LARISSA CASSEMIRO PACHECO MONTEIRO

DIVERSIDADE MICROBIANA NA RIZOSFERA DE PLANTAS EM COMPETIÇÃO

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de Magister Scientiae.

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APROVADA: 22 de fevereiro de 2016.

Marcelo Nagem Valério de Oliveira
(Coorientador)

Marcos Rogério Tótola
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Maurício Dutra Costa
(Orientador)

A Deus,

Aos meus pais,

Aos meus irmãos,

Dedico.

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BIOGRAFIA

LARISSA CASSEMIRO PACHECO MONTEIRO, filha de Raimundo José Monteiro e Neuza Pacheco Cassemiro Monteiro, nasceu em Caratinga, Minas Gerais, no dia 18 de Julho de 1991. Em 2009, ingressou na Universidade Federal de Viçosa, graduando-se em Ciências Biológicas em Março de 2014. No mesmo mês ingressou no Mestrado em Microbiologia Agrícola pelo Departamento de Microbiologia da Universidade Federal de Viçosa, submetendo-se à defesa da dissertação em 22 de fevereiro de 2016.

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RESUMO

MONTEIRO, Larissa Cassemiro Pacheco, M.Sc, Universidade Federal de Viçosa, Fevereiro de 2016. **DIVERSIDADE MICROBIANA NA RIZOSFERA DE PLANTAS EM COMPETIÇÃO.** Orientador: Maurício Dutra Costa. Coorientadores: Marcelo Nagem Valério de Oliveira e Marcos Rogério Tótola.

As plantas exercem influência sobre as populações microbianas associadas às raízes e, por meio da exsudação radicular, promovem mudanças na comunidade microbiana do solo. Quando duas plantas encontram-se em competição, a estrutura da comunidade microbiana rizosférica difere daquela observada nas monoculturas. Os diversos grupos de microrganismos presentes na rizosfera desempenham papéis importantes que podem influenciar positiva ou negativamente o desenvolvimento das plantas e as interações por elas estabelecidas. Assim, o objetivo deste trabalho foi avaliar os grupos taxonômicos microbianos presentes no solo rizosférico de plantas em competição e em monocultivo e relacioná-los com as atividades de promoção de crescimento já relatadas. Os experimentos foram realizados em casa de vegetação do Departamento de Microbiologia, da Universidade Federal de Viçosa. Duas espécies de culturas, Zea mays L. e Glycine max (L.) Merr., e três espécies de plantas daninhas, Ageratum conyzoides L., Ipomoea ramosissima (Poir.) Choisy, e Bidens pilosa L., foram avaliadas. O DNA de células presentes no solo rizosférico foi extraído e, em seguida, foi realizada a amplificação por PCR do gene rRNA 16S e da região ITS de fungos, seguida do sequenciamento conduzido na plataforma Illumina MiSeq. Para as amostras de solo rizosférico de Zea mays em monocultura e em competição com plantas daninhas foram obtidas 200.408 sequências de bactérias, 3.062 sequências de arqueias e 528.560 sequências de fungos. Já para as amostras de solo rizosférico de Glycine max, foram obtidos 210.376, 3.107 e 371.526 sequências de bactérias, arqueias e fungos, respectivamente. Em todos os tratamentos avaliados o maior número de Unidades Taxonômicas Operacionais (UTOs) foi registrado para bactérias, seguido de fungos e por último de arqueias. Muitas das UTOs encontradas são compartilhadas por todos os tratamentos. No entanto foi possível classificar taxonomicamente aquelas que são exclusivas em cada tratamento. Os resultados mostram que os filos de Bacteria mais abundantes foram Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia e Cloroflexi. Crenarchaeota foi o filo mais abundante de Archaea e Ascomycota o filo mais abundante de Fungi. O filo Glomeromycota, que compreende todos os fungos

micorrízicos arbusculares, também foi observado. Esses fungos apresentam importantes papéis ecológicos, incluindo a promoção de crescimentos de plantas. Bactérias envolvidas na ciclagem do nitrogênio também foram identificadas, como as oxidantes de amônia pertencentes ao Filo Nitrospirae e as arquéias pertencentes ao grupo Thaumarchaeota (Filo Crenarchaeota), e as bactérias fixadoras de nitrogênio pertencentes às ordens Rhodobacterales e Rhizobiales, e à família Frankiaceae. A competição entre culturas e plantas daninhas altera os perfis da comunidade microbiana associada à rizosfera dessas plantas, ficando evidente a capacidade que as plantas possuem em influenciar a comunidade microbiana a ela associada, recrutando microrganismos específicos a depender da condição de competição. A comunidade microbiana rizosférica, por sua vez, pode interferir na modulação dessas interações, afetando a habilidade competitiva das plantas.

ABSTRACT

MONTEIRO, Larissa Cassemiro Pacheco, M.Sc, Universidade Federal de Viçosa, February, 2016. **MICROBIAL DIVERSITY IN THE RHIZOSPHERE OF PLANTS IN COMPETITON.** Adviser: Maurício Dutra Costa. Co-advisers: Marcelo Nagem Valério de Oliveira and Marcos Rogério Tótola.

