HR had great impact on SOM properties after 3 yr. of experiment. Changes in C content and δ^{13}C were more expressive in 0-1 cm layer and POM fraction. Overall, HR presence increased C content and decreased δ^{13}C values, indicating a higher fraction derived from HR in SOC. +B tended to increase HR contribution, but its effect was more distinguished in -N treatments. Changes in N content followed the same trend observed for C. Mineral-N fraction was always higher when HR were present, particularly in +B treatments, but its contribution to SOM fractions was always smaller than 4 %. Py-GC/MS products revealed a distinct formation pathway of SOM fractions. HR have a direct role on POM formation, but their effect on MAOM seems to be indirect by altering microbial composition and its products. Lignin disappeared in 3 yr. MAOM – 1 -5 samples, indicating that it's not stable even associated with minerals and aboveground litter it's not efficient to keep its content below top-soil layer. In summary, we showed how HR management drives SOM fractions chemistry and we conclude that a sustainable management of HR can enhance soil C accrual.

Keywords: Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS); ^{13}C and ^{15}N stable isotopes; soil organic matter formation.

1. INTRODUCTION

Brazil is among the biggest pulp and sawn wood producers in the world. Planted forests cover more than 7.5 million hectares in the country and reduce the pressure over native forests, particularly the Amazon (IBÁ, 2015). With an average productivity of 40 m^3 ha^{-1} yr^{-1} in 7 yr. rotations, *Eucalyptus* plantations are the main responsible for wood production and occupy ~ 72 % of forest planted area (Colodette et al., 2014; IBÁ, 2015). Sustainable management of these forests usually relies on practices that maintain or increase soil organic matter (SOM) stocks, since it has a major role on nutrient dynamics in the tropics. Plants are the major carbon (C) source for SOM and particularly in these forests, litter, root turnover and harvest residues (HR) are the main inputs.

Basically, HR are non-or-less-commercial components composed by leaves, branches and twigs, non-commercial stem (small diameter at thinner end) and bark. Around 30 t ha^{-1} of HR are generated in an average eucalypt rotation in Brazil (Gatto et al., 2010). Maintaining HR in the field has proven to provide several benefits on soil physical (Jesus et al., 2015; Silva et al., 2007) and chemical properties (Kumaraswamy et al., 2014; Laclau et al., 2010b; Mendham et al., 2003), and thus tree growth (Laclau et al., 2010a; Mendham et al., 2014; Rocha et al., 2016; Versini et al., 2013). HR impacts on SOM stocks, though, is still debated (Kumaraswamy et al., 2014; Rocha et al., 2016) and possible effects on SOM chemistry are even less studied (Mathers et al., 2003). Actually, studies addressing changes in SOM quality due to *Eucalyptus* plantations introduction are scarce (Sanderman et al., 2014; Santana et al., 2015; Soares et al., 2013).

Several authors have tried to understand the pathway of SOM formation by litter input (Bradford et al., 2013; Cotrufo et al., 2015; Gunina and Kuzyakov, 2014; Hatton et al., 2015; Mambelli et al., 2011). The presence of more complex and lignified woody materials among HR components might change their decomposition pattern and therefore their role on C cycle (Bradford et al., 2014; Ferreira et al., 2016; Magnússon et al., 2016). Management practices like changes in HR mix and increasing nitrogen (N) fertilization aiming at higher productivity might also affect C dynamics (Berg and Matzner, 1997; Souza et al., 2016; Vivanco and Austin, 2011). Particularly, the possible interaction between lignified compounds and tannins of eucalypt HR with N requires further investigation (Knorr et al., 2005; Kraus et al., 2003).

To understand the dynamics of transference from plant-litter to SOM, separate SOM into different functional pools is crucial. Physical fractionation is based on the premises that the association with finer mineral particles (silt and clay) play a key role on

the turnover and stability of SOM (Dungait et al., 2012; von Lützow et al., 2007). Particulate Organic Matter (POM), *i.e.* OM not associated with minerals, may be the entry of plant litter into SOM pool and is characterized for fast turnover time and low stability (Grandy et al., 2007; Gunina and Kuzyakov, 2014), although some authors recently have shown a direct flow of soluble components of litter to stable mineral associated pools (MAOM) (Cotrufo et al., 2015; Sanderman et al., 2014). MAOM, in turn, comprises the major part of SOM, where the protection against microbial accessibility and/or an organo-mineral association grant stability to this pool. Whilst POM is formed basically by plant inputs (Grandy et al., 2007), microbial products dominate MAOM fraction and plant signal, even from recalcitrant compounds, is almost absent in this fraction (Grandy and Neff, 2008; Klotzbücher et al., 2016). We argue if this behavior would be the same with more lignified inputs like HR or could more lignified materials create a breakdown continuum and be stabilized into stable pools (Sanderman et al., 2014)?

In this study, we tried to understand the fate of eucalypt HR on SOM dynamics. We took advantage of an experimental network set up across representative areas for eucalypt production (Ferreira et al., 2016; Souza et al., 2016). A unique site where *Eucalyptus* afforestation over natural grassland was just happening was selected, so natural differences in the ^{13}C isotope signature of C_3 (eucalypt) and C_4 (grassland) plants could be used to quantify HR contribution to SOM pools. Pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) was used to create a fingerprint of SOM fractions and assess similarities between them in a time sequence. Treatments were applied based on two hypotheses: (i) residues mix, particularly bark presence, would increase HR complexity and C stabilization; (ii) increasing nitrogen availability would alter C transference to more stabilized pools either by eliminating any possible constraint due to eucalypt HR low N content or by suppressing lignin decomposers activity.

2. MATERIAL AND METHODS

2.1. Site description

Experiment was carried out in Southern Brazil (30°26'S; 54°31'W), in Rio Grande do Sul State, near Brazil-Uruguay border. We selected a representative site in a region that is considered a new frontier of *Eucalyptus* plantation in Brazil, where an expansion of these forests over natural grasslands of Pampa Biome has been occurring. The natural grassland (C_4) of selected site was replaced by *Eucalyptus* (C_3) planted forests

at the same time at experiment set up, so we could use and maximize differences in natural isotope signature and chemistry between fresh input (C_3 harvest residues) and former SOM. Grassland was eliminated using a non-selective herbicide (glyphosate) and *Eucalyptus dunnii* seedlings were planted at 2.2 x 3.3 m spacing.

Site is located in a sub-tropical climate area, with mean annual temperature (MAT) of 18 ºC and annual rainfall of 1351 mm during experiment years (Data from Brazilian National Institute of Spatial Research – INPE – S. Gabriel/RS station, located at ~17 km away from experimental site at similar altitude). Soils from the region are formed from sedimentary material mainly composed by arenite, siltite and argillite (MAPA, 1973; Roesch et al., 2009). Soil site was classified as Inceptsol (U.S. Taxonomy), its main properties are shown in Table 1 and SOM characterization is presented in Table 2.

Table 1 – Soil properties (0 -10 cm layer) at experiment beginning.

pH[(1)]	**SB**[(2)]	**SBD**[(3)]	**TP**[(4)]	**Particle-Size**[(5)]		
				Clay	**Silt**	**Sand**
-	$cmol_c\ dm^{-3}$	$g\ cm^{-3}$	$m^3\ m^{-3}$		%	
4.71	7.71	1.15	0.53	28	55	17

[(1)]Determined in water, 1:2.5 soil:water ratio; [(2)]Sum of Bases, Ca^{2+} and Mg^{2+} determined in KCl 1 mol L^{-1}, K^+ and Na^+ determined with Melich-1 extractor. [(3)]SBD: Soil Bulk Density; [(4)]TP: Total Porosity; [(5)]determined following Ruiz (2005).

Table 2 – SOM properties of topsoil layers (0 -1 and 1 -5 cm) at experiment beginning.

Properties	**POM**[†(1)]		**MAOM**[†(2)]	
	0-1 cm	1-5 cm	0-1 cm	1-5 cm
C ($g\ kg^{-1}$)	11.66	6.75	26.79	25.08
N ($g\ kg^{-1}$)	0.80	0.51	2.78	2.67
$\delta^{13}C$ (‰)	-13.72	-13.01	-13.40	-12.77
Aromatic (%)	0.15	0.12	0.11	0.10
Lignin (%)	0.13	0.14	0.03	0.03
Lipid (%)	0.15	0.16	0.25	0.20
N-Bearing (%)	0.07	0.09	0.06	0.05
Phenol (%)	0.00	0.00	0.00	0.00
Polysaccharide (%)	0.21	0.21	0.21	0.20
Protein (%)	0.05	0.08	0.11	0.13
Unknown Origin (%)	0.23	0.20	0.24	0.29

[†]SOM fractionation following Cambardella and Elliott (1992) protocol; [(1)]POM: Particulate Organic Matter; [(2)]MAOM: Mineral Associated Organic Matter; C, N and $\delta^{13}C$ determined using an elemental isotope ratio mass spectrometer (EA-IRMS GSL 20-20, Sercon, Crewe, UK); Chemical composition determined with Pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) analysis and presented as relative abundance.

2.2. Experimental design

Harvest residues used in this experiment came from a commercial *E. urograndis* hybrid stand harvested at two-year age. These are the same residues used in an experimental network set up across representative sites in Brazil aiming at understanding decomposition processes of eucalypt harvest residues under different management practices and its effect on soil carbon and properties (Ferreira et al., 2016; Souza et al., 2016). Trees were separated into leaves, branches, non-commercial stem, bark, and roots. Residues were dried at 45 ^{o}C in a forced draft oven, chopped in smaller pieces varying from 4 to 8 cm (leaves were left uncut) and dry stored until experiment set up. Proportions of each component used to represent harvest residue and its total composition were based on observations of harvested commercial plantations with a mean annual increment of 50 m^3 ha^{-1} yr^{-1} (Ferreira et al., 2016).

Micro-plots made by PVC tubes of 15 x 15 cm (diameter x height) were used to study HR decomposition and C transfer to the soil. Tubes were introduced 10 cm into the soil between planting rows. Six lateral holes (20-mm diameter) were made in the tube wall to allow fauna movement and water flow. Residues were placed on soil surface inside the tubes and then tubes were covered after treatment application with a 1-mm mesh net to avoid external litter input. The use of this type of micro-plot has been proved to be efficient to study decomposition, minimizing some problems found in litter bag studies such poor contact between litter and soil, fauna exclusion, stolen or lost bags, or higher previous processing and litter fragmentation (Ferreira et al., 2016; Powers et al., 2009; Shorohova and Kapitsa, 2014; Souza et al., 2016).

Harvest residues composition and the effect of an external N source were studied in a 3 x 2 full-factorial, split-plot in time randomized block design with four replications. We tested the effect of residue removal (-R), removal of bark only (-B), and maintenance of all residues (+B), combined (+N) or not (-N) with an external N source on decomposition (-B and +B treatments, only) and SOM dynamics. Residues totaled an equivalent amount of 21.7 and 31.7 t ha^{-1} of dry mass, in treatments -B and +B, respectively. As N source, we used a solution consisted of NH_4NO_3 and $(NH_4)_2SO_4$ (19:1) in an equivalent dose of 200 kg ha^{-1} of N. The ammonium sulphate solution was enriched in ^{15}N and provided a 10 % enrichment in the solution to be used as a mineral N tracer. N was applied only once at experiment beginning.

The whole experimental unit (EU) of all replications were sampled five times during decomposition, *i.e.* 0, 3, 6, 12 and 36 months. At each time, 24 EU were collected,

sealed and transported to the lab, where residues were carefully hand-picked, stripped from any adhered mineral particles and taken to oven at 45 ^{o}C to obtain remaining dry mass. Soils were carefully separated in 0 -1, 1 -5 and 5 -10 cm layers, 2-mm sieved and air-dried to further analysis. For this study, residues from all sampling time were used to build decomposition curves, and soil analysis focused on 0 -1 and 1 -5 cm layers, of 12 and 36 mo., to investigate SOM dynamics.

2.3. Soil analysis

2.3.1. SOM fractionation

2-mm sieved, air dried and free from visible plant material mineral soils from the two different depths studied here were physically fractionated in particulate organic matter (POM) and mineral associated organic matter (MAOM) following a similar protocol as described by Cambardella & Elliott (1992). Briefly, 5 g of soil were dispersed using 15 mL of a 15g L^{-1} solution of sodium hexametaphosphate and one small glass bed and shaken for 15 h at 200 rpm in a horizontal shaker. Afterwards, fractions were separated by a 53 µm sieve by gently adding deionized water until the flush through the sieve was completely clear. All the material and water that flushed through the sieve (MAOM) was recovered and taken to the oven at 60 ^{o}C until completely dry. The material remained on the sieve (POM) was removed by rinsing deionized water again, recovered in a glass and also taken to oven at 60 ^{o}C until dry. This fractionation procedure results in no loss of any C fraction, as all the soil, dispersing solution and water is recovered, at the same time that both fractions are thoroughly washed from the dispersing salt with the deionized water used to get a complete fraction separation. After dry, POM and MAOM were then weighted and finely ground with a ball mill for C, ^{13}C, N, ^{15}N and Py-GC/MS analysis.

2.3.2. C, δ^{13}C, N and δ^{15}N

No carbonate presence has ever been reported for the soils in the region where study is located, therefore all C content obtained in samples were considered to be organic C (MAPA 1973; Tornquist et al. 2009). Total C, ^{13}C, total N and ^{15}N were determined on dried and finely ground soil samples from both fractions using an elemental isotope ratio mass spectrometer (EA-IRMS GSL 20-20, Sercon, Crewe, UK). Reference gas was calibrated with Pee-Dee-Belemnite (PDB) and atmospheric air N_2 certified standards for

isotope signature calculations. The abundance in samples of ^{13}C and ^{15}N was calculated as follows:

$$\delta^{13}C\ (‰) = \left({R_{sample}}/{R_{standard}} - 1\right) \times 100 \quad (1)$$

$$\delta^{15}N\ (‰) = \left({R_{sample}}/{R_{standard}} - 1\right) \times 100 \quad (2)$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios.

Based on $\delta^{13}C$ and $\delta^{15}N$ obtained, we could calculate the proportion of C in each fraction that is derived from residues (fC_{HR}) and the amount of N in each fraction that is derived from the fertilizer (fN_m) as follows:

$$fC_{HR} = {\left(\delta^{13}C_f - \delta^{13}C_{Ref}\right)}/{\left(\delta^{13}C_{HR} - \delta^{13}C_{Ref}\right)} \quad (3)$$

where $\delta^{13}C_f$ is the $\delta^{13}C$ result obtained in each fraction, $\delta^{13}C_{Ref}$ is the reference value, that we adopted the $\delta^{13}C$ of the treatments without residues (-R), fertilized or not, of each fraction, depending on the treatment compared, and $\delta^{13}C_{HR}$ is the average $\delta^{13}C$ of the HR used. And

$$fN_m = {\left(\delta^{15}N_f - 0.3663\right)}/{\left(\delta^{15}N_m - 0.3663\right)} \quad (4)$$

where $\delta^{15}N_f$ is the $\delta^{15}N$ result obtained in each fraction for the treatments amended with N, 0.3663 is the reference value for N_2 air, and $\delta^{15}N_m$ is the enrichment of the solution used (10 %).

2.3.3. Pyrolysis-Gas Chromatography/Mass Spectrometry

Chemical composition of SOM fractions was analyzed using Py-GC/MS. We used samples from time 0, 12 and 36 mo. to see SOM dynamics and chemical changes in the two fractions in order to test a pathway of litter transformation into SOC (Cotrufo et al., 2015). Py-GC/MS analysis followed previously described protocols (Grandy et al., 2007;

Rinkes et al., 2016). Briefly, samples were pyrolyzed with a CDS Pyroprobe 5150 pyrolizer (CDS Analytical, Inc., Oxford, PA, USA) at 600 ^{o}C for 20 s and then transferred to a Thermo Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Austin, TX, USA) where they were separated on a fused silica column over the course of approximately 60 min with a starting temperature of 40 ^{o}C and final temperature of 310 ^{o}C. Compounds were then ionized via ion trap on a Polaris Q mass spectrometer (Thermo Fisher Scientific).

The Automated Mass Spectral Deconvolution and Identification System (AMDIS, V 2.65) and the National Institute of Standards and Technology (NIST) compound library were used to analyze and identify peaks. Peaks of each sample were expressed as relative abundance of total sample peak area. Py-GC/MS products were grouped into eight classes, *i.e.* Aromatics, Lignin, Lipids, N-Bearing, Phenol, Polysaccharides, Protein and Unknown Origin.

2.4. Data analysis

Successive sampling allowed us to estimate decomposition rate in treatments with residues presence. Remaining mass at each time was used to fit the single exponential decay model (Olson, 1963) as follows:

$$X = X_0 e^{-kt} \qquad (5)$$

Where X_0 = initial mass (%), X = remaining mass (%) at a time t, in days, and k = decomposition rate per day. The fitted model allowed us to estimate half-life (hl), in days, *i.e.* the required to time to decompose 50 % of initial mass, of residues in each treatment through the following equation:

$$hl = \ln(2)/k \qquad (6)$$

Data were tested for normality with Shapiro-Wilk test and natural log or box-cox transformed in cases where data for ANOVA did not meet normality assumptions. Afterwards, a three-way ANOVA was performed with Depth, Residues and N as main factors, and all interactions, for soil SOM variables, and a two-way ANOVA with Residues (Bark) and N was performed to compare residues decomposition in the different treatments.

A multivariate description of Py-GC/MS products was performed using Principal Components Analysis (PCA) to investigate their distribution and obtain a chemical fingerprint of SOM fraction at each treatment, depth and time. We calculated Euclidean distance matrixes from the eight classes that Py-GC/MS products were grouped to create PCA plot. All analysis were performed with R 3.02 (R Development Core Team, 2013).

3. RESULTS

3.1. Decomposition

Bark notably reduced decomposition of residues ($p<0.05$), while Nitrogen addition had a tendency ($p = 0.0663$) to slow down HR decomposition (Fig. 1). +B treatments showed an average *hl* of ~ 80 days longer than -B. +N presented an average *hl* of ~ 60 days longer than -N. +B had a higher influence in +N treatments, and N addition had bigger effect in +B treatments, although *N x Res.* interaction was not significant ($p=0.7999$).

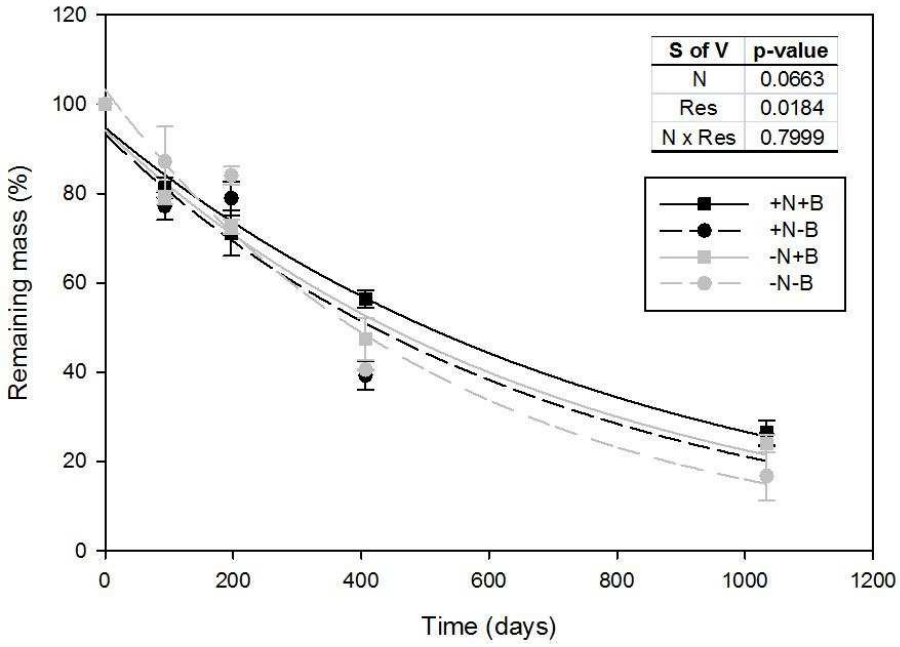

Figure 1 – Decomposition curve of the different residues treatments during experiment. Symbols indicate observed values at each sampling time. Lines represent the single exponential model ($X=X_0e^{-kt}$) fitted to observed values. *+N*: presence of external N supply*; -N*: absence of external N supply; *+B*: presence of bark; *-B*: absence of bark. *+N+B: hl=523 d., R^2=0.96; +N-B: hl=430 d., R^2= 0.91; -N+B: hl=448 d., R^2=0.97; -N-B: hl=387 d., R^2=0.91.* ANOVA was performed with estimated half-life values.