Plants have a strong influence on the microbial populations associated with their roots and, by radicular exudation, they promote changes in the soil microbial community. When two plants are in competition, the structure of the rhizospheric microbial community differs from that observed in monocultures. Several groups of microorganisms present in the rhizosphere play important roles that can positively or negatively influence the growth of plants and the interactions established by them. The objective of this work was to evaluate the microbial taxa present in the rhizosphere of plants in competition and in monoculture and relate them with growth-promoting activities already reported. The experiments were conducted in a greenhouse, at the Department of Microbiology, Universidade Federal de Viçosa. Two crop species Zea mays L. e Glycine max (L.) Merr., and three species of weed Ageratum conyzoides L., Ipomoea ramosissima (Poir.) Choisy, and Bidens pilosa L., were evaluated. The DNA from cells present in the rhizospheric soil was extracted, and PCR amplification of the bacterial 16S rRNA gene and fungal ITS region were carried out to perform sequencing through the Illumina MiSeq platform. For Zea mays rhizosphere soil in monoculture and in competition with weeds 200,408 sequences were obtained for bacteria, 3,062 for archaea and 528,560 for fungal. For Glycine max rizospheric soil, 210,376; 3,107 and 371,526 sequences were obtained for bacteria, archaea and fungi, respectively. In all treatments evaluated, most of the Operational Taxonomic Units (OTUs) where identified within bacteria, followed by fungi and archaea. Many OTUs are shared by all treatments, however it was possible to classify taxonomically those that are exclusive for each treatment. Our results show that the most abundant phyla of Bacteria were Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia, and Cloroflexi. Crenarchaeota was the most abundant phylum of Archaea and Ascomycota the most abundant phylum of Fungi. The Glomeromycota phylum, which comprises all mycorrhizal fungi, was also reported. These fungi have important ecological roles, including plant growth promotion. Bacteria involved in nitrogen cycling were also identified, such as the ammonia-oxidizer bacteria belonging to the Nitrospirae phylum

and archaea belonging to Thaumarchaeota group (Crenarchaeota Phylum); and nitrogen-fixing bacteria belonging to Rhodobacterales and Rhizobiales orders and Frankiaceae family. The competition between crops and weeds caused changes in the microbial communities in the rhizosphere associated with these plants highlighting the ability of plants to influence the associated microbial community by recruiting specific microorganisms, depending on the competition conditions. The rhizospheric microbial community, in turn, can interfere in the modulation of this interaction, affecting the competitive ability of plants.

INTRODUÇÃO GERAL

O Brasil é um dos líderes mundiais na produção e exportação de vários produtos agropecuários, liderando o ranking das vendas externas de soja (MAPA, 2015). A soja é a cultura agrícola brasileira que mais cresceu nas últimas três décadas e corresponde a 49 % da área plantada com grãos. O país é também o terceiro maior produtor mundial de milho, com mais de 66 milhões de toneladas produzidas na safra 2011/2012 (CARVALHO et al., 2014). No entanto essas culturas são amplamente afetadas pela presença de plantas daninhas.

As plantas daninhas competem diretamente com as culturas por nutrientes, água, espaço e luz e, também, indiretamente, quando são hospedeiras alternativas para patógenos e insetos, podendo ainda dificultar ou impedir a colheita mecânica (FARIA, et al., 2014). A competição por nutrientes é um dos principais fatores que limitam o crescimento e a produção das plantas cultivadas (CARVALHO et al., 2014).

A competição entre plantas é influenciada pela espécie da planta daninha e sua densidade nas áreas cultivadas (FARIA et al., 2014). As plantas daninhas também podem interferir na produção das culturas pela produção de compostos alelopáticos, que são metabólitos secundários produzidos pelas plantas que interferem no crescimento das plantas competidoras, sendo uma das principais ferramentas no estabelecimento bem-sucedido das plantas daninhas no ambiente (SAFDAR et al., 2014).

A cultura do milho, apesar de ser considerada competitiva por promover um sombreamento intenso do solo, é afetada quando em competição com plantas daninhas, o que ocasiona redução do crescimento e da produtividade dos grãos (MELO, 2012). Estima-se que as perdas na cultura, em função da competição, podem chegar a 85 % no sistema de plantio convencional e até 100 % no sistema de plantio direto (CARVALHO et al., 2011), com reduções de até 90 % do rendimento dos grãos (CARVALHO et al., 2007).

A cultura da soja é também afetada pela interferência de plantas daninhas, acarretando perdas de qualidade e produtividade dos grãos (FIALHO, 2013). Quando as plantas de soja se encontram sob competição com plantas daninhas, a cultura tende, de maneira geral, a incrementar sua altura como forma de aumentar a captação da radiação solar e sombrear as plantas daninhas (SILVA et al., 2009). No entanto, o acúmulo de massa seca, a área foliar e a relação folhas/ramos são reduzidos (SILVA et al., 2009).

A rizosfera é atualmente definida como a região do solo que recebe a influência direta das raízes, possibilitando a proliferação microbiana. Essa região no entorno das

raízes funciona como um habitat dinâmico que apresenta comunidades microbianas complexas, que desempenham muitas funções importantes, tanto em sistemas naturais como em sistemas agrícolas, participando das transformações da matéria orgânica e dos ciclos biogeoquímicos (MELO, 2012).

As raízes das plantas são responsáveis pela incorporação de compostos de pequeno peso molecular que são potencialmente valiosos na rizosfera, permitindo o estabelecimento de interações entre as raízes e os microrganismos presentes no solo. Essas interações podem influenciar positivamente o crescimento da planta por uma variedade de mecanismos (BAIS, 2006).

A identificação das possíveis interações entre plantas e os microrganismos do solo, que podem beneficiar ou prejudicar a convivência entre plantas daninhas e culturas, são de extrema importância para o sucesso da implantação e manutenção da lavoura. O conhecimento de como se dão as interações entre os microrganismos do solo e as plantas, bem como quais são os grupos microbianos predominantes e seus papéis na manutenção e sobrevivência das espécies vegetais, pode contribuir para o sucesso dos programas de manejo de plantas daninhas nas culturas do milho e da soja.

Assim, o objetivo deste estudo foi avaliar a diversidade da comunidade microbiana presente na rizosfera de Zea mays e Glycine max cultivadas em monocultura e em competição com plantas daninhas, e relacioná-la com atividades de promoção de crescimento anteriormente relatadas.

REVISÃO DE LITERATURA

2.1 – PLANTAS DANINHAS E PLANTAS INVASORAS

As espécies de plantas consideradas "invasoras" são designadas como uma pequena porção de espécies exóticas, que quando introduzidas, tornam-se localmente dominantes, com a capacidade de alterar comunidades de plantas associadas às plantas cultivadas (REINHART & CALLAWAY, 2006). No entanto, os termos "plantas invasoras" ou "plantas daninhas" têm sido mais comumente empregados para designar plantas que são consideradas indesejáveis em relação a alguma atividade humana (BRIGHENTI & OLIVEIRA, 2011).

Em um conceito mais amplo, uma espécie é considerada daninha se estiver direta ou indiretamente prejudicando determinada atividade humana, como por exemplo, plantas interferindo no desenvolvimento de culturas comerciais, plantas tóxicas em pastagens, plantas estranhas em jardins, dentre outras (SILVA et al., 2007).

As plantas daninhas existem em elevadas densidades de espécies e possuem grande capacidade competitiva e adaptativa. As principais espécies de plantas daninhas em plantações no Brasil são altamente agressivas, apresentam rápida capacidade de adaptação, estabelecimento e perpetuação (SANTOS et al., 2012). Essas espécies possuem ainda grande capacidade de germinação e produção de sementes, desenvolvimento inicial rápido e sementes que permanecem viáveis por longos períodos de tempo podendo ser dispersas por diversos mecanismos (SILVA et al., 2007; SANTOS et al., 2012).

As plantas daninhas interferem na saúde do homem e em suas atividades, causando sérios prejuízos. Na agricultura, cerca de 20 a 30 % do custo de produção deve-se ao controle de plantas daninhas (SILVA et al., 2007). Outros prejuízos diretos causados pelas plantas daninhas incluem: a redução da qualidade do produto comercial; o parasitismo de fruteiras, milho e plantas ornamentais; impedimento da operação da colheita, como nas infestações severas de corda-de-viola (Ipomoea spp.), por diminuírem a eficiência das máquinas; redução do valor da cultura pela presença de sementes de plantas daninhas, dentre outros. Além desses prejuízos diretos, a presença das plantas daninhas reduz a eficiência agrícola, aumentando os custos da produção (SILVA et al., 2007; BRIGHENTI & OLIVEIRA, 2011).

Em comunidades de plantas já estabelecidas, as plantas daninhas possuem baixa capacidade de competir por recursos do meio tais como nutrientes, luz, água e espaço,

sendo encontradas principalmente em áreas nas quais a vegetação natural foi retirada (BRIGHENTI & OLIVEIRA, 2011). No entanto, as plantas daninhas apresentam maior habilidade no recrutamento de recursos do meio do que as plantas cultivadas (CARVALHO et al., 2007; BRIGHENTI & OLIVEIRA, 2011). Quando em competição, plantas daninhas e culturas utilizam os mesmos recursos e o sucesso de uma sobre a outra vai depender da capacidade da planta em acessá-los. No entanto, essa capacidade competitiva vai depender da espécie vegetal e das associações que esta é capaz de estabelecer com os microrganismos do solo (FIALHO, 2013).