3.2. C content, $\delta^{13}C$, N content and fN_m of SOM fractions

All variables varied between the two-depth studied, except C:N ratio of both fractions (Table 3). Harvest residues (HR) and N had influenced SOM properties after 3 yr. of experiment. Changes in C content and $\delta^{13}C$ were more expressive in 0 -1 cm layer and POM fraction (Fig. 2). Overall, HR presence increased C content and decreased $\delta^{13}C$ values, indicating a higher fraction derived from HR in SOM. +B tended to increase HR contribution, but its effect was more distinguished in -N treatments. Changes in N content followed similar trend observed for C (Fig. 3). +N showed higher N content in POM fraction than -N treatments, except in +B treatments where this behavior was not observed. Contrary, +N resulted in smaller N content in MAOM than -N treatments. Therefore, C:N ratio – POM was higher in -N (13.65) treatments than +N (11.49) treatments, and C:N ratio – MAOM was higher in +N (9.48) treatments than -N (9.07) treatments. Mineral N fraction was always higher when HR were present, and even higher when bark was kept among residues. However, mineral N contribution to SOM fractions was always smaller than 4 %.

Table 3 – ANOVA results (*p*-value) of SOM fractions properties after 36 mo. of experiment. All *p*-values > *0.10* were omitted for simplicity.

Variable	***N***	***Res.***	***Depth***	***N*x*Res.***	***N*x*Depth***	***Res.*x*Depth***	***N*x*Res.*x*Depth***
C-POM		< 0.0001	0.0013	0.0013		0.0616	
δ^{13}C-POM		< 0.0001	0.0045	0.0165			
N-POM	0.0945	0.0012	0.0034	0.0098			
C:N-POM	0.0059	0.0089					
fN_m-POM	-	0.0039	0.0093	-	-		-
C-MAOM	0.0184	0.0068	0.0047				
δ^{13}C-MAOM	0.0299	0.0489	0.0004	0.0903			
N-MAOM	0.0062		0.0449				
C:N-MAOM	0.0171					0.0375	
fN_m-MAOM	-	0.0527	0.0035	-	-		-

Res.: Residues; C: Carbon; N: Nitrogen; $\delta^{13}C$: ^{13}C abundance; C:N: C:N ratio; fN_m: fraction derived from mineral-N calculated based on ^{15}N content; POM: Particulate Organic Matter; MAOM: Mineral Associated Organic Matter.

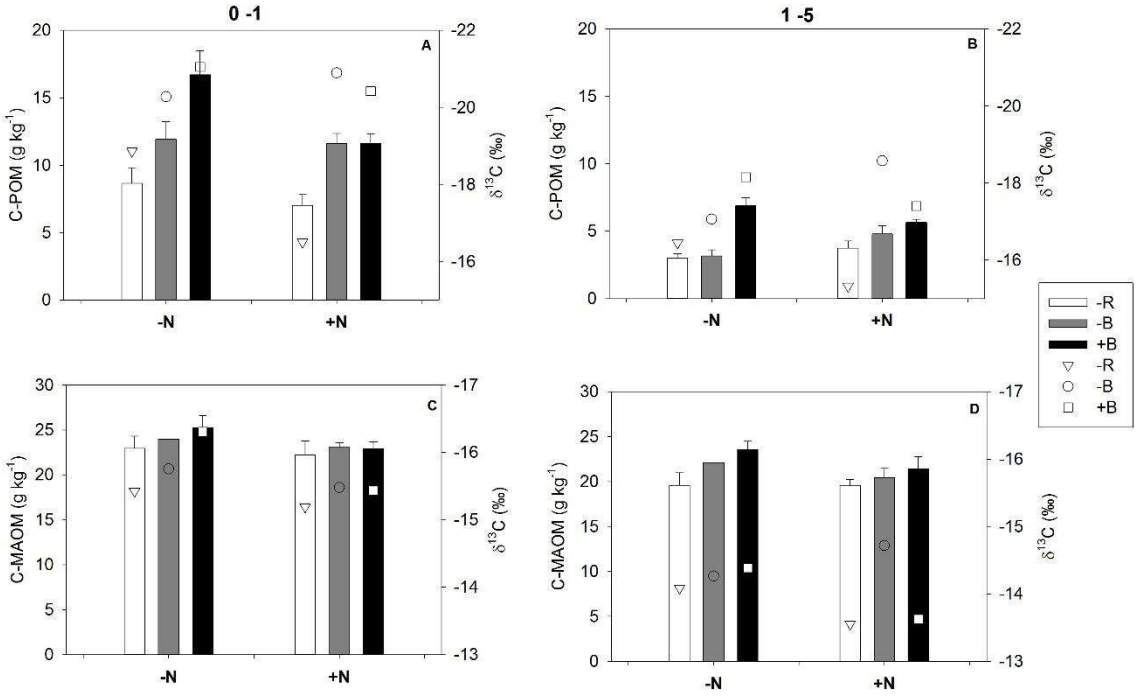

Figure 2 – C content (g kg^{-1} – bars) and $\delta^{13}C$ (‰ – symbols) in both fractions (POM and MAOM) at the end of experiment (36 mo.). **-N**: 0 kg ha^{-1} of N; **+N**: 200 kg ha^{-1} of N. **A** and **B** refer to C-POM, 0-1 and 1-5 cm layers, respectively. **C** and **D** refer to C-MAOM, 0-1 and 1-5 cm layers, respectively.

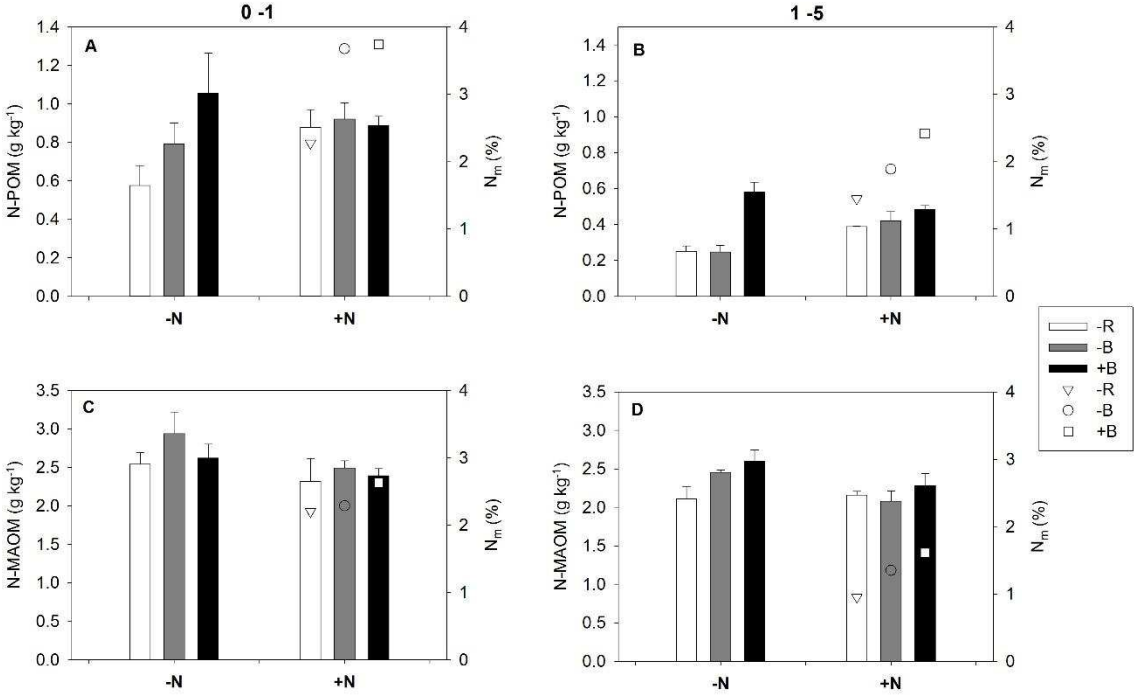

Figure 3 – N content (g kg^{-1} – bars) and fraction of mineral-N (N_m - % - symbols) in both fractions (POM and MAOM) at the end of experiment (36 mo.). **-N**: 0 kg ha^{-1} of N; **+N**: 200 kg ha^{-1} of N. **A** and **B** refer to N-POM, 0-1 and 1-5 cm layers, respectively. **C** and **D** refer to N-MAOM, 0-1 and 1-5 cm layers, respectively.

3.3. Py-GC/MS of SOM fractions

The relative abundance of Py-GC/MS products summarized in the eight classes are presented in Table 4 (0 -1 soil cm soil depth) and Table 5 (1 -5 cm soil depth). Depths were highly different in SOM chemistry ($p < 0.001$), so we organized their representation separately to simplify the understanding of their dynamics (Fig. 4). The PCA plot obtained using these eight classes showed a clear separation of fractions inside both depths and shifts in chemistry of fractions overtime (Fig. 4). Higher contribution of lignin to total chemical composition was observed in treatments with HR presence in POM fraction of 0 -1 cm depth, particularly when bark was present. In this layer and POM fraction, from 1 yr. to 3 yr., Lignin drecreased from 15 % to 7 %, and from 23 % to 15 %, in -R treatments, -N and +N, respectively (Table 4). When HR were present, it increased from 16 to 23 % and from 29 to 32 %, in -N treatments, -B and +B, respectively. In +N treatments, Lignin drecreased from 21 to 16 % and from 27 to 21 %, in -B and +B treatmentes, respectively (Table 4). Despite little or no lignin contribution in MAOM fraction in 1 -5 depth (ranging from 0 to 2 %), Lignin seems to be driving the separation between MAOM 1 and 3 yr., basically due to native lignin disappearance in the 3 yr. samples (Table 5).

Lipid contribution is higher in POM of 1 -5 than in POM 0 -1 cm layer. In MAOM fraction, it seems to be similar between the two depths. Aromatics compounds increased overtime in POM and decreased in MAOM fraction of both depths (ranging from 10 to 18 %). Polyssacharides and Protein showed similar behavior overtime. They seem to decrease in POM fraction overtime, while increase in MAOM, independently of treatment. In MAOM 3 yr. samples of both depths, Polyssacharides were higher in -N treatments, reaching more than 30 %. Protein peaked around 17 % in 3 yr. MAOM samples in residues presence (Tables 4 and 5). Phenol compounds were mostly absent in our samples.

Treatments with N input (+N) showed a tendency of staying closer in MAOM 0 -1 cm depth, although a good separation of treatmens happened only in POM 0 -1 depth and it was due to HR presence, indicating a constant flow of HR input to this fraction (Fig. 4).

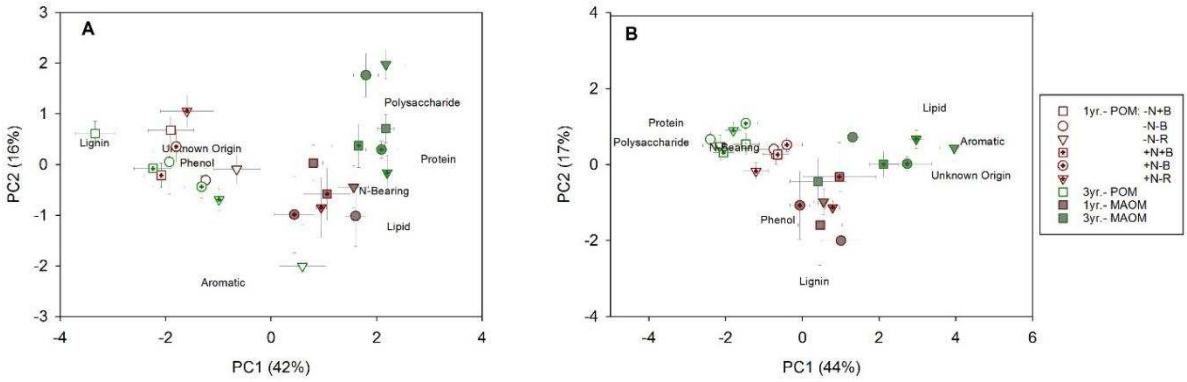

Figure 4 – PCA plot of SOM fractions (white: POM; grey: MAOM) chemical composition based on Euclidean distance matrix of the pyrolysis products grouped in the eight classes regarding their likely origin. Symbols represent mean values of the different treatments (empty: -N; dotted: +N; square: with bark (+B); circle: without bark (-B); triangle down: residues removal) and edge colors represent different sampling time (red: 1 yr.; green: 3 yr.). Position of the different variables represents their PCA score. Fig. **A** shows 0 -1 cm layer and Fig. **B** represents 1 -5 cm layer plot.

Table 4 – Relative abundance Py-G/MS products grouped in eight classes in 0 -1 cm soil layer, in both SOM fractions (POM and MAOM) and sampling time (1 yr. and 3 yr.) evaluated, and respective two-way ANOVA results.

0 - 1 soil depth	**POM**									**MAOM**								
	-N			**+N**			*p-value*			**-N**			**+N**			*p-value*		
1yr.	**-R**	**-B**	**+B**	**-R**	**-B**	**+B**	***N***	***Res.***	***N x Res.***	**-R**	**-B**	**+B**	**-R**	**-B**	**+B**	***N***	***Res.***	***N x Res.***
Aromatic	0.14	0.15	0.11	0.11	0.14	0.13			*	0.13	0.16	0.15	0.15	0.15	0.15			
Lignin	0.16	0.16	0.27	0.23	0.22	0.26	**	***	**	0.02	0.01	0.03	0.03	0.03	0.03	**		
Lipid	0.13	0.10	0.09	0.09	0.09	0.12			*	0.15	0.14	0.10	0.16	0.16	0.13	**	***	
N-Bearing	0.07	0.07	0.05	0.07	0.05	0.06				0.11	0.10	0.08	0.09	0.07	0.10	*		*
Phenol	0.00	0.01	0.00	0.00	0.00	0.00			***	0.00	0.00	0.00	0.00	0.01	0.00			
Polysac.†	0.20	0.18	0.16	0.20	0.17	0.12		**		0.22	0.22	0.25	0.21	0.19	0.24			
Protein	0.07	0.08	0.07	0.05	0.07	0.06	**	**		0.13	0.13	0.12	0.11	0.11	0.11	*		
Unkn. Or.‡	0.24	0.25	0.24	0.24	0.26	0.25				0.24	0.24	0.26	0.27	0.27	0.23			**
3yr.																		
Aromatic	0.19	0.14	0.14	0.16	0.16	0.15				0.08	0.09	0.11	0.12	0.12	0.13	***		
Lignin	0.07	0.23	0.32	0.14	0.16	0.21		***	***	0.01	0.02	0.02	0.01	0.01	0.01			
Lipid	0.18	0.09	0.05	0.11	0.09	0.08		***	**	0.12	0.12	0.13	0.17	0.15	0.13	**		*
N-Bearing	0.06	0.06	0.03	0.09	0.07	0.06	***	***		0.08	0.07	0.08	0.08	0.06	0.08			
Phenol	0.00	0.00	0.00	0.02	0.00	0.02	*	*	**	0.00	0.00	0.00	0.00	0.00	0.00	*	**	
Polysac.†	0.19	0.16	0.13	0.16	0.18	0.16			**	0.33	0.32	0.28	0.24	0.27	0.28	***		
Protein	0.12	0.07	0.06	0.11	0.07	0.08		***	**	0.14	0.13	0.16	0.15	0.17	0.13			**
Unkn. Or.‡	0.19	0.25	0.26	0.22	0.26	0.24		***		0.24	0.24	0.22	0.24	0.22	0.24			

†: Polysaccharides; ‡: Unknown Origin.

Table 5 – Relative abundance Py-G/MS products grouped in eight classes in 1 -5 cm soil layer, in both SOM fractions (POM and MAOM) and sampling time (1 yr. and 3 yr.) evaluated, and respective two-way ANOVA results.

1 - 5 soil depth	**POM**									**MAOM**								
	-N			**+N**			*p-value*			**-N**			**+N**			*p-value*		
1yr.	**-R**	**-B**	**+B**	**-R**	**-B**	**+B**	***N***	***Res.***	***N x Res.***	**-R**	**-B**	**+B**	**-R**	**-B**	**+B**	***N***	***Res.***	***N x Res.***
Aromatic	0.17	0.15	0.17	0.18	0.15	0.19				0.16	0.18	0.14	0.15	0.17	0.16			
Lignin	0.06	0.10	0.08	0.06	0.08	0.03				0.01	0.01	0.02	0.01	0.01	0.02			
Lipid	0.14	0.14	0.14	0.13	0.14	0.19				0.12	0.14	0.15	0.11	0.15	0.15		**	
N-Bearing	0.05	0.04	0.06	0.06	0.08	0.05			**	0.10	0.08	0.07	0.08	0.09	0.07		*	
Phenol	0.00	0.00	0.00	0.00	0.00	0.00				0.00	0.00	0.00	0.00	0.00	0.00			
Polysac.†	0.19	0.16	0.17	0.16	0.19	0.13				0.28	0.23	0.24	0.28	0.20	0.24		*	
Protein	0.08	0.07	0.08	0.07	0.09	0.12				0.16	0.12	0.12	0.11	0.12	0.13	**		**
Unkn. Or.‡	0.30	0.34	0.30	0.33	0.27	0.29			*	0.17	0.23	0.25	0.26	0.26	0.24			**
3yr.																		
Aromatic	0.25	0.20	0.18	0.28	0.19	0.22		***		0.10	0.12	0.10	0.11	0.11	0.11			
Lignin	0.00	0.00	0.04	0.00	0.00	0.00	***	***	***	0.00	0.00	0.00	0.00	0.00	0.00			
Lipid	0.21	0.18	0.14	0.20	0.15	0.15		**		0.12	0.11	0.13	0.15	0.16	0.13	*		
N-Bearing	0.06	0.05	0.07	0.05	0.07	0.07		***	***	0.07	0.07	0.06	0.09	0.07	0.06			
Phenol	0.00	0.00	0.00	0.00	0.00	0.00				0.00	0.00	0.00	0.00	0.00	0.00			
Polysac.†	0.07	0.17	0.15	0.06	0.07	0.11	***	***	**	0.33	0.32	0.29	0.28	0.28	0.30			
Protein	0.00	0.10	0.11	0.07	0.05	0.04		***		0.15	0.16	0.14	0.15	0.16	0.17			
Unkn. Or.‡	0.41	0.30	0.30	0.34	0.47	0.41	*		**	0.23	0.21	0.27	0.22	0.23	0.23			

†: Polysaccharides; ‡: Unknown Origin.

4. DISCUSSION

We found a clear effect of residue mix on the decomposition rate (Fig.1). Although the mix composition of litter is known to influence decomposition behavior (Hernández et al., 2009; Vivanco and Austin, 2011), our results were the opposite from similar but broader studies of eucalypt harvest residues decomposition in Brazil (Ferreira et al., 2016; Souza et al., 2016). Studying several sites in the country, the referred authors found out that, overall, bark presence accelerated decomposition and nutrient release from eucalypt harvest residues. Indeed, the site where our study is located had a peculiar behavior when compared to the others (Ferreira et al., 2016). They hypothesized that this effect was due to the high nutrient content in bark, specially Calcium, and the probable higher physical protection against any disturb when bark was present. Our site is located in a subtropical area of the country and as it is a nutrient-rich soil, microorganism did not need to scavenge the material for nutrients for their processes, so this could explain why bark slowed down decomposition here and elsewhere (Epron et al., 2006; Hernández et al., 2009; Shammas et al., 2003), as it is a more complex material and presents a higher lignin and phenol content (García et al., 2014; Kögel-Knabner, 2002).

As observed in the other sites, nitrogen effect on decomposition was not significant (Ferreira et al., 2016; Souza et al., 2016). However, +N here presented a trend to slow down decomposition, and its greater effect when bark was present, or the greater effect of +B in +N treatments, although not significant, might indicate a possible interaction between the tannins present in bark and N, enhancing their toxicity and unpalatability to decomposers or by binding with dietary proteins and digestive enzymes (Coulis et al., 2009; Dong et al., 2016; Kraus et al., 2003; Makkonen et al., 2012).

Harvest residues (HR) had a positive impact on C and N of both SOM fractions, increasing the content and C derived from C_3 plants (Fig. 2 and Fig.3). Effects of HR on soil C were higher on POM than MAOM fraction (Fig.2). +B particularly had greater impact on -N treatments than on +N treatments. This could be a result of the slower decomposition of this treatment, since there is less decomposed material "ready" to become SOM. However, considering the proportion of SOM derived from HR, we noticed a different behavior in -N and +N treatments, while changes in decomposition were not that expressive, being another indicator of possible interaction between tannins and N, slowing down decomposition and reducing C transfer and stabilization. Moreover, the reduction of +B effect on POM-N and MAOM-N content in +N treatments is intriguing. Changes in N of SOM fractions were similar to that happened to soil C. The

lack of effect +N treatments in N content, although recovery of mineral N in SOM fractions (Fig. 3), might be explained by C:N ratio of our soils fraction. Ratios varied from 9 to 14. This narrow range is not likely to offer any constraint to microbial activity (Manzoni et al., 2010, 2008). Therefore, little +N effect on decomposition could either be attributed to the optimum stoichiometric range of our soil C:N ratio or the ability of decomposers to decompose N-poor materials (Ferreira et al., 2016; Manzoni et al., 2008).

Although HR and N impact on soil C happened in both layers, they were more expressive on 0 -1 cm soil layer. Sampling this thin layer is not usual and hard to do in field experiments. However, the use of micro-plots allowed us to have better control of sampling process and better study the dynamics of C transference from litter to soil. Albeit this layer might not be representative of soil C stocks and be too susceptible to impacts, it seems to concentrate the first steps of litter transformation (Cotrufo et al. 2015) and the effects on it might be reflected in deeper layers in smaller scales later (Figs. 2 and 3).

Several authors have studied the impact of eucalypt HR on soil properties (Epron et al., 2015, 2006; Jesus et al., 2015; Kumaraswamy et al., 2014; Mendham et al., 2003; Rocha et al., 2016). HR usually have a positive impact on soil physics (Jesus et al., 2015; Silva et al., 2007), as they protect the soil from compaction during harvesting operations; soil chemistry, by increasing the amount of nutrients that remain in the field and thus nutrient cycling (Ferreira et al., 2016; Hernández et al., 2009; Laclau et al., 2010b); and soil organic properties, helping to keep or increase soil C stocks (Epron et al., 2015, 2006; Kumaraswamy et al., 2014; Rocha et al., 2016). Their effect on soil C is, however, highly debated (Epron et al., 2006; Kumaraswamy et al., 2014; Mendham et al., 2003). Even though several factors could affect soil C dynamics, especially when trying to see the effect of more lignified litter as HR (Magnússon et al., 2016), we hypothesize that this could be a matter of soil sampling depth, as transference occurs first in a thin top-soil layer, and the method used to measure soil C. Setting up the experiment in the beginning of a rotation allowed us to quantify the transference, as the gap between soil and residues ^{13}C signature was expressive and we believe the results here are quite representative of HR impact on SOM "*per se*", any direct input from root was avoided using the micro-plots here described. Indirect contribution happened due to land use change, as $\delta^{13}C$ of control plots became more and more negative overtime (Table 2; Fig. 2). This considerable $\delta^{13}C$ change in control plots highlights the difficulty of using isotope in perennial culture to study HR impact on SOM, as it could be hampered by the greater root input during growth until HR become available (Binkley et al., 2004; Binkley and Resh, 1999; Christina et al., 2011; Jourdan et al., 2008). Moreover, HR effects seem to happen

first in the POM fraction (Epron et al., 2015), so measuring bulk soil C could prevent a better analysis of HR impact on soil C, especially because the major part of soil C is associated with minerals, and effects on this fraction seems to be indirect or take longer to happen. A positive impact on SOC by HR, as seen here or elsewhere, should be interpreted with careful, as it could be happening only in non-stable fractions of soil C.

Using a chronosequence (0, 1 and 3 yr.) of residue decomposition, we aimed at understanding the pathway of SOM formation, *i.e.* litter becoming soil C and stabilizing. We tried to keep track by identifying similarities among SOM fractions with different turnover times, accessibility by microbes, and thus stability and role on soil processes (Schulze et al., 2009; von Lützow et al., 2007). Several authors have shown that SOM fractions have different chemistry and their formation processes are not the same (Buurman and Roscoe, 2011; Grandy and Neff, 2008; Schnecker et al., 2016). However, to our knowledge, we were the first to try this approach in a time scale. We hypothesized that if POM was the first step of litter transformation and it would become a stabilized C, 3 yr. MAOM chemistry would be similar to 1 yr. POM. That could not be seen by Py-GC/MS outputs (Fig. 4). It was seen that POM and MAOM are chemically different, and therefore we infer they have different formation pathways. Analyzing together changes in C content and C transference ($\delta^{13}C$) and Py-GC/MS, HR were an important source to POM (higher lignin content), while MAOM was formed mainly by microbial products (Grandy and Neff, 2008). HR still had, however, some effects on MAOM, leading us to think that HR have an indirect, but still crucial, role on C stabilization, since it is an important source of C and N for the POM fraction, and this is an important source of energy for microbial community (Bradford et al., 2013; Cotrufo et al., 2013). Indeed, the importance of microbial products to SOM under *Eucalyptus* ecosystems has been highlighted elsewhere (Soares et al., 2013).

Oliveira (2015) showed significant changes in soil community in the same experiment studied here through analysis of soil phospholipids fatty acids (PLFA) after one year of experiment. Changes happened in the amount and proportions of different PLFA classes with HR management. HR removal resulted in increases in Gram + bacteria proportion, while HR presence increased the amount of PLFA and most likely microbial biomass (Frostegard et al., 1996), raised the proportion of Gram – bacteria and bark presence caused an increase in Fungi concentrations. Changes were, however, not drastic. Therefore, changes in microbial products signature in MAOM fraction would take a much longer time to be seen and might be seen clearer once there are constant input of litter and HR in following rotations.

The fate of lignin in soils converted to *Eucalyptus* plantations in Brazil is still unclear, either decreasing (Soares et al., 2013) or increasing (Santana et al., 2015) with afforestation. Comparing initial lignin content of fractions and the differences in lignin content from POM to MAOM after 3 yr. of only litter input (Tables 2, 4 and 5), indicates that root turnover and exudates inside aggregates may supply lignin to MAOM, and almost no lignin is directly transferred from HR to stable fractions. The almost complete absence in lignin of 3 yr. samples from 1 -5 cm depth of MAOM fraction (Table 5) points out to the fact that without a constant supply, lignin is not stable even associated with minerals. The idea that lignin is not that recalcitrant in soils has been growing lately (Klotzbücher et al., 2016; Lobe et al., 2002; Rasse et al., 2006; Thevenot et al., 2010). However, the idea that when associated with minerals it could remain in soils for decades still persists (Dungait et al., 2012; Gaudinski et al., 2001; Rasse et al., 2006; Thevenot et al., 2010). Our data support the idea that may not resist even few years if there is no other input (Grandy et al., 2007).

5. CONCLUSIONS

In summary, we showed that more sustainable management of eucalypt HR, *i.e.* keeping HR in the field, can enhance C sequestration in these forests. Eucalypt HR proved to be a significant source to particulate fraction of SOM and may be an important source of energy for soil microorganisms, driving microbial community dynamics (Oliveira, 2015) therefore changing microbial products, chemistry of mineral associated fraction and C stabilization (Kallenbach et al., 2016, 2015). N addition, although less expressive, alters decomposition and reduce C stabilization of tannin-rich material (bark). SOM fractions chemistry differed each other, indicating distinct formation pathway for each fraction and different role on C cycle. Above-ground litter may not be a source for lignin in deeper layers and lignin may not be as stable as thought even associated with minerals.

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7. Supplementary material

Table S1 – List of compounds identified with Py-GC/MS, its probable source and number of samples where it was observed (*n=193*).

Compound	Source†	N. Samples
Propanoic acid, 2-hydroxy-, methyl ester, (Ò)-	Unknown Or.	4
1 H-Pyrrole, 2-ethyl-	Protein	17
1,2-Cyclopentanedione	Polysaccharide	130
1,3,5,7-Cyclooctatetraene	Unknown Or.	185
1,3,5-Cycloheptatriene	Unknown Or.	115
1,3,5-Cyclooctatriene	Unknown Or.	1
1,3-Butadiene	Lipid	27
1,3-Octadiene	Lipid	21
1,4-Benzenediamine	N-Bearing	148
1-Butyne, 3,3-dimethyl-	Lipid	9
1-Heptene	Lipid	146
1-Hexadecene	Lipid	179
1-Hexene, 3-methyl-	Lipid	90
1H-Inden-1-one, 2,3-dihydro-	Unknown Or.	144
1H-Pyrazole, 4,5-dihydro-1,5-dimethyl-	N-Bearing	1
1H-Pyrrole, 1-methyl-	Protein	16
1H-Pyrrole, 2-methyl-	Protein	166
1H-Pyrrole, 3-methyl-	N-Bearing	1
1H-Pyrrole-2-carboxaldehyde	N-Bearing	97
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	N-Bearing	22
1H-Tetrazole, 1-methyl-	N-Bearing	11
1-Pentene, 3-ethyl-2-methyl-	Lipid	147
1-Propene, 3-azido-	Unknown Or.	1
1-Undecanol	Unknown Or.	186
2(3H)-Benzofuranone, 3-methyl-	Polysaccharide	6
2(3H)-Furanone, 5-methyl-	Polysaccharide	94
2(5H)-Furanone	Polysaccharide	175
2(5H)-Furanone, 5-methyl-	Polysaccharide	42
2,3,5-Trimethylnaphthalene	Unknown Or.	125
2,3,6-Trimethylnaphthalene	Unknown Or.	96
2,5-Furandione, 3-methyl-	N-Bearing	9
2-Acetylfuran	Polysaccharide	111
2-Butanone, 3,3-dimethyl-	Unknown Or.	15
2-Butenedioic acid, 2-methyl-, (E)-	Lipid	2
2-Butenoic acid, methyl ester, (E)-	Unknown Or.	30
2-Cyclohexen-1-one	Unknown Or.	4
2-Cyclopenten-1-one, 2,3,4-trimethyl-	Polysaccharide	1
2-Cyclopenten-1-one, 2,3-dimethyl-	Polysaccharide	119
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	Polysaccharide	91
2-Cyclopenten-1-one, 2-methyl-	Polysaccharide	178
2-Cyclopenten-1-one, 3-methyl-	Polysaccharide	84
2-Furanmethanol	Polysaccharide	6
2H-1-Benzopyran-2-one	Aromatic	2
2H-1-Benzopyran-3,4-diol, 2-(3,4-dimethoxyphenyl)	Unknown Or.	76

To be continued…

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2H-Pyran-2-one	Polysaccharide	8
2-Methoxy-5-methylphenol	Unknown Or.	11
2-Naphthalenol, 3-methoxy-	Unknown Or.	1
2-Pentanone	Unknown Or.	41
2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)- (Guaiacylacetone)	Lignin	39
2-Propen-1-ol	Unknown Or.	2
2-Propenenitrile, 2-methyl-	N-Bearing	2
2-Propenoic acid, anhydride	Unknown Or.	3
2-Propyn-1-amine	Lipid	1
2-Pyrimidinamine	N-Bearing	35
3-Decene	Lipid	181
3-Furaldehyde	Polysaccharide	11
3-Methylindole	Protein	124
3-Methylpyridazine	N-Bearing	4
3-Methylthiophene	Unknown Or.	25
3-Methylthiophene-2-carbonitrile	Unknown Or.	3
3-Penten-2-one, (E)-	Unknown Or.	66
3-Phenylpyridine	N-Bearing	99
3-Pyridinol	N-Bearing	3
4(1H)-Pyridinone, 2,3-dihydro-1-methyl-	N-Bearing	27
4-Amino-2(1H)-pyridinone	N-Bearing	3
4H-Pyran-4-one, 3-hydroxy-2-methyl-	Polysaccharide	31
4-Hydroxy-2-methylacetophenone	Unknown Or.	24
4-Pyridinamine	Protein	136
4-Pyridinecarboxaldehyde	N-Bearing	1
5-Dimethylaminopyrimidine	N-Bearing	27
5-Ethyl-2-furaldehyde	Polysaccharide	14
5H-1-Pyrindine	N-Bearing	106
6-Methyl-1,2,3,4-tetrahydroquinoline	Unknown Or.	10
7-Methylindan-1-one	Unknown Or.	1
7-Hexadecenoic acid, methyl ester, (Z)	Lipid	2
7-Tetradecene	Lipid	163
Acenaphthene	Unknown Or.	13
Acenaphthylene	Unknown Or.	6
Acetamide, N-(2,4-dihydroxyphenyl)-	N-Bearing	2
Acetamide, N-hydroxy	N-Bearing	2
Acetic acid	Polysaccharide	2
Acetic anhydride	Polysaccharide	54
Acetonitrile, (dimethylamino)-	N-Bearing	6
Acetophenone	Aromatic	169
à-Cubebene	Unknown Or.	1
Alpha-amino-gamma-butyrolactone	N-Bearing	18
Aniline	N-Bearing	180
Anthracene	Aromatic	137
Asarone	Unknown Or.	5
Benzaldehyde	Aromatic	71
Benzaldehyde, 4-hydroxy-3,5-dimethoxy- (Syringaldehyde)	Lignin	15
Benzaldehyde, 4-hydroxy-3-methoxy- (Vanillin)	Lignin	19
Benzene	Aromatic	11

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Benzene	Aromatic	190
Benzene, (1,3-dimethylbutyl)-	Aromatic	128
Benzene, (1-methylethyl)-	Aromatic	109
Benzene, 1,2,3,4-tetramethyl-	Aromatic	55
Benzene, 1,2,3-trimethoxy- (Methylsyringol)	Lignin	1
Benzene, 1,2,3-trimethyl-	Aromatic	188
Benzene, 1,2-diethyl-	Aromatic	65
Benzene, 1,2-dimethoxy-	Lignin	5
Benzene, 1,3-bis(1,1-dimethylethyl)-	Aromatic	13
Benzene, 1,4-dimethoxy-	Phenol	10
Benzene, 1,4-dimethoxy-2,3,5,6-tetramethyl-	Unknown Or.	21
Benzene, 1-ethenyl-3-methyl-	Aromatic	101
Benzene, 1-ethenyl-4-methoxy-	Lignin	115
Benzene, 1-ethyl-4-methoxy-	Phenol	22
Benzene, 1-methoxy-4-methyl-	Lignin	34
Benzene, 2-propenyl-	Aromatic	164
Benzene, 4-ethenyl-1,2-dimethoxy-	Lignin	42
Benzene, butyl-	Aromatic	17
Benzene, heptyl-	Unknown Or.	130
Benzene, hexyl-	Aromatic	164
Benzene, methoxy-	Lignin	7
Benzene, pentyl-	Unknown Or.	173
Benzene, propyl-	Aromatic	186
Benzenepropanenitrile	Protein	11
Benzofuran	Aromatic	169
Benzofuran, 2,3-dihydro-	Polysaccharide	19
Benzofuran, 2-methyl-	Polysaccharide	181
Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester (Vanillic Acid, methyl ester)	Lignin	43
Benzoic acid, 4-hydroxy-3-methoxy-Vanillic acid)	Lignin	49
Benzoic acid, methyl ester	Lignin	11
Benzonitrile	Protein	144
Benzyl nitrile	Protein	134
Beta-Pinene	Unknown Or.	33
Biphenyl	Aromatic	180
Butanal, 2-methyl-	Polysaccharide	111
Butanoic acid, 3-methyl-, methyl ester	Lipid	2
C11_H12	Unknown Or.	47
C14_alkene_#3	Lipid	114
C8_H16	Unknown Or.	93
C9_H8	Unknown Or.	171
Calamenene (Naphthalene)	Unknown Or.	11
Cyclobut-1-enylmethanol	Unknown Or.	9
Cyclopent-2-ene-1-one, 2,3,4-trimethyl-	Polysaccharide	51
Cyclopentanone	Polysaccharide	72
Cyclopropanecarboxaldehyde, methylene-	Polysaccharide	5
Dimethylbenzofuran	Unknown Or.	173
Disulfide, dimethyl	Unknown Or.	5
D-Limonene	Unknown Or.	22
Dodecene	Lipid	44

To be continued…

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Ethanone, 1-(1-methyl-1H-pyrrol-2-yl)-	N-Bearing	29
Ethanone, 1-(3,4,5-trimethoxyphenyl)-	Lignin	1
Ethanone, 1-(3,4-dimethoxyphenyl)-	Lignin	12
Ethanone, 1-(3-hydroxy-4-methoxyphenyl)-	Unknown Or.	68
Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)- (Acetosyringone)	Lignin	43
Ethylbenzene	Protein	70
Ethylphenol	Lignin	18
Fluoranthene	Unknown Or.	11
Fluorene	Aromatic	183
Furan, 2,3,5-trimethyl-	Polysaccharide	153
Furan, 2,4-dimethyl-	Polysaccharide	11
Furan, 2,5-dimethyl-	Polysaccharide	193
Furan, 2-ethyl-	Polysaccharide	185
Furan, 2-ethyl-5-methyl-	Polysaccharide	188
Furan, 3-methyl-	Polysaccharide	5
Furfural	Polysaccharide	169
Furfural, 5-methyl-	Polysaccharide	82
Hex-2-yn-4-one, 2-methyl-	Lipid	7
Hexadecanoic acid, methyl ester (Palmitic acid-C16)	Lipid	74
Hexanedinitrile	N-Bearing	132
Hydroquinone	Unknown Or.	15
Indane	Unknown Or.	182
Indole	Protein	106
Levoglucosenone	Polysaccharide	92
Mequinol	Unknown Or.	153
Methanesulfonic acid, methyl ester	Unknown Or.	6
Methyl 2-furoate	Polysaccharide	1
Monobenzone	Unknown Or.	22
m-xylene	Aromatic	192
Naphthalene	Aromatic	187
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	Unknown Or.	1
Naphthalene, 1,2-dihydro-4-methyl-	Aromatic	168
Naphthalene, decahydro-4a-methyl-1-methylene-7-…	Unknown Or.	1
N-Butyl-tert-butylamine	N-Bearing	25
n-Decane	Lipid	167
n-Docosane (C22)	Lipid	12
n-Dodecane	Lipid	186
n-Dotriacontane	Lipid	15
n-Eicosane	Lipid	28
n-Heneicosane (C21)	Lipid	36
n-Hentriacontane (C31)	Lipid	1
n-Heptacosane (C27)	Lipid	15
n-Heptadecane	Lipid	176
n-Heptane	Lipid	182
n-Hexacosane (C26)	Lipid	6
n-Hexadecane	Lipid	189
n-Nonacosane (C29)	Lipid	5
n-Nonane	Lipid	191
n-Octacosane	Lipid	3

To be continued…

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n-Octadecane	Lipid	158
n-Octane	Lipid	193
n-Pentacosane (C25)	Lipid	3
n-Pentadecane	Lipid	191
n-Tetracosane	Lipid	3
n-Tetradecane	Lipid	173
n-Tricosane (C23)	Lipid	11
n-Tridecane	Lipid	190
n-Undecane	Lipid	186
Oxirane, ethenyl-	Aromatic	37
o-xylene	Phenol	4
p-Aminotoluene	N-Bearing	163
Pentadecane, 7-methyl	Lipid	6
PENTANAL	Polysaccharide	8
Phenanthrene	Unknown Or.	1
Phenol	Phenol	42
Phenol, 2,4-bis(1,1-dimethylethyl)-	Aromatic	84
Phenol, 2,6-dimethoxy- (Syringol)	Lignin	66
Phenol, 2,6-dimethoxy-4-(2-propenyl)- (Methoxyeugenol)	Lignin	62
Phenol, 2-methoxy- (Guaiacol)	Lignin	8
Phenol, 2-methoxy-4-(1-propenyl)- (4-Isoeugenol)	Lignin	12
Phenol, 2-methoxy-4-(1Z)-1-propen-1-yl- (cis-Isoeugenol)	Lignin	2
Phenol, 2-methoxy-4-(2-propenyl)- (4-Eugenol)	Lignin	11
Phenol, 2-methoxy-4-methyl- (4-Methylguaiacol)	Lignin	77
Phenol, 2-methoxy-4-propyl- (4-Guaiacylpropane)	Lignin	22
Phenol, 2-Methoxy-4-vinyl- (Vinylguaiacol)	Lignin	135
Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	Unknown Or.	58
Phenol, 3,4-dimethyl-	Aromatic	75
Phenol, 3-methyl-	Aromatic	130
Phenol, 4-ethyl-2,6-dimethoxy- (Ethylsyringol)	Lignin	49
Phenol, 4-ethyl-2-methoxy- (Ethylguaiacol)	Lignin	99
Phenol, 4-methyl-	Phenol	14
Phosphonic acid, (p-hydroxyphenyl)-	Unknown Or.	78
Piperidine-2,5-dione	N-Bearing	29
Propane, 2-nitro-	N-Bearing	187
Propene	Lipid	2
p-Xylene	Aromatic	1
Pyrazolo[5,1-c][1,2,4]benzotriazin-8-ol	N-Bearing	79
Pyridine	Protein	41
Pyridine	Protein	183
Pyridine 3-methyl	N-Bearing	11
Pyridine, 2-ethyl	N-Bearing	131
Pyridine, 3,5-dimethyl-	Protein	155
Pyridine, 4-methoxy-	N-Bearing	20
Pyrimidine	N-Bearing	79
Pyrrole	Protein	48
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	N-Bearing	5
Pyruvaldehyde	Unknown Or.	81

To be continued…

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Resorcinol (Dihydroxybenzene)	Unknown Or.	6
S-Tetrazine, 3-methyl-6-(2-thienyl)-	N-Bearing	2
Styrene	Protein	1
Thiophene	Unknown Or.	6
Toluene	Unknown Or.	193
Trimethylphenol	Unknown Or.	46
Vinyl crotonate	Unknown Or.	1
Vinylfuran	Polysaccharide	191

†Unknown Or.: Unknown Origin.