2.2 - COMPETIÇÃO ENTRE ESPÉCIES DE PLANTAS

A competição pode ser definida como a interação negativa que ocorre quando os indivíduos ou plantas competem por recursos ambientais, o que leva à redução do crescimento ou da sobrevivência das espécies menos adaptadas (RIZZARDI & WANDSCHEER, 2014). A habilidade competitiva de uma espécie vegetal está relacionada com a utilização eficiente dos recursos do meio no qual se encontra (RIZZARDI et al., 2001), e a capacidade de manter a produtividade ou suprimir o crescimento das plantas concorrentes (CARVALHO et al., 2011).

As plantas daninhas competem com as culturas por água, luz e nutrientes, além de interferirem na colheita. Dentre as características conferidas às plantas daninhas que as fazem mais eficientes no uso dos fatores do ambiente e se sobressaiam sobre as culturas, estão incluídas: a elevada capacidade de produção de sementes ou outros dissemínulos; a capacidade diferenciada de germinação; o rápido desenvolvimento inicial; a manutenção da viabilidade das sementes no solo por longo tempo; a produção e liberação de substâncias alelopáticas no solo e a adaptação a variadas condições ambientais (SILVA et al., 2007; MELO, 2012).

Por outro lado, culturas comerciais tendem a apresentar baixa capacidade competitiva como consequência dos programas de melhoramento que geraram mudanças expressivas não só na produtividade das principais culturas como também na maior adaptabilidade ao estresse hídrico, maior capacidade de resposta à adubação e maior resistência às pragas e doenças (MELO, 2012). Tais mudanças podem ter levado à seleção de cultivares menos dependentes de processos ecológicos e altamente adaptadas a condições artificiais, resultando no baixo potencial competitivo das culturas (MASSENSSINI, 2014a).

A competição entre plantas daninhas e culturas é responsável por perdas significativas na produtividade agrícola, e depende de vários fatores, tais como a cultivar, o período de competição, as espécies de plantas daninhas envolvidas, densidade da cultura e da planta daninha, condições ambientais, bem como as práticas agrícolas aplicadas (CARVALHO et al., 2007; RYAN et al., 2009; SILVIA et al., 2009; RIZZARDI & WANDSCHEER, 2014). Carvalho et al. (2011), ao avaliarem os efeitos da competição entre três cultivares de milho e seis espécies de plantas daninhas sobre o crescimento inicial e a alocação de matéria seca pelas plantas, observaram que as cultivares de milho testadas apresentaram menor acúmulo de matéria seca quando em competição, sendo que a folha e o caule foram os principais órgãos afetados negativamente, e o grau de interferência variou com a espécie da planta daninha e com as diferentes cultivares de milho.

Aspectos físicos e químicos do ambiente podem alterar a intensidade e o balanço das interações entre as plantas. A disponibilidade de recursos é um dos fatores que mais afeta a competição entre as plantas, uma vez que quanto mais limitante for determinado recurso (água, luz e nutrientes), mais intensa será a competição (BERGER et al., 2008). Os fatores abióticos, além de exercerem papel decisivo na composição das comunidades vegetais, possuem papel indireto no desenvolvimento das interações entre as plantas (MASSENSSINI et al., 2014a). As condições climáticas extremas e a limitação de espaço, aéreo ou subterrâneo, promovido pelas plantas daninhas, podem também afetar o desenvolvimento das espécies cultivadas (SILVA et al., 2007). Além disso, aspectos biológicos do ambiente podem influenciar na intensidade das relações entre as plantas. Fialho (2013), observou que as espécies de Bidens pilosa L. e Eleusine indica (L.) Gaertn. apresentaram maior colonização por fungos micorrízicos quando em competição com Zea mays L. Esse resultado foi atribuído à estratégia competitiva das espécies, sendo que as plantas daninhas podem apresentar interações positivas com diversos grupos microbianos do solo.

Parcela significativa da competição entre as plantas ocorre abaixo da superfície do solo onde as raízes exercem papel fundamental no processo competitivo (RIZZARDI et al., 2001) e na determinação do resultado das interações planta-planta (BEVER, 2003). Por meio da liberação de exsudatos radiculares que possuem diferentes combinações de compostos orgânicos, as plantas promovem alterações na estrutura da comunidade microbiana do solo (BAIS et al., 2006), e os microrganismos do solo apresentam papel importante na determinação da habilidade competitiva de plantas

daninhas e cultivares, podendo alterar os resultados da competição (MASSENSSINI et al., 2014a).

Os organismos presentes no solo possuem efeitos diretos ou indiretos no crescimento das plantas, que podem variar entre efeitos fortemente positivos, que favorecem o crescimento, como a presença de fungos micorrízicos, ou efeitos negativos, como a presença de patógenos (BEVER, 2003). Embora vários estudos tenham demonstrado os efeitos prejudiciais das plantas daninhas à produtividade agrícola, pouca atenção tem sido dada aos aspectos microbiológicos envolvidos.