III. CHAPTER 2

Assessing harvest and silvicultural systems effects on soil physical properties, CO_2 efflux, soil C fractions and early tree growth in two *Eucalyptus* stands in Minas Gerais State, Brazil

ABSTRACT

Harvesting and logging are probably the most impactful operations in eucalypt forest ecosystems and may define silvicultural operations needed for a successful following rotation. Changes in soil physical properties are usually critic and may be reflected in soil CO_2 efflux, soil organic matter (SOM) properties and plant development. In this study, we aimed at understanding the effect of two different harvesting systems (Feller + Forwarder – F+F; and Feller + Skidder – F+S) on soil physical and organic properties, soil CO_2 efflux, and how these properties would be recovered and trees would respond in two different silvicultural systems (Coppice or replanting). Hence, two homogeneous and adjacent stands were selected and soil physical properties [Soil Bulk Density (*Ds*), Micro (*Mi*), Macro (*Ma*) and Total Porosity (*TP*), and Penetration Resistance (*PR*)] until 60 cm depth, SOM properties [Labile-C, C associated with Particulate (C-POM) and Mineral (C-MAOM) fractions] until 20 cm depth, soil CO_2 efflux and stump mortality rate, were assessed after harvesting operations and compared with reference (before harvesting). After harvesting, each stand was divided in coppice and replanting, and we followed soil CO_2 efflux right after planting and one year later, when trees height (*H*) and SOM properties were also measured in both areas and system to evaluate how areas would recover from harvesting impacts. All soil variables were assessed at row and inter-row positions. Both systems affected soil bulk density and soil pore configuration, but in different ways. F+F concentrates traffic at inter-planting row position, and therefore caused a slightly higher compaction in this region, while F+S does not follow traffic routes and impacted the planting-row similarly, resulting in higher stump mortality. Harvesting increased *Ds*, *Mi* and *PR* and reduced *Ma* and *TP* at all layers. Effects were more pronounced on superficial (until 20 cm) layers. Soil CO_2 efflux was not affected and presented similar values before and post harvesting. Differences in soil CO_2 were found after planting, when it was higher in F+S system, that also presented higher labile-C and C-POM, probably due to greater disturbance. One year after planting, mortality rate was still higher under coppice in F+S system, that also presented lower trees. In turn, trees were higher in replanting in this area. Soil respiration behaved similarly, *i.e.*, higher in F+F under coppice and lower under replanting. Changes in C-MAOM only happened one year after planting, where it was higher in coppice systems. Overall, after one year coppice system presented higher Labile-C, C-POM and C-MAOM and we could not attribute any differences to different harvest system. We conclude that forest floor removal by skidding operations does not seem to be harmful to SOM properties in a single operation and SOM may take longer to respond to this intensive removal. But different harvesting systems affect row and inter-row positions differently, creating different soil functional zones, that might be enhanced by the next silvicultural system chosen, and should be observed when assessing ecosystem services and site condition.

Keywords: Eucalypt harvest system**;** soil compaction; soil respiration; soil organic matter fractions.

1. INTRODUCTION

Harvesting and logging are among the most expensive operations in planted forests and the most impactful on soil physical properties. The possible soil degradation due to traffic of heavy machines is a matter of concern regarding ecosystem functions and sustainability of future production. Mechanization of these operations is inevitable as it is extremely more efficient. With the advance of technology more powerful vehicles were developed to meet productivity increases, but at the same time, it may increase soil compaction due to heavier machinery carrying larger amounts of wood at a time (Horn et al., 2007, 2004; Vossbrink and Horn, 2004). Stresses due to compaction may be long-lasting or even irreversible and are usually concentrated on soil bulk density and porosity, that are closely linked with soil and plant vital processes (Bottinelli et al., 2014; Cambi et al., 2015).

Reductions in porosity and increases in soil bulk density because of compaction may modify water and air flow in the soil, and therefore impact plant root development and soil biological activity. Decreases in productivity of subsequent rotations are often associated with compaction caused by harvesting of previous rotations (Ampoorter et al., 2011; Jesus et al., 2015). The extent of soil disturbance varies according to several factors such as machinery used, amount of wood logged, slope, number of passes in the same area, soil clay and organic matter content, soil compaction status and soil humidity during operation (Ampoorter et al., 2012, 2010; Cambi et al., 2015; Seixas and Souza, 2007; Silva et al., 2010). In summary, the more trafficked areas, the finer-textured soils with low organic matter content, the lower soil bulk density and the farthest from optimum moisture are the most susceptible conditions to harmful impacts.

Clear-cut of *Eucalyptus* stands is usually done with *Harvester* + *Forwarder* (H+F) or *Feller Buncher* + *Skidder* (F+S) systems. The first is usually known as “cut-to-length” system, where each tree is harvested and processed in small logs individually, and pilled by the harvester, and then the forwarder is used for logging. It usually follows pre-defined routes, concentrating the compaction in these areas, and all the non-commercial components remain in the area, softening the impact direct to the soil and therefore decreasing compaction. However, it is less efficient and compaction may increase with the number of passes (Seixas and Souza, 2007). The second, in turn, is used in the “whole-tree” harvesting process, where the *Feller Buncher* harvests a group of trees together and pile them, and *Skidder* drags them to the edge of the plot where the tree is processed with a harvester or a grapple saw. Albeit is a faster and lighter harvesting system, all the non-

commercial residues and the forest floor are removed, which may have further implications in nutrient cycling and carbon (C) budget (Achat et al., 2015; Epron et al., 2015). Currently, the use of *Feller Buncher* + *Forwarder* (F+F) has risen trying to obtain the most of both systems. Basically, the *Feller* would harvest a group of trees and pile them, that would be processed inside the plot before being logged with the *Forwarder* following the same track the *Feller* used. This system would create some "residues islands" where the trees were processed, but the consequences still need to be studied.

As said, the choice of the harvesting system may define harvest residue management and therefore have directly consequences on soil C stocks. Although the impacts on soil C may not be immediate or short-term, it could have significant mid-or-long-term effect on carbon cycle (Achat et al., 2015; Epron et al., 2006), and therefore affect the role of these planted forests in climate change mitigation as recognized by the "Clean Development Mechanism" of Kyoto Protocol. Hence, the use of SOM indicators that are sensible to soil management, such soil CO_2 efflux or labile-C (Goutal et al., 2012; Hurisso et al., 2016), is needed to support management decisions. Likewise, the harvest system may define the silvicultural management adopted for the next rotation, *i.e.* coppice or high-forest (replanting), based on damage and mortality of strains. The silvicultural system would modify ecosystem resilience, since coppice would allow the system to recover itself and replanting would require further operations such as subsoiling. Tree growth rates are different in both systems and tillage operations might accelerate recovery from compaction but at the expenses of breaking down aggregates, releasing stable soil C (Fialho, 2016; Nouvellon et al., 2008).

Based on this, we set up an experiment in two adjacent commercial eucalypt stands that were harvested with a *Feller Buncher*, but one was logged with a *Forwarder*, while the other was logged with a *Skidder*. After logging, areas were divided in coppice and high-forest managements. We measured soil bulk density, micro-macro-and-total porosity, penetration resistance, soil CO_2 efflux, soil C fractions and initial tree survival and growth over 1 yr. to: (i) assess the effect of different harvesting system on soil physical and organic properties and how could this changes affect soil CO_2 respiration; (ii) evaluate how different silvicultural management change the resilience of eucalypt plantation harvested with different systems; (iii) evaluate how all of these management combinations will impact on initial forest growth.

2. MATERIAL AND METHODS

2.1. Study site and experimental design

The study was conducted in two adjacent eucalypt commercial stands in Estrela do Sul, Minas Gerais State, Southeast of Brazil (19°01'S; 47°40'W), within the *Cerrado* (native savannah) domain. The two stands were selected based on homogeneity of previous operation and productivity, soil type and properties. Selected sites were covered by forest plantations for the last 40 years. *Cerrado* conversion had started with *Pinus* sp. introduction and changed to *Eucalyptus* sp. eight years ago. Last rotation averaged 44 m^3 ha^{-1} yr^{-1} of wood in both stands.

Soil of areas were classified as Oxisol (U.S. taxonomy) and its properties are presented in Table 1. Both areas are considered flat, easy for any mechanical operations. Area is located in a tropical climate region (Aw, Köppen classification), with dry winter and wet summer, and presented mean annual temperature (MAT) of 22 °C and average annual rainfall of 1410 mm during study years.

Table 1 – Soil site attributes before harvesting.

Area	**Depth**	**pH**(1)	**Clay**(2)	**Silt**(2)	**Sand**(2)	**Al^{3+}**(3)	**SB**(4)
	cm	-		%		$mmol_c$ dm^{-3}	
F+F	0-20	3.86	76	7	17	4.44	4.50
F+S	0-20	3.90	80	5	15	3.56	4.69

(1) Determined in $CaCl_2$ 0.01 mol L^{-1}; (2)Determined following EMBRAPA (1997); (3)Determined in KCl 1 mol L^{-1}; SB: Sum of Bases: Ca^{2+} + Mg^{2+} + K^+ + Na^+.

Both stands were harvested using a Feller Buncher (John Deere 903J), but logged using different machines. One was logged with Forwarder (John Deere 1710D ECOIII, 6x6) and the other with a Skidder (Tigercat 635D). Therefore, systems differ in logging and wood processing, as Feller+Forwarder (F+F) system has the wood processed inside the area with a grapple saw, before loading the Forwarder, keeping all residues (leaves and branches and twigs) spread, while in Feller+Skidder (F+S) system the wood is processed in the edge of the area, also using a grapple saw, before loading the trucks, and it concentrates all residues in this edge area.

After harvesting and logging, areas were divided in two silvicultural systems. Half of each area was conducted under coppice and the other half was conducted under high forest management, *i.e.* replanting. For replanting management, the inter-rows of area halves were subsoiled until 60 cm depth. Standard fertilization was applied in both systems and areas aiming at 45 m^3 ha^{-1} yr^{-1} of wood yield based on NUTREECALC® software estimations. Operations were all mechanized.

2.2. Sampling and data analysis

Areas were sampled four times: before harvesting [(Pre) – Feb./14], after harvesting and logging [(Post) – Apr./14], after planting and fertilizing [(AP) – Jul./14], and one year after planting [(1 yr.) – May/15]. Sampling were done each time aiming to identify changes in soil physical attributes, soil CO_2 efflux, soil organic matter properties and plant growth. For each variable, sample occurred following different procedures that are detailed below.

2.2.1. Soil physics attributes: *Soil bulk density (Ds), Macro (Ma), Micro (Mi), Total Porosity (TP) and Soil Penetration Resistance (PR)*

Ds, *Ma*, *Mi* and *TP* were assessed before and post whole harvesting and logging operations. These properties were obtained through intact soil core samples collected in the planting row and in the inter-planting row. At each sampling time, five replications were used for each position in each area. Soil cores were collected in four depths: 0 – 10, 10 – 20, 20 – 40 and 40 – 60 cm.

Mass and volume of soil core sampler were obtained previously. After sampling, samples were tightly packed with a plastic film to avoid any possible loss of soil and sent to the lab. At the lab, samples were weighted to obtain current soil moisture content. Soil cores were placed in trays with water up to 80% of each soil cylinder height for 48 h to complete saturation. Afterwards they were taken to a tension table for 48 h with 60 cm of water column pressure and then weighted to determine microporosity. Soil core were then dried at 105 ^{o}C until constant weight to obtain soil dry mass.

Soil particle density (*Dp*) was determined through the volumetric flask method and total porosity with $TP = 1 - Ds/Dp$ equation. Macroporosity was determined by math difference between *TP* and *Mi*.

PR was evaluated before and post whole harvesting and logging operations simultaneously with soil physics properties in a systematic way (Tavares Filho and Ribon, 2008), at planting and inter-planting row, with 15 replications each. *PR* was also assessed in systematic way after replanting (only at replanted areas), but with 8 replications at four distances (0.0, 0.5, 1.0 and 1.5 m) from subsoiling line.

We evaluated *PR* with an impact penetrometer (Stolf et al., 1983) until 60 cm depth. *PR* (MPa) was calculated using the following equation: $PR = (5.6 + 6.89\ N) \times 0.098$, where

N is the number of impacts per decimeter of soil, proposed by (Stolf, 1991), considering the penetrometer specifications.

Data from *Ds*, *Ma*, *Mi* and *TP* were submitted to a three-way ANOVA using SISVAR software (Ferreira, 2011), following repeated measures procedure with Harvesting System, Sampling Time and Depth as main factors. The effect of harvesting system and sampling time at each depth were compared through *F test*, and differences in soil attributes among depths were assessed by Tukey post-hoc test at 5%.

2.2.2. CO_2 efflux

Soil CO_2 surface efflux measurements were done at the four sampling times evaluated. During the first two sampling (pre-and post-harvesting), sampling was carried out in 15 replications, following a systematic distribution across the two stands, at the planting and inter-planting row. After silvicultural systems set up, *i.e.* two last sampling times, sampling occurred with 6 replications per system.

For CO_2 sampling, two static chambers placed at the planting (row) and inter-planting row (inter-row) were used. Chambers consisted of 25 x 25 cm PVC tubes, inserted 5 cm into the soil, at least 24 h prior to the sampling. Chambers were closed for 40 min and four sampling were performed during this period (0, 10, 20 and 40 min) to build the CO_2 flux. Collects were performed with 60 mL syringe coupled with closure valves that were stored under constant temperature up to 10 days until CO_2 determination. Simultaneously to gas sampling, soil temperature and moisture were determined with sensor – data logger 5 TM (Decagon Devices Inc., Pullman, WA).

CO_2 concentration at each syringe was determined in a cavity ring-down spectrometer (CRDS, Picarro Sunnyvale, CA). Flux was calculated through the following equation:

$$fCO_2 = \frac{\left(\frac{\Delta Q}{\Delta t}\right)\ x\ M\ x\ P x\ V}{R\ x\ T\ x\ A}$$

Where fCO_2 is the flux of CO_2 (mg m^{-2} h^{-1}); $\Delta Q/\Delta t$: the slope of fitted regression (µg g^{-1} s^{-1}); M: CO_2 molecular weight (g mol^{-1}); P: pressure inside the chamber (1 atm assumed); V: chamber volume (dm^3); R: molar gas constant (0.08205 L atm K^{-1} mol^{-1}); T: soil temperature (K); and A: chamber basal area (m^2).

Analysis of data was carried out with two three-way ANOVA procedures. One comparing the Harvesting System (Area), Sampling Time and Position (Row or Inter-row) effects on soil CO_2 efflux and the other comparing Area, Silvicultural System and Position on soil CO_2 efflux though F test. Box-cox and natural logarithm transformation, for the first and second dataset, respectively, were necessary to meet normality assumptions.

2.2.3. Soil organic matter properties: Labile C (C_{lab}), Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM)

SOM properties were assessed in three times of the four-sampling done: before and post harvesting, and one year after planting. At each time, a systematic sampling procedure was adopted. Three composite (*n=18*) samples were collected before and post harvesting, while in the last sampling (one year after planting) three composite (*n=12*) samples were obtained per silvicultural system, for each area at each depth and position. SOM was evaluated at 0 – 10 and 10 – 20 cm layer.

Labile C was oxidated with $KMnO_4$ 0.033 mol L^{-1} following Shang and Tiessen (1997) procedure and determined in spectrophotometer at 565 nm. POM and MAOM were determined by physical fractionation (Cambardella and Elliott, 1992). Briefly, 5 g of soil (air dried, 2 mm sieve) were dispersed with 15 mL of 5 g L^{-1} sodium hexametaphosphate solution by shaking for 15 h on a horizontal shaker. POM and MAOM were separated with a 53 µm sieve by rinsing water and recovered in different glasses. Fractions were dried at 60 ^{o}C until constant weight and then finely ground. C associated with each fraction was determined by dry combustion C analyzer.

2.2.4. Forest Inventory

We started forest inventory after harvesting, when a survivor census of each area was performed to complement soil physical data. One year after replanting/coppice, tree height (*H*) and mortality were measured at plots previous established to assess harvesting and silvicultural systems effects on trees initial growth. 32 permanent plots were established in each area and system to evaluate annual forest growth. Areas and silvicultural systems were compared through a two-way ANOVA using average tree height and mortality obtained in each plot.

3. RESULTS AND DISCUSSION

3.1.Soil physical attributes and Forest Inventory

Areas were fairly homogeneous before harvesting operations, although area under F+F harvesting system presented higher density and microporosity, and smaller macroporosity and total porosity at the inter planting-row area (Fig. 1). Both systems affected soil bulk density and soil pore configuration, but in different ways. Differences in traffic patterns could clearly be seen by different changes in soil physical properties. Results from undisturbed soil sampling and *PR* were convergent (Fig. 1 and 2). Both systems affected planting and inter-planting row, being the last the most impacted. However, while F+F system concentrates its traffic on inter-planting row areas by following pre-determined traffic routes, F+S system traffics throughout the area, impacting planting-row region likewise. This could be more harmful depending on silvicultural system adopted for the next rotation, as more strains could be damaged, increasing the mortality rate (Ampoorter et al., 2011). The smaller damage caused to the strains using F+F system (Table 2) seems to facilitate strains recovery, as a smaller mortality rate could be seen after one year under coppice (Table 3). In F+S area, almost no recovery could be seen after one year.

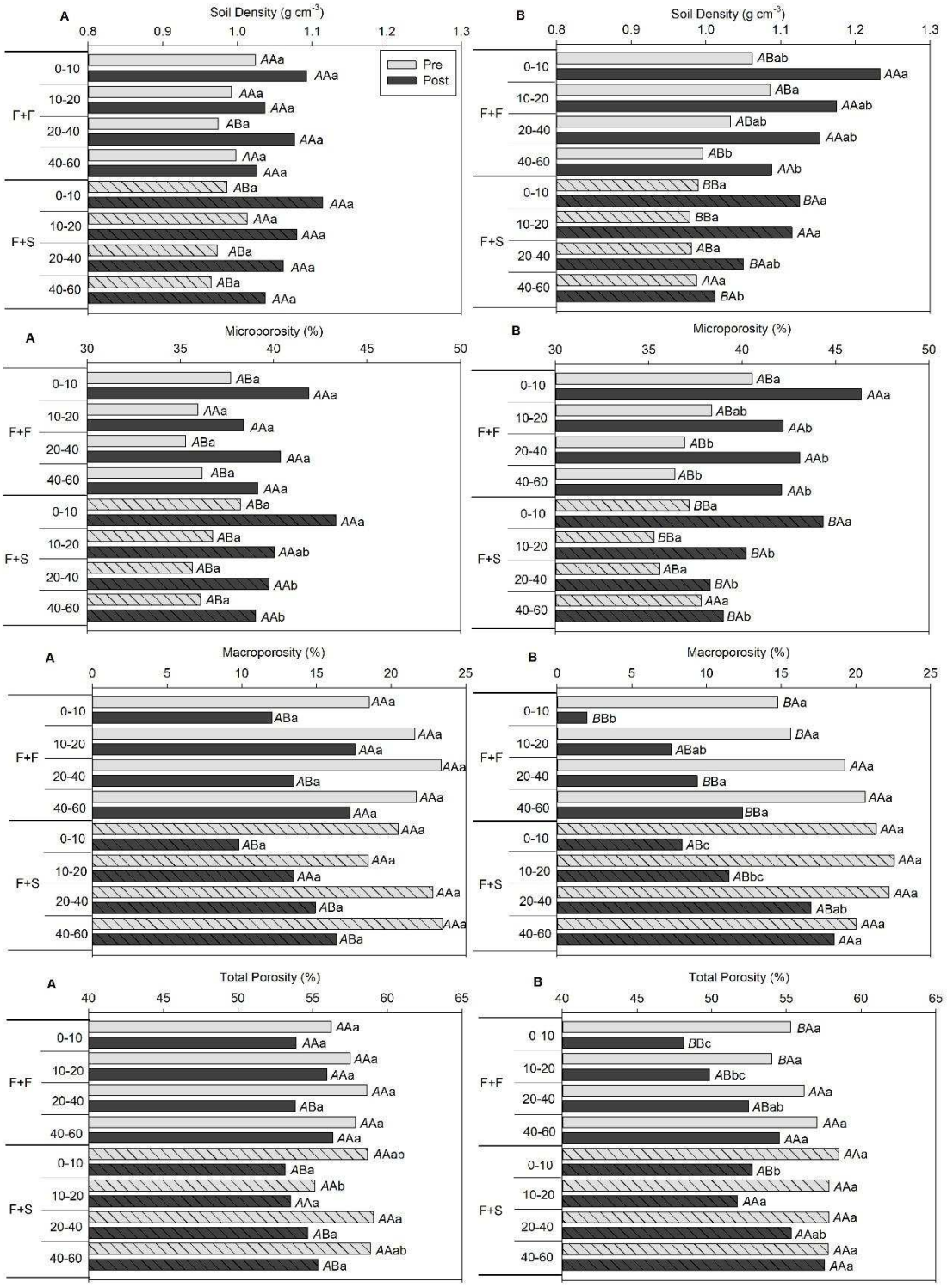

Figure 1 – Soil density (*Ds*), Microporosity (*Mi*), Macroporosity (*Ma*) and Total Porosity (*TP*) before (Pre) and post (Post) harvesting at the planting (A) and inter-planting (B) row, in each area (F+F and F+S), for the four evaluated depths (0-10, 10-20, 20-40 and 40-60 cm). Same upper case in italic inside same time and depth, and same upper case inside the same area and depth do not differ at 5% by *F test*. Same lower case inside same area and time do not differ at 5% by Tukey post-hoc test.

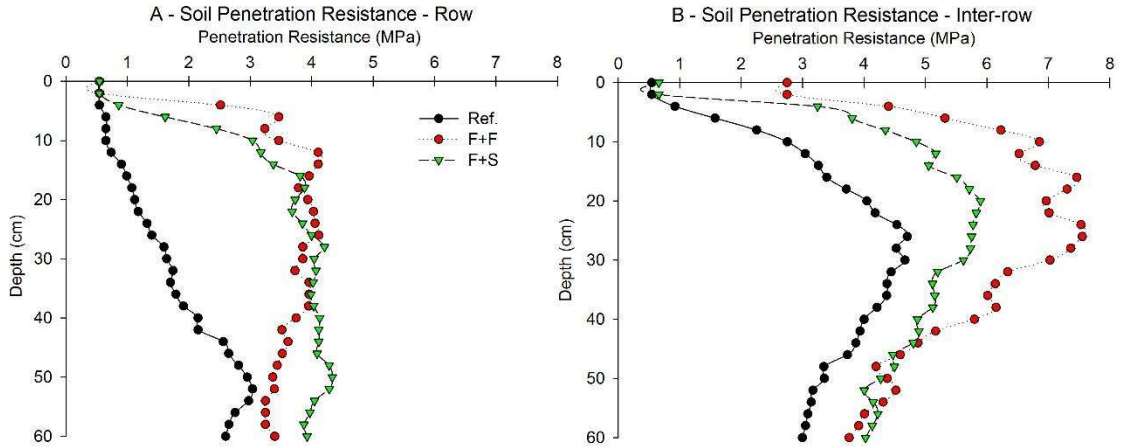

Figure 2 – Soil penetration resistance before (Ref.) and after the traffic, at planting (A) and inter-planting (B) row. "Ref." indicates Reference; "F + F" indicates Feller + Forwarder; and "F + S" indicates Feller + Skidder.

Census of the status of the remaining strain reinforce harvesting impacts as found for soil physics attributes. F+S caused higher strain mortality (Table 2). This was probably due to major traffic intensity on planting-row, as could be observed by higher alterations on its physical properties (Fig. 1). One year after planting and fertilization, trees were much higher in coppice systems. Trees under coppice were higher in F+F system, while replanted trees were higher in F+S (Table 3). Coppice systems also presented higher mortality. Mortality was higher in F+S system under coppice and did not differ between areas in replanting systems.

Table 2 – Strain survival survey after harvesting in both areas.

Harvesting system	**Area (ha)**	**Census**		
		Total strains	**Alive strains**	**Mortality (%)**
Feller + Forwarder	18.17	19440	16114	17.1
Feller + Skidder	20.07	21726	17149	21.1

Table 3 – Average height (*H*, m) and mortality (%), and standard error (*n=32*), one year after planting in both areas and silvicultural systems.

Harvesting System	**Silvicultural System**	**H (m)**	**Mortality (%)**
F+F	Coppice	8.28^{Aa} (0.09)	14.19^{Ba} (2.04)
	Replanting	4.87^{Bb} (0.07)	7.03^{Ab} (1.29)
F+S	Coppice	7.96^{Ba} (0.07)	20.31^{Aa} (2.11)
	Replanting	5.18^{Ab} (0.07)	6.51^{Ab} (1.06)

Averages followed by same upper case letter inside same silvicultural system and same lower case letter inside same harvesting system do not differ at 5 % by *F test*.

Traffic increased *Ds*, *Mi* and *PR* and reduced *Ma* and *TP* at all layers. Effects were more pronounced on superficial (until 20 cm) than deeper layers. Compared to before

traffic values, F+F caused the larger alteration in *Ds*, that was 16 % increase at inter-row in the 0-10 cm layer. At planting row, a maximum increase of 10 % was seen by this system at 20-40 cm layer. F+S, in turn, caused an increase of 13 % at planting row in the 0-10 cm layer and 14 % at inter-row 10-20 cm layer. This magnitude of change is common in eucalypt (Jesus et al., 2015; Lopes et al., 2015; Silva et al., 2007) and other forest ecosystems harvesting (Ampoorter et al., 2007; Labelle and Jaeger, 2011), and compaction usually happens in the top soil layers (Ampoorter et al., 2007; Dedecek and Gava, 2005; Jesus et al., 2015).

Other properties were, though, more affected. *PR* achieved ~ 4.0 MPa at the planting row for both systems and ~ 5.0 and 7.0 MPa at inter-planting row for F+S and F+F, respectively. As summarized by Silva et al. (2000), these levels achieved in both systems might constrain root growth, since values ranging from 1.1 to 5.0 MPa are acknowledge to affect root growth. For eucalypt, a mean value of 3.0 MPa is accepted, although other authors found values around 8.0 MPa after eucalypt harvesting and still obtained similar productivity to non-compacted soils in next rotation (Seixas and Souza, 2007). After subsoiling, the subsoiling line returned to levels before harvesting (Fig. 3). However, surrounding areas that might be used for roots exploitation remained more compacted and may be a matter of concern in F+S system, particularly when is observed high mortality rate and slower tree growth even after one year (Table 3), and this could still be reflected in the end of the rotation (Dedecek and Gava, 2005).

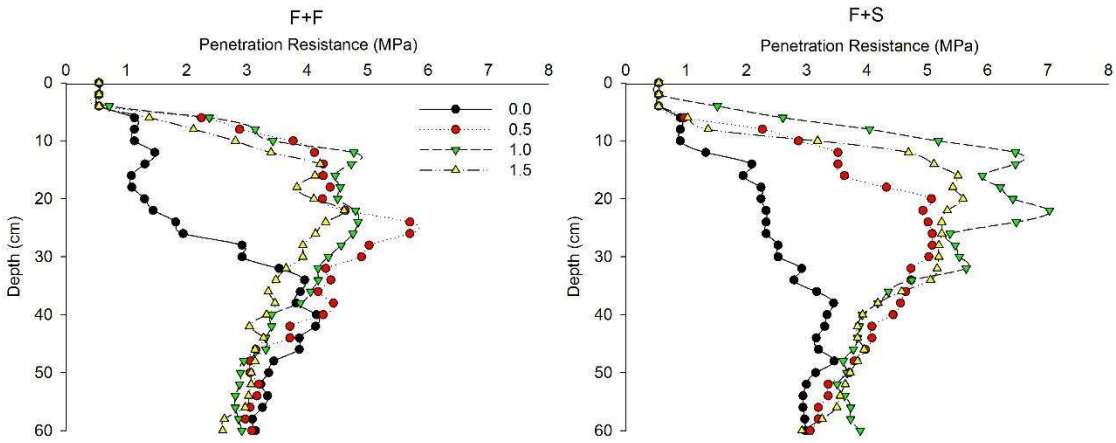

Figure 3 – Soil penetration resistance at different distances (0.0, 0.5, 1.0 and 1.5 m) from subsoiling line in both areas. “F + F” indicates Feller + Forwarder and “F + S” indicates Feller + Skidder.

Porosity is among the most important and sensitive indices to evaluate soil compaction, since it is strictly related to water movement and soil aeration (Alaoui et al.,

2011; Berisso et al., 2012; Cambi et al., 2015; Kuncoro et al., 2014). *Mi* and *Ma* were more affected than *Ds*. A reduction of 87 % in *Ma* was observed in F+F system at inter-row area in 0-10 depth (Fig. 1). This system also caused reductions higher than 50 % at the inter-row in other depths but 40-60 cm, where it lowered *Ma* in 40 %. F+S system happened to affect *Ma* more at the inter-planting row than the planting row. The biggest effect occurred in 0-10 cm layer and it was of 60 % of reduction. However, in the other depths the reductions were all lower than 50 %, being only 7 % in the deeper layer. At the planting row, in turn, F+S caused the higher changes in *Ma*. The highest decrease also happened in the top layer and it was of 52 %. Decreases in the other layer ranged from 27 % to 36 %. In F+F system, the greatest reduction (42 %) was in the 20-40 cm layer. 0-10 cm presented 35 % less *Ma* after harvesting and decreases in the other layers were smaller than 20 %.

These reductions in *Ma* caused an increase in *Mi*, however increases were in a smaller magnitude than *Ma* decreases. They were always smaller than 20 % and more concentrated at the inter-row position. *TP* was also decreased, but were even in smaller magnitude, usually around 10 %. We do not know to what extent this changes in soil porosity would affect water movement in our study soils. However, changes observed here were as expressive or more than those found in other studies that related negative effects of compaction on soil environment and soil biological properties (Ares et al., 2005; Cambi et al., 2015; Frey et al., 2009).

Our results showed that compaction happened most in top soil layer. Some authors have argued that sometimes this layer is not too affected due to its higher SOM content, which could increase aggregate stability and therefore soil elasticity, giving a higher protection against compaction (Braida et al., 2008). Hence, deeper layers would reflect more compaction status due to traffic (Jesus et al., 2015; Silva et al., 2010). Indeed, the top soil layer of our soils presented higher soil C content than deeper layers (Fig. 7). However, this higher C content also results in lower density and higher porosity (Cambi et al., 2015), enhancing the susceptibility to compaction, since this layer is the one that receives the heavy traffic impact before it could be dissipated to deeper layers. In our case, this could also be highlighted due to almost total forest floor removal in F+S system and the non-continuous residues disposal in F+F system, that would increase the pressure over the soil (Ampoorter et al., 2007; Silva et al., 2007).

3.2. Soil CO_2 efflux

Despite affecting soil pore configuration (Fig. 1), harvesting and logging did not affect soil CO_2 efflux (Fig. 4). Small changes happened, like increase in respiration in F+F system and higher inter-row respiration in F+S system. These changes were, though, not significant. We hypothesized that reductions in soil porosity would result in reduced CO_2 flux, since decreases in soil aeration could increase the production of other greenhouse gases, such CH_4 and N_2O (Frey et al., 2011; Hartmann et al., 2014). Albeit this could have happened, a possible increase in soil respiration due to disturbance and increase SOM mineralization may have offset that and our measurement was not sensible to that. A small change but not significant could be seen in F+S system where inter-planting row presented a higher respiration than planting row after traffic, probably due to greater changes in compaction of this system on this position. Also, average humidity ranged from 25 % to 28 %, after and before harvesting and logging, respectively, and did not vary between row and inter-row (Table S1). Considering the soil total porosity presented, we believe that was still enough space for aeration, therefore the compaction achieved may not be enough to hamper air flow. The possible change in pore configuration along the soil profile may have resulted in micro-sites where aeration shifted, however we could not detect that by measuring surface CO_2 efflux.

Effects of forest harvesting on soil CO_2 are variable (Goutal et al., 2012; Yashiro et al., 2008) and few are the studies addressing the effects of logging or soil preparation in eucalypt plantations (Epron et al., 2006; Fialho, 2016; Nouvellon et al., 2008). Epron el al. (2006) observed decreases in soil respiration after clear-cutting the stand in comparison to uncut stands. Fialho (2016), on the other hand, observed increases in soil CO_2 efflux after harvesting. In such study, comparing *Harvester+Forwarder* (H+F) and *Feller+Skidder* (F+S) systems, it was observed that after harvesting soil CO_2 efflux was higher at inter-planting row, while F+S clearly stimulated CO_2 efflux at the planting row. The author argued that this response was due to disturbance and aggregate breakdown, releasing protected carbon. In the present study, even with a high degree of changes in soil physical properties, we could not see clear changes in the pattern of CO_2 efflux.

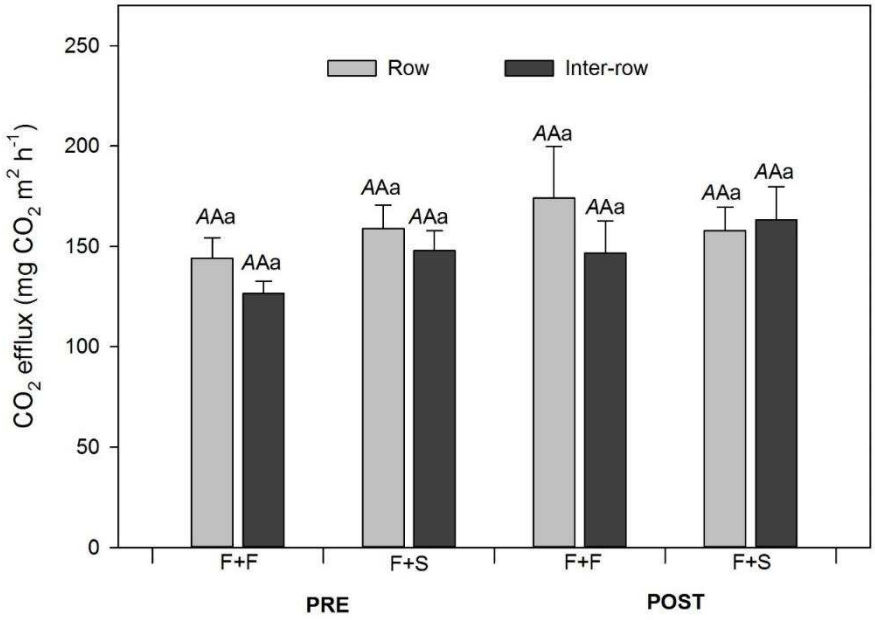

Figure 4 – Soil CO_2 efflux in both areas before (Pre) and post (Post) harvesting, at planting (row) and inter-planting (inter-row) row. Same upper case in italic inside same time and position, and same upper case inside same area and position, and same lower case inside same area and time do not differ at 5% by *F test*.

Right after planting, respiration was higher in F+S area than in F+F, independently of the silvicultural system adopted. More labile-C and POM-C (Fig. 6) was available in this area after planting, probably as result of the larger disturbance happened in the whole area and a small delay in the respiration could be captured in the next (after planting) sampling. Interestingly, subsoiling did not cause an immediate peak in respiration and respiration was lower than reference or even after harvesting and logging. At that time, respiration in coppice system was higher in F+S, while replanted areas presented a slight higher respiration than coppice on F+F system, but no significant differences were found under coppice or replanting, or between row and inter-row. As seen here, Nouvellon et al. (2008) did not see a clear effect of disturbing soil before planting on soil CO_2 efflux. Fialho (2016), in turn, observed increases in CO_2 emissions at the subsoiling line.

Overall, soil CO_2 efflux increased with time and one year after planting respiration returned to before harvesting levels, or increased, as happened in F+F area under coppice management (Fig. 5). Respiration in coppice areas were higher in F+F system independently of sampling position, respiration in replanted areas was higher at planting row in F+S system. We believe that a major part of this difference is heterotrophic respiration due to plant development as it could be seen by trees measurement at the same time, when bigger trees and smaller mortality were seen in F+F in coppice, compared to

coppice in F+S, and higher trees in replanting system were seen in F+S area, with similar mortality rates (Table 3).

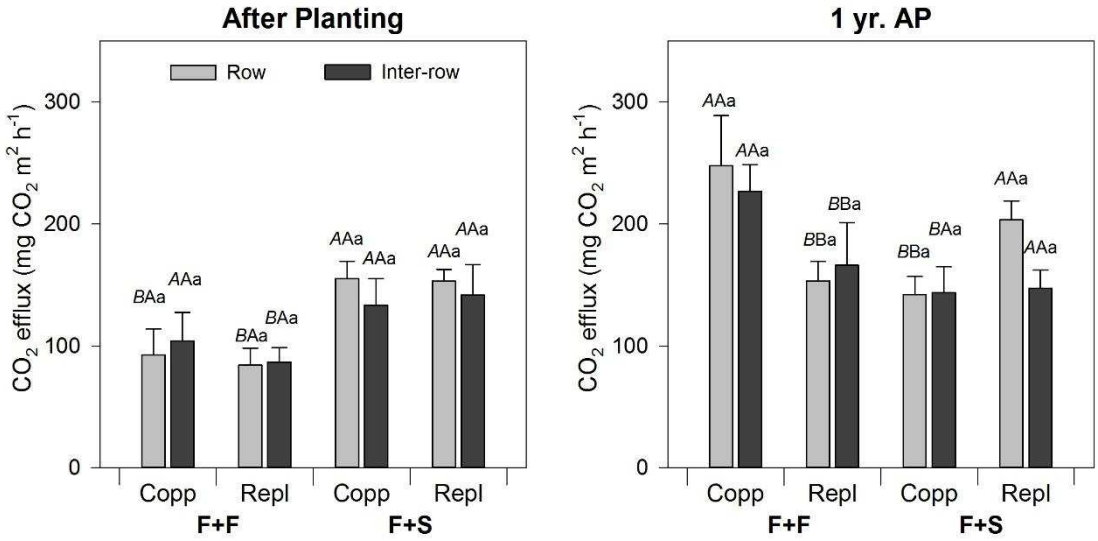

Figure 5 - Soil CO_2 efflux in both areas right after planting and one year after planting (1 yr. AP), under coppice (Copp) or replanting (Repl) managements, at planting (row) and inter-planting (inter-row) row. ANOVA was run at each time separately. Same upper case in italic inside same silvicultural system and position, and same upper case inside same area and position, and same lower case inside same area and silvicultural system do not differ at 5% by *F test*.

3.3.Soil organic matter properties

Harvesting operations usually resulted in decreases in labile C. (Fig. 6). This was not observed only in F+S system at the planting row, where an increase in labile-C was seen. Particularly before harvesting, labile C was higher at the inter-planting row areas. F+F system impacted more negatively on labile C at the inter-row area. POM-C and MAOM-C presented similar behavior as labile-C. However, changes due to harvesting were much less expressive in MAOM-C fraction. Harvesting effects on soil C are usually studied following different harvest residues management and immediate or short-term effects are not expected in stable fractions (Cambi et al., 2015; Epron et al., 2006; Johnson and Curtis, 2001). However, labile fractions of soil C may be more sensitive and earlier indicators of disturbance. The possible breakdown of aggregates due to mechanical operations would release stabilized carbon inside aggregates, enhancing respiration and decreasing soil C. Indeed, changes occurred mostly in more labile fractions. *i.e.* labile-C and POM-C.

One year after planting, labile C, POM-C and MAOM-C presented similar behavior (Fig. 7). Contents were higher at the inter-planting row. At row areas, differences regarding

depth were less expressive. Coppice usually presented higher C content than replanted areas. Differences were more expressive at inter-planting row areas, where differences among depths were also highlighted. Expressive increases in POM-C were observed at inter-planting row one year after planting. At this position, higher MAOM-C was also observed under coppice system. Significant shifts in MAOM-C was only observed at inter-row position under coppice systems. Coppice systems presented the greatest amount of soil C overall and we argue that this is due to lesser disturbance and already stablished root system, which makes recovery faster and easier, and probably the higher contribution of root system to soil C in these systems. Possible effects of harvest system and therefore residue management on soil stable pool might only happen if the same management is adopted over consecutive rotations (Achat et al., 2015).

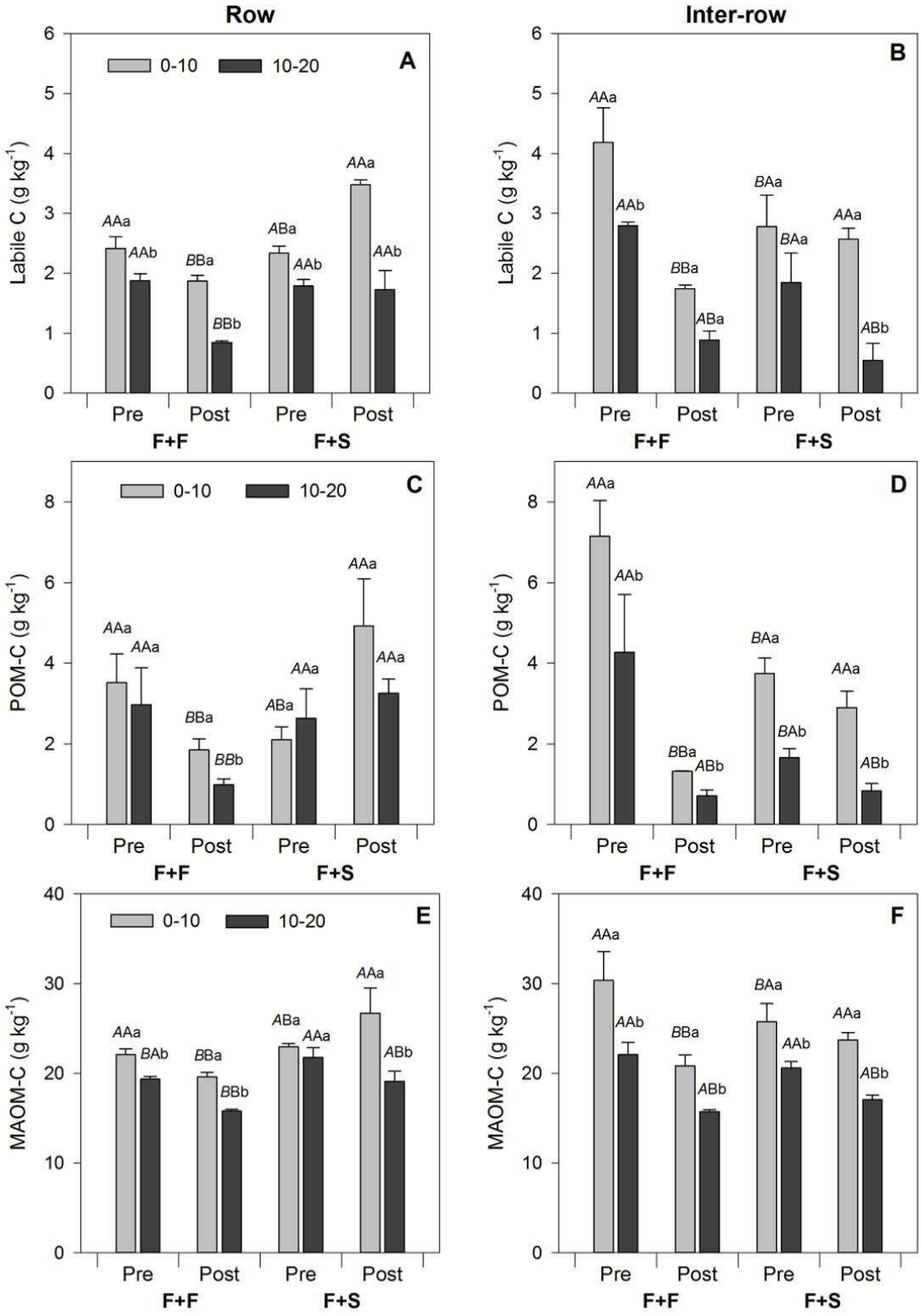

Figure 6 – Labile-C (**A, B**), POM-C (**C, D**) and MAOM-C (**E, F**) at planting (Row; **A, C, E**) and inter-planting (Inter-row; **B, D, F**) row, in both areas (F+F and F+S) before (Pre) and post (Post) harvesting, in the two depths (0-10 and 10-20 cm) studied. ANOVA was run separately in both positions (row and inter-row) evaluated. Same upper case in italic inside same time and depth, and same upper case inside same area and depth, and same lower case inside same area and time do not differ at 5% by *F test*.