2.3 - ENVOLVIMENTO DA MICROBIOTA DO SOLO NA COMPETIÇÃO

As plantas exercem forte influência sobre as populações microbianas em suas raízes. Isso pode ser explicado pela capacidade diferenciada que os microrganismos possuem em metabolizar as diferentes fontes de carbono disponíveis (MARSCHNER et al., 2004). Plantas e microrganismos do solo evoluíram concomitantemente, por meio de um sistema de sinalização química, através da secreção de aminoácidos, ácidos orgânicos e açúcares, que favorece o crescimento de microrganismos na rizosfera (SANTOS et al., 2012). Cerca de 5-21 % do carbono orgânico total produzido pela fotossíntese são transferidos para a rizosfera através dos exsudatos radiculares (HAICHAR et al., 2014) e utilizado na manutenção dos microrganismos ali presentes. Assim, os exsudatos radiculares são capazes de afetar a estrutura da comunidade microbiana (BAIS et al., 2006). Além disso, certos componentes dos exsudatos apresentam influência seletiva sobre os microrganismos da rizosfera, afetando negativamente algumas espécies e aumentando a competitividade de outras (MARSCHNER et al., 2004).

Os diversos grupos de microrganismos presentes na rizosfera desempenham papéis importantes que podem influenciar positiva ou negativamente o desenvolvimento das plantas e as interações por elas estabelecidas. O resultado geral das interações planta-planta depende das densidades locais de microrganismos mutualistas presentes no solo (ABBOTT et al., 2015). Os fungos micorrízicos arbusculares, por meio de sua influência direta ou indireta na absorção de nutrientes e na transferência desses às plantas com as quais se associam, também influenciam a invasividade e a habilidade competitiva de certas plantas (SHAH et al., 2008; KLABI et al., 2014). Já foi demonstrado que as relações de competição entre as plantas podem ser influenciadas

tanto pela presença como pelo tipo de fungo micorrízico (SHAH et al., 2008). Além disso, em um estudo realizado com 50 espécies de plantas daninhas, foi observado que 41 delas estavam colonizadas com fungos micorrízicos arbusculares e os autores sugerem que essas associações conferem vantagens competitivas a essas plantas sobre as culturas nos ecossistemas agrícolas (MASSENSSINI, et al., 2014b).

A vantagem competitiva das plantas daninhas sobre as culturas pode ser, em parte, resultante da interação dessas espécies de plantas com diversos grupos de microrganismos do solo (REINHART & CALLAWAY, 2006) e as alterações que as plantas daninhas promovem nas comunidades microbianas do solo a elas associadas, podem resultar na diminuição da interferência com as culturas (MELO, 2012). Essas associações podem proporcionar às espécies vegetais envolvidas maior eficiência no uso dos recursos disponíveis, principalmente água e nutrientes, tornando-as mais aptas a competir com as culturas.

Assim, a estrutura da comunidade microbiana do solo vai mudar como resultado da associação com diferentes espécies de plantas. A planta, ao recrutar uma comunidade microbiana específica para a sua rizosfera, pode favorecer seu próprio crescimento e alterar o crescimento das espécies de plantas competidoras (BEVER, 2003). Nesse contexto, plantas invasoras podem liberar compostos singulares no solo, acarretando a alteração da estrutura e função da comunidade microbiana do solo, o que pode em parte explicar as vantagens conferidas às plantas daninhas sobre as culturas (WOLFE & KLIRONOMOS, 2005).

As relações entre as espécies de plantas e os microrganismos do solo são complexas e as características de adaptabilidade das plantas daninhas podem ser influenciadas Bdiretamente pelos microrganismos do solo (SANTOS et al., 2012). Santos et al. (2012), ao avaliarem o crescimento e o acúmulo de macro- e micronutrientes em oito espécies de plantas daninhas e nas culturas de milho e feijão, usando substrato fumigado com brometo de metila, observaram que as culturas do milho e do feijão foram menos afetadas pela esterilização do solo, se comparado aos efeitos causados nas plantas daninhas. Acredita-se que os processos de melhoramento genético das culturas nos últimos anos contribuíram com esse resultado, demonstrando que estas são menos dependentes das associações com os microrganismos edáficos do solo se comparadas às plantas daninhas.

A competição entre as plantas promove alterações na estrutura da comunidade microbiana do solo, tornando-a diferente de quando as plantas estão em monocultura

(MASSESSINI et al., 2014a). Massenssini (2014) avaliou o impacto da competição entre duas culturas (Zea mays e Glycine max) e três espécies de plantas daninhas (Ageratum conyzoides, Ipomoea ramosissima e Bidens pilosa) sobre a estrutura da comunidade microbiana do solo. Após a determinação dos perfis da comunidade microbiana pelo método do T-RFLP, o autor concluiu que a estrutura da comunidade de microrganismos do solo nos tratamentos de competição difere significativamente do observado nas monoculturas. Além disso, foi possível verificar que a espécie de planta daninha A. conyzoides é fraco competidor, dependente de alta biodiversidade microbiana para ótimo crescimento, enquanto a cultura de Z. mays foi considerada forte competidor, com baixa dependência da biodiversidade microbiana do solo. Foi observado ainda que existe maior tendência de interações entre as raízes das plantas com bactérias, se comparadas aos outros grupos microbianos.