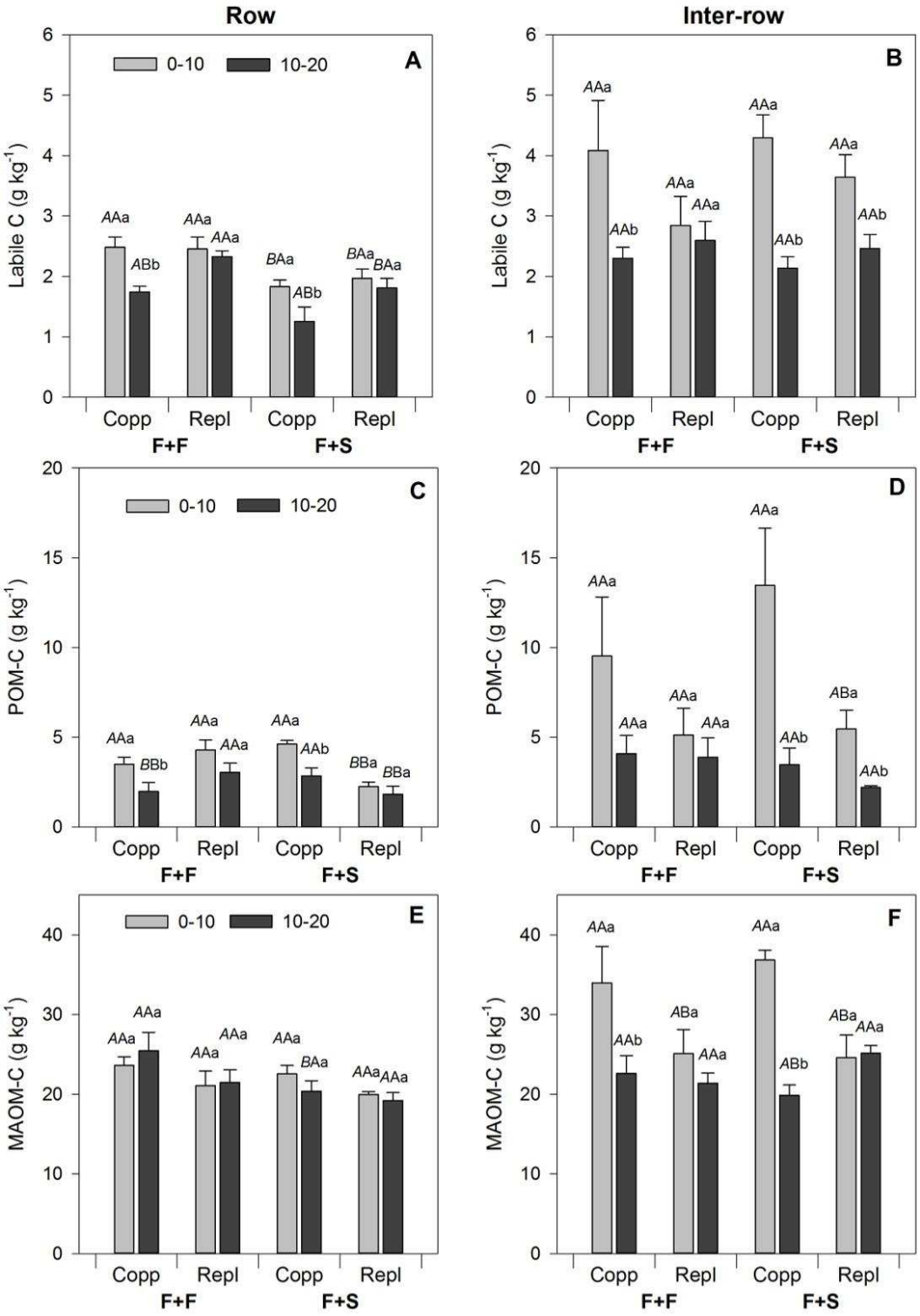

Figure 7 – Labile C (**A, B**), POM-C (**C, D**) and MAOM-C (**E, F**) one year after planting at planting (Row; **A, C, E**) and inter-planting (Inter-row; **B, D, F**) row, in both areas (F+F and F+S) under coppice (Copp) or replanting (Repl), in the two depths (0-10 and 10-20 cm) studied. ANOVA was run separately in both positions (row and inter-row) evaluated. Same upper case in italic inside same silvicultural system and depth, and same upper case inside same area and depth, and same lower case inside same area and silvicultural system do not differ at 5% by *F test*.

4. CONCLUSIONS

Combining results from shifts in soil physical and organic properties, we could see that different harvesting system affect differently row and inter-row positions, that could drive the choice of next rotation silvicultural system, due to differences in survival rate after harvesting. F+F causes higher local compaction, but trafficked area is more controlled and limited to inter-row region. F+S, in turn, system traffics throughout the area, impacting planting-row region likewise, but in lesser extent, and may require more careful recovery operations. Although expressive effects on soil density and pore configuration due to harvesting, soil CO_2 efflux seems to remain similar, which indicates that it is not a sensitive indicator to evaluate soil compaction status, since changes in soil physical properties may need to be harder to shift soil respiration. Labile-C and POM-C change rapidly and thus reflect management practices. Forest floor removal by skidding operations does not seem to be harmful to SOM properties in a single operation and SOM may take longer to respond to this intensive removal. In summary, different harvest and silvicultural systems create different soil functional zones, and soil preparation in replanting cases enhance differences between row and inter-row areas, and should be observed when assessing ecosystem services and site condition.

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6. Supplementary material

Table S1 – Soil top-layer temperature (ºC) and humidity (%) in the four-sampling time evaluated in this study, across the two stands at planting and inter-planting row.

Area	Date	Sampling time	Position	Temp. (ºC)	Humid. (%)
F+F	Feb.-14	Pre-Harvesting	Row	29.6	27.2
			Inter-row	29.2	28.2
	Apr.-14	Post-Harvesting	Row	23.3	25.6
			Inter-row	24.2	28.2
	Jul.-14	After Planting	Row	18.0	23.8
			Inter-row	18.4	26.6
	May-15	1 yr. After Planting	Row	19.8	11.9
			Inter-row	20.0	15.5
F+S	Feb.-14	Pre-Harvesting	Row	28.0	28.8
			Inter-row	29.0	28.1
	Apr.-14	Post-Harvesting	Row	24.2	26.7
			Inter-row	24.8	27.0
	Jul.-14	After Planting	Row	24.0	20.7
			Inter-row	25.0	24.0
	May-15	1 yr. After Planting	Row	20.2	13.3
			Inter-row	20.4	13.2

IV. CHAPTER 3

Nitrogen alters initial growth, fine-root biomass and spatial distribution, and soil organic matter properties of *Eucalyptus dunnii* plantation in Southern Brazil

ABSTRACT

Eucalyptus planted forests are expanding in Brazil and the effects of this land use change are still not fully understood. Particularly, nitrogen (N) fertilization effects on *Eucalyptus* growth and the effect of afforestation on soil carbon (C) are still controversial. We set up a N fertilization experiment in Southern Brazil, a new frontier of eucalypt expansion over natural grassland, to evaluate initial tree growth and changes in soil C. We also evaluated fine-root biomass (*FRB*), its spatial distribution and relation to tillage practices (ridge tillage) adopted there. Four N levels (24 – Reference, 36, 48 and 108 kg ha^{-1} of N) as cover fertilization, *i.e.* applied when trees were 1-year-old, were tested and tree growth was assessed during the two first years. Afterwards, representative trees were chosen to evaluate *FRB* and its distribution, and soil samples until 40 cm depth were collected. Soil samples were separated in 0 -10, 10 -20 and 20 -40 cm and soil organic matter (SOM) was fractionated in Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM), and C and N content, and shifts in $\delta^{13}C$, were determined in these fractions. Positive effect of N on tree growth was highlighted right after fertilization, *i.e.*, 1.5 yr., whereas at 2 yr. N effects were seen only at trees height. Highest N dose resulted in higher diameter and height, but also higher mortality, therefore greater basal area was seen using 48 kg ha^{-1} of N dose. Increasing N until 56 kg ha^{-1} resulted in higher *FRB*, but after that level *FRB* decreased and the highest N dose resulted in the lowest *FRB* observed. N does not seem to drive fine-root spatial distribution, and both horizontal and vertical anisotropy in fine-root distribution were observed. Overall, afforestation had positive effects on soil C. Changes were more expressive in top-soil layers. 36 kg ha^{-1} resulted in higher C-POM in 0 -10, while 48 kg ha^{-1} resulted in higher C-MAOM in this layer. C and N dynamics were tightly correlated, especially in MAOM fractions. $\delta^{13}C$ of both SOM fractions became more negative overtime, particularly of POM fraction. C-POM was positively correlated with *FRB*. $\delta^{13}C$-POM was more negative with depth, while $\delta^{13}C$-MAOM became less negative with depth, but changes in $\delta^{13}C$-MAOM were not significant. Tillage had a strong control on soil C and N stocks, enhancing C deposition and turnover at row (ridge) region, most likely for favoring roots development in this region. In summary, we showed that N fertilization may alter initial tree growth, but its effects do not seem to last longer. Nevertheless, N effects can be reflected on fine-root biomass and C and N of SOM fractions.

Keywords: Land use change; Nitrogen fertilization; *Eucalyptus* root system; soil organic matter

1. INTRODUCTION

The growing demand for forest products over the last years and the need for cleaner sources to mitigate increasing atmospheric CO_2 concentrations have increased the importance of planted forests. Brazil is among the world's biggest sawn wood and pulp producers, with highest average productivity. Planted forests cover more than 7.5 million

hectares in the country and ~ 72 % of this area is dominated by *Eucalyptus* genus plantations (IBÁ, 2015). Southern areas in the country, particularly Rio Grande do Sul State, is a new frontier of *Eucalyptus* expansion and planted area in the state with the genus has increased 68 % over the last 10 years (ABRAF, 2013; IBÁ, 2015). Most of this area is located in the only subtropical region in the country and special attention should be given due to its particular characteristics (Alvares et al., 2013; Gonçalves et al., 2013).

Low temperatures and the risk of frost damage are probably the main concerns for eucalypt development in the Brazilian subtropical region. Therefore, tolerance to these conditions is essential for a successful planting. Tolerance usually happens at the expenses of a higher productivity. *E. dunnii* showed good adaptability to Southern Brazil climatic conditions and is among the most planted species in the region (Floriani et al., 2011; Gonçalves et al., 2013; Jovanovic et al., 2000). However, few are the studies addressing this specie in the region helping to find suitable managements to boost this specie productivity as happened with other *Eucalyptus* species in other areas of the country to achieve current levels (Gonçalves et al., 2008; Guimaraes et al., 2015; Hernández et al., 2009; Higa et al., 2000; Stape et al., 2010).

The current high eucalypt productivity in Brazil was achieved mainly due to improvements in genetics and silvicultural practices. The relationship between productivity and fertilization is well known in agriculture and this is not different in forest plantations (Stape et al., 2010, 2006). Forest growth is limited mainly by N and P in tropical regions. However, the response of eucalypt plantation to nitrogen (N) fertilization is largely discussed and contradictory in the literature (Gazola et al., 2015; Gonçalves et al., 2008, 2004; Jesus et al., 2012; Melo et al., 2015; Pulito et al., 2015; Silva et al., 2013; Smethurst et al., 2004, 2003). Some authors have shown a clear response to N fertilization (Gonçalves et al., 2008; Jesus et al., 2012; Silva et al., 2013), especially on initial phases of growth (Pulito et al., 2015), while others showed no response to N supply, suggesting that the organic mineralizable N is the main source and sufficient to support trees demand (Costa et al., 2005; Gonçalves et al., 2004; Versini et al., 2013). Regarding below-ground growth, studies are far more scarce associating N and root growth in eucalypt ecosystem (Jourdan et al., 2008). Nitrogen may also be important to sustain stable soil C formation, either by forming more complex compounds or increasing biomass input and turnover (Dijkstra et al., 2004; Moran et al., 2005; Neff et al., 2002).

In Brazil, soil C stocks have presented different responses to afforestation (Cook et al., 2016; Jesus et al., 2012; Lima et al., 2006; Santana et al., 2015) and might be dependent on prior land use, edaphoclimatic conditions, tree species planted and tillage

practices adopted (Li et al., 2012). Studies assessing the effect of afforestation on soil organic matter (SOM) properties in Southern Brazil are scarce (Fialho and Zinn, 2014; Santana et al., 2015; Soares et al., 2013) and this is particularly necessary as expansion are often taking place over a fragile ecosystem dominated by sandy-textured soils prone to wind and water erosion (Roesch et al., 2009). As sustainability of *Eucalyptus* forests is highly associated with SOM stocks, understanding the consequences of eucalypt afforestation on SOM of these areas is crucial (Barros and Comerford, 2002).

To investigate the effects of eucalypt afforestation and fertilization on initial growth and SOM properties, we set up a N fertilization experiment at the beginning of conversion of natural grasslands of Pampa Biome to *E. dunnii* plantations in Southern Brazil. We followed tree growth over the first two years, evaluating above and below-ground growth, and assessed early changes in SOM properties by fractionation in Particulate Organic Matter (POM) and Mineral Associated Organic Matter (MAOM), two pools with different turnover time and sensitivity to land use change (Cambardella and Elliott, 1992; Lehmann et al., 2001), determining C and N content, and the proportion of C-derived from eucalypt (C_3) using natural differences in ^{13}C isotope, associated with these SOM fractions.

2. MATERIAL AND METHODS

2.1. Site description and experimental design

The study was located in Rio Grande do Sul State, Southern Brazil, near Brazil-Uruguay border (30°26'S; 54°31'W). The site is within Pampa Biome, which is characterized by grass domain with many herbs and shrub species co-occurring with the grass matrix. It is a new frontier of *Eucalyptus* plantation expansion in Brazil (IBÁ, 2015). Site is located in a sub-tropical climate area (Cfa, Köppen classification), with mean annual temperature (MAT) of 18 ^{o}C and average annual rainfall of 1351 mm during experiment years (Data from Brazilian National Institute of Spatial Research – INPE – S. Gabriel/RS station, located at ~17 km away from experimental site at similar altitude). Soils were classified as Inceptsol (U.S. Taxonomy) and properties at the beginning of the experiment are presented in Table 1.

Table 1 – Soil properties at the beginning of the experiment.

Depth	pH[(1)]	SB[(2)]	ρ_b [(3)]	Clay[(4)]	POM[(5)]			MAOM[(5)]		
					C	$\delta^{13}C$	N	C	$\delta^{13}C$	N
cm	-	$cmol_c\ dm^{-3}$	$g\ cm^{-3}$	%	$g\ kg^{-1}$	‰	$g\ kg^{-1}$	$g\ kg^{-1}$	‰	$g\ kg^{-1}$
0-10	4.71	7.71	1.15	28	2.35	-14.75	0.14	19.22	-13.52	2.22
10-20	4.82	6.83	1.32	32	1.32	-15.74	0.07	14.37	-13.50	1.60
20-40	5.07	7.74	1.36	23	0.81	-16.11	0.03	10.79	-13.41	1.22

[(1)]pH determined in H_2O, 1:2.5 soil:water solution; [(2)]Sum of Bases, Ca^{2+} and Mg^{2+} determined in KCl 1 mol L^{-1}, K^+ and Na^+ determined with Melich-1 extractor. [(3)]Soil Bulk Density; [(4)]determined following Ruiz (2005); [(5)]Fractionation following Cambardella and Elliott (1992) protocol and content and ^{13}C determinations using an elemental isotope ratio mass spectrometer (EA-IRMS GSL 20-20, Sercon, Crewe, UK).

Eucalyptus dunnii seedling were planted in October/2012 using a 3.3 x 2.2 m spacing. Before planting, soil was subsoiled until 40 cm depth and then prepared in zonal tillage, *i.e.* ridge tillage management was used at planting rows. Overall, planting row consisted of ~ 20 x 40 cm (height x width) ridges.

At planting, 12 kg ha^{-1} of N were added as base fertilization. Using a randomized block design with four replications, the stand was divided in four treatments (totaling 16 plots) that consisted of four N doses (Source: Urea), applied as cover fertilization one year after planting: 12, 24, 36 and 96 kg ha^{-1} of N, totaling 24 (Reference, commercial application used by forest companies in the area), 36, 48 and 108 kg ha^{-1} of N applied. Each plot contained 140 trees, divided in 20 trees for 7 different rows, occupying an area of ~ 1000 m^2.

2.2. Variables assessed

2.2.1. Tree growth

Trees measurement [*Diameter at breast height (DBH)* and *Height (H)*] started one year (1 yr. age) after planting, *i.e.* right before the second fertilization, and were carried out six months (1.5 yr. age) and one year after (2 yr. age) second fertilization. For the first measurement (1 yr.), *DBH* and *H* of all trees were measured. For the two other measurements (1.5 and 2 yr.), *DBH* higher than 3 cm and *H* of the central 20 trees plus dominant trees (*Hd*) (Assman, 1970) were measured. Remaining *H* were estimated using a hypsometric equation developed for each age. We tried the following models: Trorey (1932) and Curtis (1967), per treatment, and the generic models Campos and Leite (2006) and Scolforo (2005) for all treatments combined. All these models have proven to be well fitted to *Eucalyptus* plantations (Ribeiro et al., 2010). The best fitted equations were chosen based on coefficient of determination (R^2), standard error of prediction (*Syx)* and graphical residues distribution. Basal area (*G*, $m^2\ ha^{-1}$) was calculated using obtained *DBH*, mortality rate and plot area.

During the 1.5 yr. age sampling, we also assessed leaf area index (*LAI*) of each treatment and leaf N content. *LAI* was measured using a Li-COR LAI2000 plant canopy analyzer. For each plot, 30 LAI measurements were obtained, simultaneously with the above canopy light measurements that were done in an open field adjacent of the eucalypt plantation. Leaves from average tree of each plot were collected for leaf N content. They were dried and finely ground before sulfur digestion for N determination by Kjedahl method.

2.2.2. Fine-root biomass and architecture

Fine-root (*Ø* < 2 mm) biomass (*FRB*) and its architecture were sampled at 2.75 yr. age, *i.e.* July 2015. After getting data from trees measurement, average representative trees were defined for each plot and root sampling was taken on those trees. We selected one average tree for plot, *i.e.* for each treatment and replication. Root sampling was carried out in a quarter of each tree area. We sampled nine points in pre-defined locations based on planting spacing for each tree until 40 cm depth, as almost 90 % of fine-roots are expected to be concentrated in shallower layers (Bouillet et al., 2002; Rodrigues, 2013). Points were collected in three different directions: row (*r*), inter-row (*i*) and a diagonal (*d*) at 45º between *r* and *i* axis, as shown in Figure 1. A 6-cm diameter auger was used for sampling.

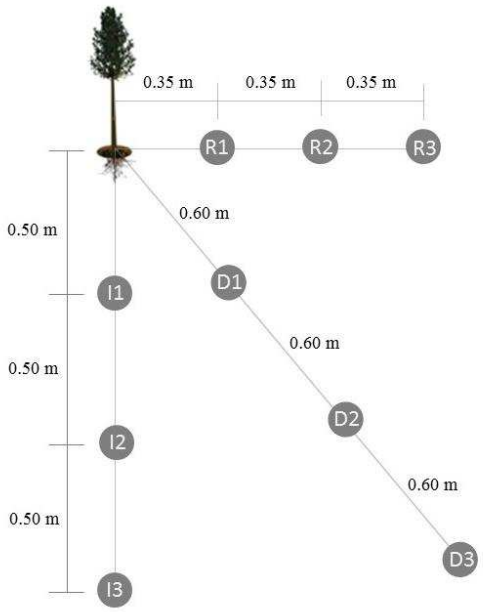

Figure 1 – Representation of sampling design used for a singular tree. Area shown represents ¼ of total tree area. **R**: indicates row positions; **I**: indicates inter-row positions; and **D**: indicates diagonal positions at 45º between row and inter-row.

Samples were separated in 0 – 10, 10 – 20, 20 – 30 and 30 – 40 cm depth. For each point and depth, a soil cylinder was collected and roots gently separated from soil by hand. Roots were stored in plastic bags and taken to the lab further cleaning, drying and weighting. Roots from one replication were stored in a 20 % hydro-alcoholic solution to keep its fresh properties. In the lab, roots were thoroughly washed, and fine-root were separated from the others with a sieve and taken to the oven at 50 ^{o}C until dry to obtain total dry mass. Roots that were stored in the hydro-alcoholic solution were scanned at 300 dpi resolution before being taken to the oven. We processed root image with *Safira* software (Jorge and Rodrigues, 2008) to obtain average length, average diameter, total surface area and therefore specific root area (*SRA*).

2.2.3. SOM properties

2.2.3.1. C and N associated with SOM fractions

Soil was collected at the same time as root sampling. Soil samples were randomly collected in five points (*n=5*) per plot until 40 cm depth, divided in 0 – 10, 10 – 20 and 20 – 40 cm. Samples were then grouped and mixed to build a composite sample per plot. Samples were air-dried and 2-mm sieved before further analysis. We took a 5 g sub-sample for SOM physical fractionation (Cambardella and Elliott, 1992) in Particulate Organic Matter (POM) and Mineral Associated Organic Mater (MAOM), and C, ^{13}C and N content associated with each fraction were determined. Briefly, 5 g of soil were dispersed using 15 mL of a 15g L^{-1} solution of sodium hexametaphosphate and one small glass bed and shaken for 15 h at 200 rpm in a horizontal shaker. Afterwards, fractions were separated by a 53 µm sieve by gently adding deionized water until the flush through the sieve was completely clear. All the material and water that flushed through the sieve (MAOM) was recovered and taken to the oven at 60 ^{o}C until completely dry. The material remained on the sieve (POM) was removed by rinsing deionized water again, recovered in a glass and taken to oven at 60 ^{o}C until dry.

After dry, POM and MAOM were then weighted and finely ground with a ball mill for C, ^{13}C and N content determination using an elemental isotope ratio mass spectrometer (EA-IRMS GSL 20-20, Sercon, Crewe, UK). Reference gas was calibrated with Pee-Dee-Belemnite (PDB) certified standard for isotope signature calculations. The abundance in samples of ^{13}C was calculated as follows:

$$\delta^{13}C\ (‰) = \left({R_{sample}}/{R_{standard}} - 1\right) \times 100 \quad (1)$$

where R = $^{13}C/^{12}C$ ratio.

2.2.3.2. Litter and tillage effects on SOM properties

Inside the smallest dose treatment (24 kg ha^{-1} of N – Reference) area, a central area occupied by 9 trees (~ 65 m^2) was permanently covered with a net (0.5-mm mesh) to avoid litter input to the soil. Net was cleaned every month and fallen litter was taken to the lab where it was dried and weighted to obtain litter input rates. Soil was collected inside this area in three different points (*n=3*) until 40 cm depth, then grouped and mixed to make one composite sample per replication. C and N content and $\delta^{13}C$ associated with SOM fractions were obtained using the same procedure described above. We compared these data with those obtained from areas not covered with the net inside the same treatment.

Soil samples were also collected inside reference dose plots at the planting row and inter-planting positions to evaluate any tillage effect on SOM properties. Five samples (*n=5*) until 40 cm depth were collected per position to form one composite sample for each position for each replication. The same SOM fractionation procedure and C, N and $\delta^{13}C$ content determination described previously were adopted to see any tillage effect on SOM properties.

2.3. Data analysis

Data was checked for normality and homoscedasticity for each variable and *ln*-transformed in necessary cases to meet normality assumptions. We fitted linear regressions for each sampling time to see increasing N fertilization effects on tree growth (*DBH*, *H*, *LAI*) and root biomass. Two-way ANOVA and post-hoc Student LSD test were used for all tree variable and SOM properties. A correlation between above and below-ground tree variables with SOM properties was also performed.

Spatial distribution of fine-roots across horizontal and vertical layers was represented using ordinary kriging of fine-root density (*FRD*). This method smoothes root distribution representation, by estimating *FRD* in areas where roots were not measured, based on the semi-variogram of variances of measured data, considering distance and

direction of obtained data. The semi-variogram model was adjusted according to the normality, stationarity, tendency and anisotropy of the samples, and its fitness was evaluated by a cross validation technique. All analysis were carried out in SISVAR® and R 3.02 software (Ferreira, 2011; R Development Core Team, 2013).

3. RESULTS

3.1. Tree growth

Trees presented similar growth pattern before cover fertilization (Fig. 2). Differences in *DBH* and *H* were not significant until 1.5 yr., when a positive effect of N fertilization on growth was seen. Differences were higher at 1.5 yr., whereas at 2 yr. differences could be seen only for *H*. A clear change in slope due to fertilization could be seen at growth curves. The highest N dose resulted in higher mortality, which explains the lower *G*, even with higher average *DBH*, than 48 kg ha^{-1} of N dose. These differences were though not significant. An increase in leaf N content and *LAI* could be seen with increasing N dose, however increases were also not significant at 5 % of probability (Table 2).

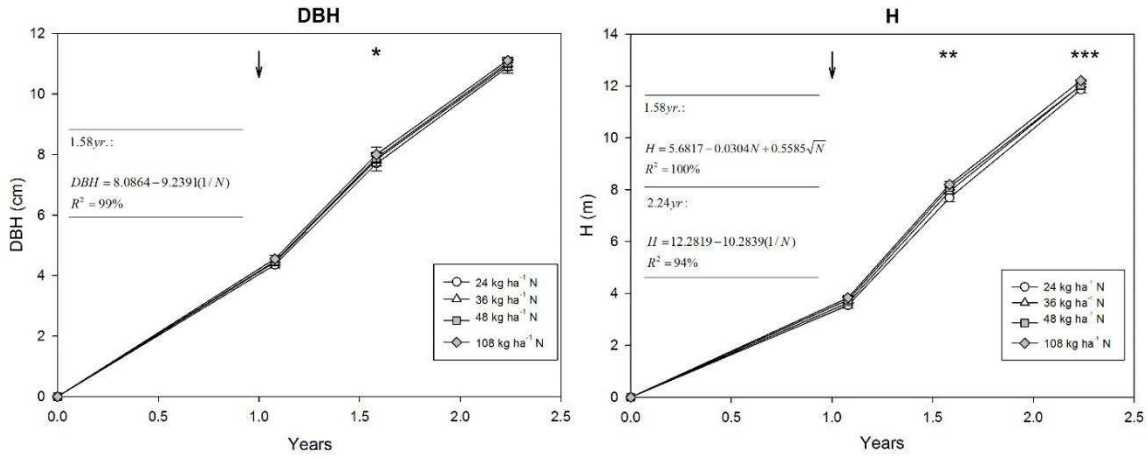

Figure 2 – Eucalyptus tree average diameter at breast height (*DBH*, cm) and height (*H*, m) as a function of N level. Arrow indicates when treatments were applied. *, ** and *** indicate significance of regression (*Growth x N*) parameters at each age at 10, 5 and 1 %, respectively.

Table 2 – Effects of N dose on average tree variables at 1, 1.5 and 2 yr. sampling.

Variables	-	Age (yr.)	Nitrogen dose (kg ha^{-1})			
			24	36	48	108
DBH	cm	1.08	4.35^{a} (0.11)	4.44^{a} (0.12)	4.47^{a} (0.10)	4.55^{a} (0.11)
		1.58	7.70^{b} (0.25)	7.83^{a} (0.22)	7.89^{a} (0.18)	8.00^{a} (0.24)
		2.24	10.89^{a} (0.20)	10.97^{a} (0.18)	11.03^{a} (0.10)	11.11^{a} (0.10)
H	m	1.08	3.54^{a} (0.11)	3.64^{a} (0.09)	3.75^{a} (0.12)	3.83^{a} (0.08)
		1.58	7.69^{b} (0.15)	7.94^{ab} (0.05)	8.09^{a} (0.10)	8.20^{a} (0.11)
		2.24	11.86^{b} (0.11)	12.02^{ab} (0.06)	12.02^{ab} (0.05)	12.21^{a} (0.03)
Hd	m	1.08	4.54^{a} (0.11)	4.71^{a} (0.08)	4.79^{a} (0.14)	4.79^{a} (0.18)
		1.58	8.83^{ab} (0.33)	8.51^{b} (0.26)	9.17^{a} (0.16)	9.15^{a} (0.27)
		2.24	12.64^{a} (0.23)	12.88^{a} (0.32)	12.94^{a} (0.16)	12.91^{a} (0.11)
Survival	%	1.08	94.82^{a} (1.71)	95.90^{a} (1.07)	95.71^{a} (1.34)	95.00^{a} (1.68)
		1.58	91.25^{a} (1.35)	92.68^{a} (1.03)	95.18^{a} (1.28)	92.14^{a} (1.62)
		2.24	92.14^{a} (0.23)	90.36^{a} (0.90)	91.96^{a} (1.63)	89.11^{a} (1.88)
G	m^2ha^{-1}	1.08	1.55^{a} (0.13)	1.73^{a} (0.13)	1.82^{a} (0.18)	1.94^{a} (0.12)
		1.58	6.21^{a} (0.41)	6.29^{a} (0.32)	6.54^{a} (0.35)	6.61^{a} (0.47)
		2.24	12.49^{a} (0.41)	12.65^{a} (0.23)	13.17^{a} (0.30)	13.14^{a} (0.31)
LAI	m^2m^{-2}	1.58	2.50^{a} (0.31)	2.72^{a} (0.13)	2.73^{a} (0.23)	2.78^{a} (0.30)
Leaf N	g kg^{-1}	1.58	21.68^{a} (1.32)	22.08^{a} (1.52)	22.33^{a} (0.99)	23.07^{a} (1.31)

DBH: Diameter at breast height; ***H***: Height; ***Hd***: Dominant height; ***G***: Basal area; ***LAI***: Leaf area index; ***Leaf N***: Foliar N content. Standard errors (*n=4*) are presented inside parenthesis. Averages followed by same lower case letter at each sampling do not differ at 5 % by LSD test.

3.2. Fine-root biomass (*FRB*) and spatial distribution

Fine-root biomass responded positively until ~ 60 kg ha^{-1} of N. After this dose, increasing N addition reduces *FRB* until 40 cm depth (Fig. 3). This behavior is a result of a positive impact of N availability on root growth until the third highest dose used, but the lowest root biomass was found at the highest N dose applied.

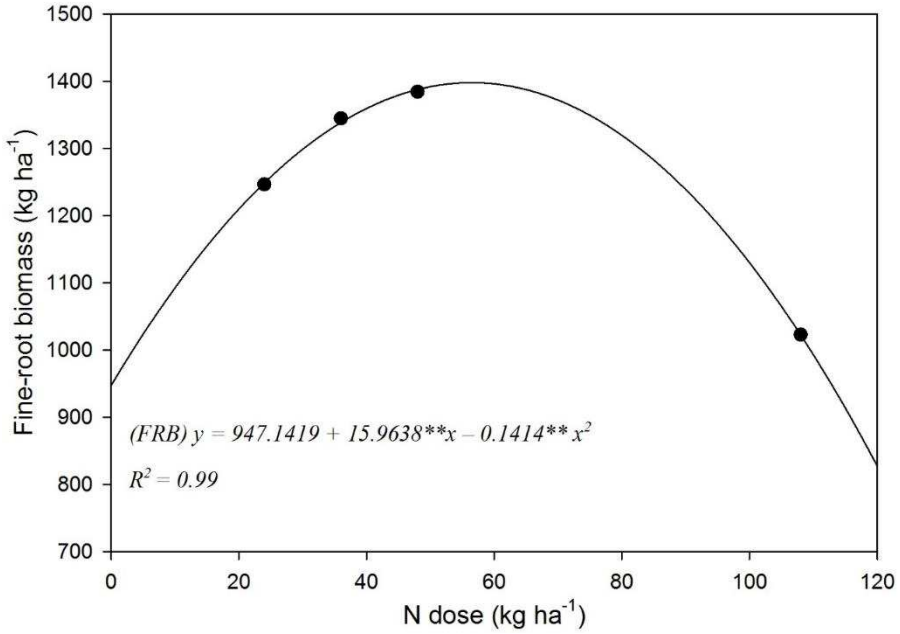

Figure 3 – Fine root biomass (*FRB*) until 40 cm depth at 2.75 yr. as affected by N fertilization. ** indicates parameter is significant at 5 %.

Tillage changed root distribution along soil surface and profile (Fig. 4). Fine-root density showed a high heterogeneity along soil profile, and there was horizontal and vertical anisotropy in root distribution. Root spatial distribution varied in the different N doses tested and in the different regions. *FRD* seems to increase along soil profile at planting row, particularly due to 20 cm ridges created. Inter-row and diagonal position presented a "more natural" behavior, *i.e.* higher root density in soil top layers (Bouillet et al., 2002; Grant et al., 2012; Laclau et al., 2001; Lourenço, 2009; Rodrigues, 2013). Overall, in these two last position the highest root concentration happened in 10 -20 cm layer. This is particularly interesting due to the higher soil density of this layer in comparison with top-soil (Table 1). That is probably the reason why more roots could be found at planting row position below 20 cm depth.

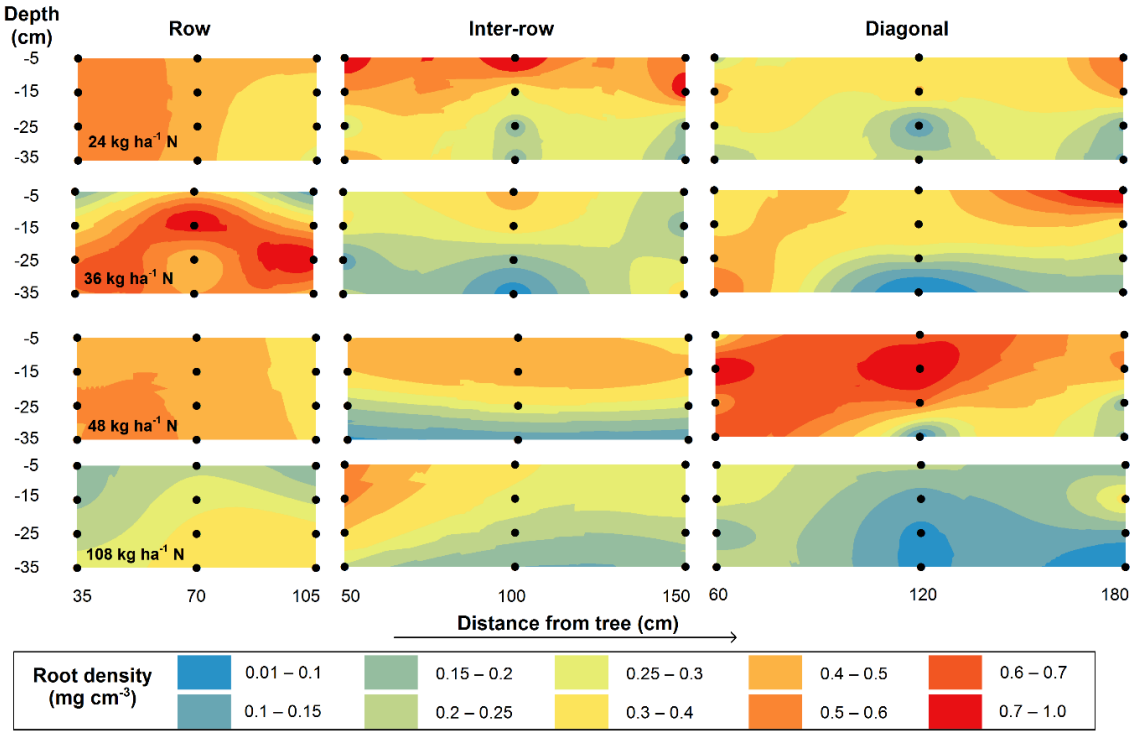

Figure 4 – Kriging interpolation of average fine-root density (*FRD*) distribution along soil profile in the three positions evaluated. Trees are placed in the top left corner of each figure. *x* axis represents increasing distance from trees in each position, and *y* axis represents depth.

Correlations among tree and soil variables are summarized in Table 3. *DBH* and *H* correlated negatively with *FRB* and positively with *SRA*. SOM properties had little or no correlation with aboveground variables (*DBH*, *H*). In turn, C-POM correlated positively with *FRB* and therefore negatively with *SRA*. C-MAOM only correlated with root length. Correlations results show a close link between POM and MAOM fractions and between soil C and N.

Table 3 – Mean correlations among trees variable and SOM fractions properties. Only significant correlations are shown for simplicity.

-	Dose	Length	RA	SRA	FRB	DBH	H	Mort.	C-POM	C-MAOM	δ^{13}C-POM	δ^{13}C-MAOM	N-POM	N-MAOM
Length		-	-	-	-	-	-	-	-	-	-	-	-	-
RA	*-0.47*	**0.93**	-	-	-	-	-	-	-	-	-	-	-	-
SRA				-	-	-	-	-	-	-	-	-	-	-
FRB	**-0.60**		**0.53**	**-0.74**	-	-	-	-	-	-	-	-	-	-
DBH				*0.49*	**-0.50**	-	-	-	-	-	-	-	-	-
H	**0.67**			*0.42*	**-0.63**	**0.84**	-	-	-	-	-	-	-	-
Mort.								-	-	-	-	-	-	-
C-POM				**-0.69**	**0.58**				-	-	-	-	-	-
C-MAOM		**0.54**	*0.45*						**0.55**	-	-	-	-	-
δ^{13}C-POM									**0.56**	*0.49*	-	-	-	-
δ^{13}C-MAOM												-	-	-
N-POM					*0.44*				**0.84**				-	-
N-MAOM									*0.46*	**0.92**			**0.66**	-

Italic values indicate $p < 0.10$ and bold values indicate $p < 0.05$. Variables: **Length**: Root length (mm); **RA**: Root area (mm^2); **SRA**: Specific root area (m^2 kg^{-1}); **DBH**: Diameter at breast height (cm); **H**: Height (m); **Mort.**: Mortality (%).

3.3. C and N associated with SOM fractions as affected by N level, litter presence and tillage

Nitrogen had influence on C associated with POM and MAOM, particularly in the 0 -10 cm soil layer. This influence was, though, non-linear and different between fractions (Table 4). 36 kg ha^{-1} of N resulted in the highest C-POM at 0 -10 cm layer. At 10 -20 cm layer, highest C-POM was found at 48 kg ha^{-1} of N dose. This dose also resulted in the highest C-MAOM in 0 -10 cm layer and N-MAOM in 10 -20 cm layer. In general, 48 kg ha^{-1} of N was the dose that provided higher C and N contents. Increasing N dose had no effect on $\delta^{13}C$ values of SOM fractions. $\delta^{13}C$-POM became more negative as it gets deeper, whilst $\delta^{13}C$-MAOM presented small and non-significant differences among depths and treatments.

Table 4 – N fertilization effects on C, $\delta^{13}C$ and N of SOM fractions properties at 2.75 yr. eucalypt plantation.