Nesse contexto, a comunidade microbiana do solo assume papel central no estabelecimento das interações entre culturas e plantas daninhas, sendo capaz de alterar a habilidade competitiva das espécies. Isso demonstra que o manejo das populações microbianas do solo deve ser levado em conta nos programas de controle de plantas daninhas e no estabelecimento de novos modelos agrícolas. Diante disso, existem ainda lacunas no conhecimento dos grupos microbianos que estão presentes na rizosfera de plantas em competição, das funções ecológicas desses microrganismos e de como eles influenciam as interações entre plantas no ambiente agrícola.

2.4 – O ESTUDO DA DIVERSIDADE MICROBIANA

O estudo das comunidades microbianas, das interações que são capazes de estabelecer com as plantas e dos efeitos da alteração sobre o estabelecimento, a adaptabilidade e, também, a capacidade competitiva, é importante para o entendimento da dinâmica dos ecossistemas agrícolas. A ecologia microbiana baseia-se no conhecimento da composição e da estrutura das comunidades microbianas como base para o entendimento dos seus papéis e das suas funções ecológicas (NELSON et al., 2014). Os métodos independentes de cultivo, que permitem o acesso a espécies microbianas que não podem ser cultivadas em laboratório, apresentam grande impacto sobre o entendimento das comunidades microbianas no ambiente.

Os avanços científicos e tecnológicos, incluindo os novos procedimentos de extração de ácidos nucléicos e o sequenciamento de nova geração (SNG), permitem análises da diversidade da comunidade microbiana, da sua abundância e das suas

funções no ambiente (PYLRO et al., 2014), caracterizando enorme avanço no acesso e no estudo dos recursos biológicos nos últimos anos. O grande desenvolvimento oferecido pelas tecnologias de SNG é a capacidade de produzir grandes volumes de dados de forma mais barata, gerando até milhões de leituras curtas por corrida (METZKER, 2010).

O sequenciamento de alto rendimento realizado na plataforma Illumina fornece maior cobertura da comunidade microbiana com menor custo por sequência, (CAPORASO et al., 2012; SCHMIDT et al., 2013; KOZICH et al., 2013) e taxas de erros inferiores (GLOOR et al., 2010; NELSON et al., 2014), quando comparado com outras plataformas (ex.: plataforma de pirossequenciamento 454). Essa tecnologia é capaz de gerar cinco milhões de leituras com cerca de 250 pb cada em uma única corrida (CAPORASO et al., 2012; KOZICH et al., 2013). Essa abordagem tem sido bastante usada para estudar a comunidade microbiana a partir de diversas amostras (MAUGHAN et al., 2012; SANTOS & BICALHO, 2012; MA et al., 2015; RODRIGUES et al., 2015; ZHANG et al., 2015), mas não há relato do uso do SNG para o estudo da comunidade microbiana associada às raízes de plantas em competição.

As tecnologias de sequenciamento desenvolvem-se rápida e continuadamente, gerando acúmulo de dados (KIM et al., 2013) que, muitas vezes, não é acompanhado pelo aumento do processamento e pela criação de algoritmos para análise dos dados. Para sanar o problema da interpretação dos dados de sequenciamento, o QIIME (Quantitative Insights Into Microbial Ecology Software) (CAPORASO et al., 2010), fornece uma ampla gama de análises e visualizações da comunidade microbiana em estudo. Essa plataforma robusta oferece análises de network, histogramas e interfaces gráficas interativas, visando facilitar a caracterização e a obtenção rápida de novos conhecimentos sobre as comunidades microbianas.

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CHAPTER 1

MICROBIAL DIVERSITY IN THE RHIZOSPHERE OF PLANTS IN COMPETITION

Abstract

Plants have a strong influence on the microbial populations associated with their roots, promoting changes in the soil microbial community by releasing root exudates. When two plants are under competition, the structure of the rhizospheric microbial community differs from that observed in monocultures. Several groups of microorganisms present in the rhizosphere play important roles that can affect positively or negatively the growth of plants and the interactions established by them. The aim of this work was to evaluate the microbial taxa present in the rhizosphere of plants under competition and in monoculture and relate them with growth-promoting activities already reported. The experiments were carried out under greenhouse conditions, at the Department of Microbiology, Universidade Federal de Viçosa. Two crop species, Zea mays L. e Glycine max (L.) Merr., and three weed species, Ageratum conyzoides L., Ipomoea ramosissima (Poir.) Choisy, e Bidens pilosa L., were evaluated. The DNA from cells present in the rhizospheric soil was extracted, following PCR amplification and sequencing using the Illumina MiSeq platform. Our results show that the most abundant phyla of Bacteria were Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia and Cloroflexi. Crenarchaeota was the most abundant phylum of Archaea and Ascomycota the most abundant phylum within Fungi. The Glomeromycota phylum, which comprises all mycorrhizal fungi, was also reported. These fungi have important ecological roles, including the promotion of plant growth. The co-culture of crops and weeds caused changes in the rhizospheric microbial communities that may interfere in the establishment of the plant-plant interaction, affecting their competitive ability.