Properties	**Nitrogen dose (kg ha^{-1})**			
	24	**36**	**48**	**108**
C-POM (*g kg^{-1}*)				
0-10	4.23^{Ba} (0.87)	7.55^{Aa} (0.65)	4.88^{ABa} (1.16)	4.30^{Ba} (0.52)
10-20	1.56^{Bb} (0.48)	1.99^{ABb} (0.52)	2.82^{Ab} (0.76)	1.90^{ABb} (0.28)
20-40	0.70^{Ac} (0.14)	0.85^{Ac} (0.09)	0.61^{Ac} (0.04)	0.67^{Ac} (0.08)
C-MAOM (*g kg^{-1}*)				
0-10	18.66^{Cc} (1.38)	21.72^{Ba} (1.74)	27.44^{Aa} (1.14)	20.41^{BCa} (0.64)
10-20	15.36^{Ab} (1.67)	16.92^{Ab} (1.32)	16.12^{Ab} (1.35)	17.46^{Ab} (1.39)
20-40	9.69^{Ac} (0.83)	11.09^{Ac} (0.96)	11.51^{Ac} (0.09)	9.05^{Ac} (1.31)
δ^{13}C-POM *(‰)*				
0-10	-15.77^{Ac} (0.31)	-15.78^{Ab} (0.41)	-16.35^{Ab} (0.89)	-16.56^{Ab} (0.36)
10-20	-17.33^{Ab} (0.34)	-16.93^{Aa} (0.36)	-16.62^{Ab} (0.58)	-17.25^{Aab} (0.26)
20-40	-18.34^{Aa} (0.16)	-17.63^{Aa} (0.29)	-17.78^{Aa} (0.36)	-17.70^{Aa} (0.23)
δ^{13}C-MAOM *(‰)*				
0-10	-13.37^{Aa} (0.11)	-14.29^{Aa} (0.28)	-14.46^{Aa} (0.35)	-14.19^{Aa} (1.05)
10-20	-14.24^{Aa} (0.06)	-14.20^{Aa} (0.26)	-14.10^{Aa} (0.43)	-13.71^{Aa} (1.24)
20-40	-13.19^{Aa} (0.97)	-13.77^{Aa} (0.20)	-13.67^{Aa} (0.53)	-14.06^{Aa} (0.66)
N-POM (*g kg^{-1}*)				
0-10	0.30^{Aa} (0.06)	0.48^{Aa} (0.12)	0.41^{Aa} (0.05)	0.31^{Aa} (0.04)
10-20	0.09^{Bb} (0.03)	0.13^{ABb} (0.03)	0.19^{Ab} (0.05)	0.12^{ABb} (0.02
20-40	0.03^{Ac} (0.01)	0.04^{Ac} (0.00)	0.04^{Ac} (0.01)	0.03^{Ac} (0.00)
N-MAOM (*g kg^{-1}*)				
0-10	1.96^{Ba} (0.16)	2.28^{Ba} (0.19)	2.69^{Aa} (0.32)	2.14^{Ba} (0.07)
10-20	1.62^{Aa} (0.17)	1.75^{Ab} (0.17)	1.69^{Ab} (0.16)	1.82^{Aa} (0.15)
20-40	0.97^{Ab} (0.12)	1.13^{Ac} (0.10)	1.18^{Ac} (0.03)	0.91^{Ab} (0.03)

Standard errors (*n=4*) are presented inside parenthesis. Averages followed by same upper case letter inside each depth and same lower case letter inside each N level do not differ at 5 % by LSD test.

Litter had no effect on C and N of SOM fractions (Table 5). Albeit slight higher content of C-POM and N-POM on soil top layer when litter was present, differences were not significant. Tillage, in turn, affected C and N content of SOM fractions (Table 5). It had little effect on $\delta^{13}C$ of these fractions, although more negative values were found at planting row areas overall. Row position presented higher C and N content in all three depths evaluated. Differences were more expressive in shallower layers.

Table 5 – Effects of litter removal and ridge tillage on C, $\delta^{13}C$ and N of SOM fractions properties at 2.75 yr. eucalypt plantation in the 24 kg ha^{-1} N dose.

Properties	**Litterfall**		**Tillage**	
	Exclusion	**Presence**	**Row**	**Inter-row**
C-POM (*g kg^{-1}*)				
0-10	3.83 (1.12)	4.23 (0.87)	***4.66 (0.95)***	***2.31 (0.57)***
10-20	1.64 (0.48)	1.56 (0.48)	2.03 (0.11)	0.97 (0.46)
20-40	0.61 (0.05)	0.70 (0.14)	1.01 (0.12)	0.59 (0.03)
C-MAOM (*g kg^{-1}*)				
0-10	19.74 (1.16)	18.66 (1.38)	***22.00 (0.54)***	***18.26 (1.46)***
10-20	16.00 (2.32)	15.36 (1.67)	18.17 (0.93)	16.16 (1.14)
20-40	9.47 (0.31)	9.69 (0.82)	11.98 (0.67)	10.23 (0.42)
δ^{13}C-POM *(‰)*				
0-10	-15.83 (1.12)	-15.77 (0.87)	-16.08 (0.78)	-16.68 (0.80)
10-20	-16.68 (0.48)	-17.33 (0.48)	-17.66 (0.79)	-17.70 (0.51)
20-40	-17.76 (0.05)	-18.33 (0.14)	-19.19 (0.25)	-18.41 (0.45)
δ^{13}C-MAOM *(‰)*				
0-10	-13.11 (1.16)	-13.37 (1.38)	-14.16 (0.29)	-13.68 (0.23)
10-20	-13.75 (2.32)	-14.24 (1.67)	-14.50 (0.15)	-13.84 (0.24)
20-40	-12.59 (0.31)	-13.19 (0.82)	***-14.14 (0.16)***	***-12.00 (0.99)***
N-POM (*g kg^{-1}*)				
0-10	0.27 (0.08)	0.31 (0.06)	***0.29 (0.06)***	***0.16 (0.04)***
10-20	0.06 (0.02)	0.09 (0.03)	***0.12 (0.01)***	***0.05 (0.03)***
20-40	0.04 (0.02)	0.03 (0.01)	0.05 (0.01)	0.02 (0.00)
N-MAOM (*g kg^{-1}*)				
0-10	2.13 (0.16)	1.96 (0.16)	***2.27 (0.08)***	***1.95 (0.14)***
10-20	1.70 (0.25)	1.62 (0.17)	1.89 (0.11)	1.68 (0.11)
20-40	0.95 (0.02)	0.97 (0.12)	1.20 (0.05)	1.04 (0.05)

Bold and italic averages indicate significant effect of litter removal or tillage on property at each depth by LSD test at 5 %.

4. DISCUSSION

4.1. Tree growth

Eucalypt growth response to N has been largely debated. Mineralizable N and the efficient internal N cycling are considered to suffice their fast growth (Gama-Rodrigues and Barros, 2002; Laclau et al., 2010). Seldom responses of eucalypt growth to N fertilization are found in literature and they are usually limited to the two first years, while

canopy is still in formation or under N limiting situations (Gazola et al., 2015; Gonçalves et al., 2008; Jesus et al., 2012; Melo et al., 2015; Pulito et al., 2015; Silva et al., 2013; Smethurst et al., 2004, 2003). Here, tree growth responded positively to N fertilization during the two years evaluated. N effects on growth was greater at 1.5 yr. age, *i.e.* six months after cover fertilization and treatment application, when *DBH* and *H* were positively influenced by N (Fig. 2). At 2 yr., only *H* was positively influenced.

The highest N dose resulted in the highest *LAI* and leaf N content, although not significant, which could explain this higher growth (Table 2). Albeit differences in eucalypt *LAI* and leaf nutrient content due to fertilization may respond in lower extent than growth (Melo et al., 2015), they are considered good indicators of continuous forest growth (Albaugh et al., 2004; Jesus et al., 2012; Smethurst et al., 2003; Stape et al., 2004). *LAI* smaller than 3 $m^2 m^{-2}$ as found here have already been reported for eucalypt tropical plantations (Binkley et al., 2002; Smethurst et al., 2003), but it still considered a low *LAI* and is expected to increase as *LAI* tends to peak when eucalypt forest is around 4-year-old (Beadle, 1997).

4.2.Root biomass and spatial distribution

The highest N dose also resulted in the lowest root biomass (Fig. 3). All trees sampled for roots were not located side by side with dead trees, so it is more likely that the lower *FRB* found here is at least in part responsible for the higher mortality, even though support trees on the soil is not the major function of fine-roots. Considering the site soil is a shallow Inceptsol with higher density in deeper layers (Table 1), a well-developed lateral root system would be essential to protect trees against wind. Whilst coarse roots are mainly located near the stump, fine and medium roots spread laterally and have good biomass and distribution correlation (Laclau et al., 2013, 2001; Teixeira et al., 2002), and may be helping lateral support of those trees, especially because roots until 2-mm *Ø* were sampled here.

The high nutrient availability may have resulted in needless root expansion under the highest dose. In contrast to what we found here, Jourdan et al. (2008) found no effect of N fertilization on fine root growth of *Eucalyptus grandis* plantations in Brazil. They used a 120 kg ha^{-1} N dose and found negligible differences in *FRB* in the first 0 -30 cm of soil compared to control (0 kg ha^{-1} of N). Fabião et al. (1995) also found no differences in fine root eucalypt production due to fertilization or irrigation. Coarse roots and total below-ground biomass, in turn, were affected by their treatments.

As fine roots are specialized in acquiring water and nutrient, other variables such as length and *SRA* may be as or more important than total amount of *FRB*. Indeed, mean correlations showed that specific root area was positively correlated with tree growth (Table 3). *FRB* was negatively correlated with tree growth. This mean result needs to be evaluated carefully, as it was mostly obtained due to root behavior under 108 kg ha^{-1} of N dose, since *FRB, DBH* and *H* increased with increasing N in the smaller doses. So, we do not argue that N was limiting and trees had to choose where to allocate C.

As observed in other studies with eucalypt roots distribution, a horizontal and vertical anisotropy was observed (Bouillet et al., 2002; Laclau et al., 2013, 2001), and this might have resulted in some fine-root clustering (Fig. 4). Root spatial distribution presented a strong behavior driven by ridge tillage. Although this tillage control, total amount of *FRB* found here are in agreement to other studies that adressed eucalypt roots of similar age plantations (Fabião et al., 1995; Jourdan et al., 2008; O'Grady et al., 2005) or older (Teixeira et al., 2002; Witschoreck et al., 2003). This indicate that eucalypt fine root biomass may stabilize in certain age and vary in different seasons due to their rapid turnover (Jourdan et al., 2008; Kätterer et al., 1995; Mello et al., 2007; Teixeira et al., 2002).

Overall, eucalypt fine roots tend to concentrate in shallower layers, and sharply decrease with depth (Bouillet et al., 2002; Laclau et al., 2001; Lourenço, 2009; Rodrigues, 2013; Teixeira et al., 2002; Witschoreck et al., 2003). Here, roots growing on the ridge increased with depth. At planting row position, soil was subsoiled and a ridge of ~ 20 cm height was created that might have driven and changed pattern of root distribution. Moreover, trees probably invested in deeper roots in ridge area due to the elevated density in surrounding areas. In the other positions, *FRD* peaked in 10 -20 cm layer and then decreased. This is probably a result of obstructions found in deeper layers as plinthite and other cohesive structure formations were observed. Although differences in *FRB* among N-levels were observed, we could not see a pattern of root distribution being driven by N fertilization.

4.3. SOM properties as affected by N levels, litter and tillage

Nitrogen had influence on C associated with POM and MAOM, particularly in the 0 -10 cm soil layer. C and N associated with SOM fractions presented similar responses to N fertilization regimes, although effect on N content was less expressive (Table 4).

Their dynamics was tightly correlated (Li et al., 2012), particularly when associated with mineral soil fraction (Table 3).

Overall, eucalypt afforestation resulted in C and N accrual when compared to previous land use. Accrual happened in the top soil layer, especially in POM fractions and noteworthy for N-POM, whereas deeper layer (20 -40 cm) remained unchanged or presented lower C and N content. Both δ^{13}C-POM and δ^{13}C-MAOM became more negative, with δ^{13}C-POM changing faster. Interestingly, while top soil layers presented higher C-POM content with eucalypt afforestation, it also presented a less negative δ^{13}C-POM. This could be a dilution effect, since a slightly decrease of C-POM in 20 -40 cm layer was observed, but at the same time more eucalypt contribution (more negative δ^{13}C) was observed (Table1; Table 4). This is an evidence that fresh root input may destabilize old deep soil C (Fontaine et al., 2007), and the overall lower root growth and slower turnover in deeper layer can explain this decrease of C-POM in deeper layers, since *FRB* was positively correlated with C-POM (Table 3).

Afforestation effects on soil C is largely debated in the literature (Binkley et al., 2004; Binkley and Resh, 1999; Li et al., 2012; Lima et al., 2006; Santana et al., 2015). The positive effect of N on soil C pools showed here are in disagreement with some other authors who found no effect of N fertilization on soil C pools under eucalypt plantations (Binkley et al., 2004; Jesus et al., 2012). Binkley et al. 2004 tested much higher N doses (300 – 1600 kg ha^{-1} of N) on development of first-rotation eucalypt forest in Hawaii and found no evidence of N altering soil C accrual because losses of old C_4-derived C offset C_3-derived C inputs throughout an 8-year rotation. Here, at 2.75 yr. rotation we observed a positive effect of N particularly with 36 kg ha^{-1} and 48 kg ha^{-1} of N doses for C-POM and C-MAOM, respectively. Although this behavior might change until the end of rotation, we hypothesize that effects might be even enhanced, since a nutrient-richer cycling is expected (Silva et al., 2013) and therefore a positive effect of combined mineral and organic N on soil C pools (Moran et al., 2005).

Litterfall exclusion had no effect on soil C and N, although some minor changes could be observed. Eucalypt litterfall influence on soil C is poorly studied thus making difficult any comparison. Litter might have a positive influence on soil C, but it should be restricted to the very top-soil layer (Cotrufo et al., 2015; Souza, 2012) and the 0 -10 cm sampling used here may have hampered any possible litter effects. Litterfall production tends to peak when eucalypt forest is around 4-year-old (Nouvellon et al., 2012) and may achieve up to 8 Mg ha^{-1} in a single year (Cizungu et al., 2014; Corrêa et al., 2013; Inkotte et al., 2015). The amount of litterfall produced here was around 3.7 Mg

ha^{-1} yr^{-1}. Hence, it may even double the amount of litter entering the soil and the constant input throughout a rotation might alter SOM dynamics and differ from what we found here.

Differently of litterfall exclusion influence on SOM properties, the adoption of ridge tillage notably altered soil C and N pools. It might be a consequence of changing root distribution (Fig. 4) and likely breakdown of soil aggregates, changing temperature, humidity and therefore C and N accrual and turnover (Fialho, 2016; Oliveira, 2015; Williams et al., 2016a, 2016b). Higher C content was observed in row position in both POM and MAOM fractions and differences were remarkable especially in 0 -10 cm layer (Table 5). It also resulted in more eucalypt-derived C, with significant differences in the deeper layer (20 -40 cm), that is probably the consequence of the higher fine-root biomass and turnover. This result shows that adoption of a zonal management technique such ridge tillage is likely to create different functional zones in the area and therefore drive ecosystem functioning.

5. CONCLUSIONS

Our results showed that N fertilization alters tree growth and SOM fractions properties. A positive change in the slope of growth curve with N addition was observed, but differences do not seem to last longer. This indicate that a yearly N supply may be the best approach to enhance forest productivity over Inceptsols in Southern Brazil, at least during the first years. Differences in SOM fractions properties due to N fertilizations were higher, but non-linear, and were correlated with fine-root biomass production and turnover. Most of soil C eucalypt-derived came from roots, with negligible effects of litter input. Zonal tillage management, *i.e.* ridge tillage management adopted here, had significant influence on soil C and N, probably due to changes in soil aggregation, aeration, temperature and humidity and to be driving root distribution along soil surface and profile. The best results were achieved with slightly increases (12 and 24 kg ha^{-1} of N) in reference dose (24 kg ha^{-1}), being the 48 kg ha^{-1} of N the dose that resulted in higher stand basal area, fine-root biomass and stable C and N, indicating that it is probably the most suitable one to be used under the evaluated conditions.

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7. Supplementary material

Table S1 – Root variables obtained from root imaging and *Safira*® software processing (*n=1*).

N level	**Root variables**		
kg ha^{-1}	**Length** (mm)	**Area** (mm^2)	**SRA** ($m^2\ kg^{-1}$)
24	844.55	1721.11	19.89
36	812.58	1598.43	17.35
48	1054.53	1991.68	20.38
108	829.96	1504.43	21.29

SRA: Specific root area.

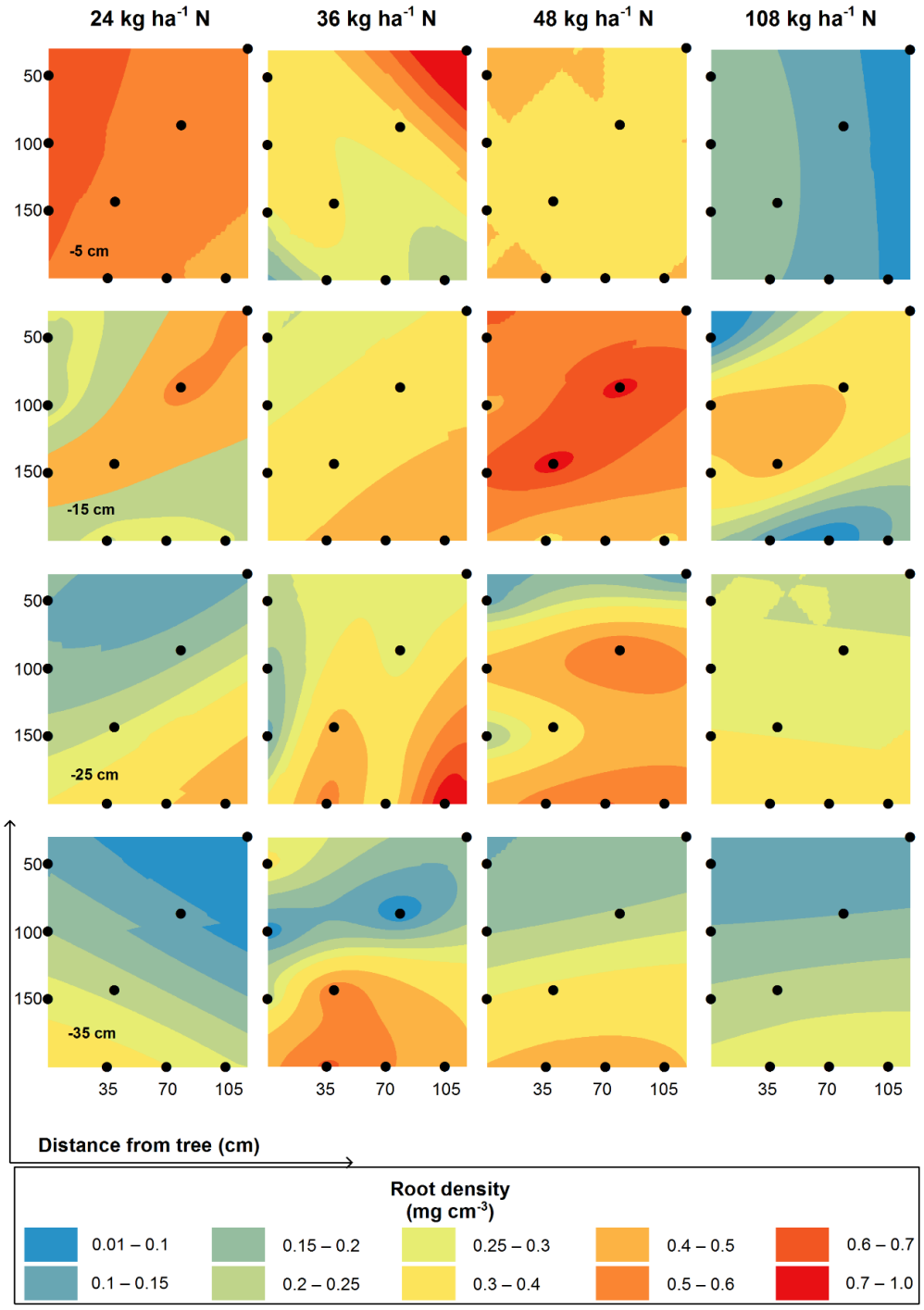

Figure S1 – Kriging interpolation of average fine-root density (*FRD*) distribution in the four N levels across the four depths evaluated (0 -10: -5 cm; 10 -20: -15 cm; 20 -30: -25 cm; 30 -40: -35 cm). Trees are placed in the bottom left corner of each figure. Axis represent increasing distance from trees. *x* axis represents Row position, *y* axis represents inter-row position and diagonal is represented at 45º between row and inter-row.

V. FINAL CONSIDERATIONS

This thesis tried to approach some existing gaps regarding the impact of forest operations on soil physical and organic properties, and early stand status in planted *Eucalyptus* plantations in Brazil. We hope that results found here can be used to guide more accurate decisions regarding sustainability of eucalypt plantation, particularly in forest expansion areas where studies are still scarce.

In summary, it is shown that:

- Harvest residues (HR) sustainable management could enhance soil C stocks and directly alter soil organic matter (SOM) particulate fraction chemistry. HR are could also be an important energy source for soil microorganisms and therefore increase C sequestration in SOM stable pools. HR directly effects are, however, restricted to the very top-soil layer;

- The choice of harvesting system and next rotation silvicultural system should be done together, since harvesting impacts vary from system to system and might be harmful for next rotation development, requiring specific silvicultural techniques to grant forest success. Different harvest and silvicultural system might create different soil functional zones, enhancing differences between row an inter-row areas, that shall be observed when evaluating operations impacts;

- Lastly, it's shown positive although short impacts of N fertilization on initial growth of *Eucalyptus* trees in a new forest expansion area in the country. N addition had also positive effects on fine-root biomass and C and N content of SOM fractions. Hence, the use of slightly higher N doses than the ones adopted currently may be beneficial. C and N content were positively correlated with fine-root biomass. Litter input had no effect on SOM properties. Planting operations seems to drive fine-root distribution and create soil favorable zones for C input and cycling.