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Keywords: Crop, Weeds, Competition, Microbial Diversity, Archaea, Bacteria, Fungi, Illumina MiSeq sequencing.

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Introduction

Competition between weeds and crops is responsible for significant losses in agricultural production (RYAN et al., 2009; SILVIA et al., 2009; CARVALHO et al., 2011; RIZZARDI & WANDSCHEER, 2014). When two plants grow together, they compete for environmental resources and the success of one over the other will depend on the plant's ability to gain access to such resources. Generally, the weeds have greater ability to explore environmental resources than crops (CARVALHO et al., 2007; BRIGHENTI & OLIVEIRA, 2011). Competition results in a decrease of growth and survival of the less adapted species (RIZZARDI & WANDSCHEER, 2014) and the competitive capacity of plants depends on the plant species and the associations established with soil microorganisms (FIALHO, 2013).

The structure and dynamics of plant communities are affected by the direct impact of a wide range of biotic and abiotic factors. It is believed that biotic interactions that occur below ground have an effect equal to, if not greater than, those above ground in the outcome of plant-plant interactions (BEVER, 2003). The roots of plants are important players in competition (RIZZARDI et al., 2001), through root exudates, plants can affect the structure of the microbial community of rhizosphere (MARSCHNER et al., 2004, BAIS et al., 2006; HAICHAR et al., 2014).

Soil microganisms play key roles in ecological processes, such as the biogeochemical transformations of nutrients and the establishment of interdependent relationships between plants (BEVER, 2003; MASSENSSINI et al., 2015). The microorganisms in the rhizosphere have direct and indirect effects on the plants and can influence positively (e.g. mycorrhizal fungi) or negatively (e.g. pathogens) their development, affecting their invasiveness and competitive ability (KLIRONOMOS, 2002; BEVER, 2003; WOLFE & KLIRONOMOS, 2005; REINHART & CALLAWAY, 2006; SHAH et al., 2008; MASSENSSINI et al., 2014a).

The competitive advantage of the weeds over crops may be partly a result of the interaction of these species with different groups of soil microrganisms (REINHART & CALLAWAY, 2006), and the changes that weeds promote in the soil microbial communities associated with them, may result in decreases interference with cultures (MELO, 2012). These plant-microrganism associations can provide a more efficient use of available resources for the plant species involved, especially water and nutrients, improving their ability to compete with crops.

The competition between weeds and crops changes the structure of the soil microbial community, making it different from that observed when plants are in monoculture (MASSESSINI et al., 2014a). Plants can recruit specific microbial communities into their rhizosphere that may favor their own growth and decrease the growth of competing plant species (BEVER, 2003; MASSESSINI et al., 2014a).

The association with arbuscular mycorrhizal fungi can influence the competitive ability and invasiveness of certain plants (SHAH et al., 2008; KLABI et al., 2014). These fungi play either direct or indirect roles on the uptake of nutrients, transferring them to the host plants, besides other growth-promoting activities. The presence of arbuscular mycorrhizal fungi in combination with weeds has also been reported (MASSENSSINI et al., 2014b), raising the hypothesis that these associations can provide competitive advantages for the weeds over crops in agricultural ecosystems.

Several studies have shown the harmful effects of weeds for the agricultural productivity of certain crops, but little attention has been given to microorganisms as modulators of the interactions between them. There is a lack of knowledge on which microbial groups are present in the plant rhizosphere when individuals are under competition, which are the putative ecological roles of the rhizosphere microbial community, and how specific microbial taxa may possibly contribute to the outcome of plant competition. Thus, the aim of this study was to evaluate the microbial diversity present in the rhizosphere of Zea mays and Glycine max grown in monoculture and in competition with weeds and to establish putative growth-promoting activities to the resident microbiota based on previous literature reports.

Materials and Methods

The experiments were conducted under greenhouse conditions at the Department of Microbiology, Universidade Federal de Viçosa, Minas Gerais, Brazil. The DNA samples used for carrying out this study were kindly provided by André Marcos Massenssini (MASSENSSINI, 2014). The experimental design and soil sampling procedures were as described below.

Assembly of the experiment

Two crop species, Zea mays variety DKB 390 VT Pro of Dekalb® and Glycine max variety MG/BR 46 of EMBRAPA, and three weed species, Ageratum conyzoides, Ipomoea ramosissima, and Bidens pilosa, were chosen according to the different interactions that these plants establish with the soil microbial community (SANTOS et al., 2012). The soil used in the experiment was an oxisol collected at an experimental area belonging to the Universidade Federal de Viçosa. The chemical and textural characteristics are shown in Table 1.

The plants were cultivated in plastic pots (29-cm diameter and 26-cm height) containing 10 kg of previously sieved soil, and were maintained for 80 days in the greenhouse during the period of November/2012 to January/2013. The experiment was conducted in a randomized block design with 15 treatments corresponding to 5 monocultures and 10 competition treatments, with three replicates. In the monoculture treatments, the plant species were grown separately, with two individuals per pot. In the competition treatments, two plants of one species were combined with two plants of the competitor species. The seeds were sown at the corners of a square with 15-cm sides marked on the soil surface. After emergence, seedlings were watered with tap water. Then the growth period, the plants were cut at soil level and dried at 65 °C, for 5 days, to the determination of dry weight. The rhizospheric soil, defined as soil that remains attached to the roots after gentle agitation, was collected and stored at - 4 °C until analyses. In the competition treatments, the soil was collected in regions where the roots were in contact with each other. In other words, where there was a rhizosphere common to both species.

To evaluate the composition of the microbial community in the rhizosphere of plants in monoculture and competition, only the treatments involving Zea mays and Glycine max in monoculture and in all combinations with weeds were used.

Table 1: Chemical and textural characteristics of the oxisol collected at an experimental area belonging to the Universidade Federal de Viçosa and used in the experiment (MASSENSSINI, 2014).

pH[1] /	P[2/]	K[2] /	Ca[3] /	Mg[3] /	Al[3+3] /	H+Al[4] /	SB	t	T	V	m	MO[5/]	Prem[6] /
	mg/dm^3	----------	----------	----------	----------	cmol$_c$/dm^3 ----------	----------	----------	----------	-----%	----	dag/kg	mg/L
4,67	1,3	54	1,33	0,36	0,57	7,1	1,83	2,40	8,93	20,5	23,8	3,29	26,2

Coarse sand	Fine sand	Silt	Clay	Textural class
----------	dag/kg	----------		
19	14	5	62	Clay

[1/] pH in water (1:2,5). [2/] Extractor Mehlich-1(RICHARDSON et al., 2009). [3/] Extractor KCl 1 mol L^{-1} (VETTORI, 1969). [4/] Extractor calcium acetate 0,5 mol L^{-1}, pH 7,0 (VETTORI, 1969). [5/] Method of Walkey & Black (JACKSON, 1958). [6/] P concentration in equilibrium solution after 1 h shaking, with 0,01 mol L^{-1} of $CaCl_2$, containing 60 mg L^{-1} of P, soil:solution ratio of 1:10 (ALVAREZ et al., 2000).

Competition Balance Index (C_b)

The Competition Balance Index (C_b) for the evaluated plants was calculated Massenssin (2014), according to Wilson (1988). It was observed that the variation in the competitive ability of the plant species studied depending on the plant combinations. In the treatments Z. mays vs G. max, Z. mays vs B. pilosa, G. max vs B. pilosa, the species showed similar competitive abilities. Already in the treatments Z. mays vs A. conyzoides, Z. mays vs I. ramosissima, G. max vs A. conyzoides, the cultures showed greater competitive abilities than weeds. In treatments of competition between G. max vs I. ramosissima, the weed showed a greater competitive ability (MASSENSSINI, 2014).

DNA extraction and PCR amplification

To determine the composition of the rhizosphere microbial community, soil DNA was extracted using the DNA Isolation PowerSoil® Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) following the manufacturer´s instructions (MASSENSSINI,

2014). The DNA samples were quantified using Qubit® 2.0 Fluorometer (Invitrogen by Life Technologies), lyophilized and sent to the Argonne National Laboratory, USA, where PCR amplification and sequencing using Illumina MiSeq.

The V4 region of 16S rRNA gene was amplified with the specific primers 515F and 806R (Table 2), and the data generated were sufficient to analyze bacteria and archaea. The PCR reaction was prepared to a final volume of 25 µL, containing 10 µL 5x Prime HotMasterMix, 1 µL of each primer (200 pM), 1 µL of total DNA and 12 µL of water for PCR Mobio (Mobio Laboratories Inc., Carlsbad, CA, USA). The PCR was performed under the following conditions: 94 °C for 3 min to denature the DNA, followed by 35 cycles corresponding to 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; with a final extension of 10 min at 72 °C.

The fungal ITS region was amplified with primers ITS2 and ITS1f (Table 2). The PCR conditions were the same as described above for the amplification of the 16S rRNA.

Table 2: Primers used in the PCR reaction for Bacteria, Archeae and Fungi.

NAME OF PRIMER	TARGET REGION	SEQUENCE 5'-3'	REFERENCE
515F	16S rRNA	GTGCCAGCMGCCGCGGTAA	CAPORASO et al., 2012.
806R*	16S rRNA	GGACTACHVGGGTWTCTAAT	CAPORASO et al., 2012.
ITS1f	ITS	CTTGGTCATTTAGAGGAAGTAA	SMITH & PEAY, 2014.
ITS2*	ITS	GCTGCGTTCTTCATCGATGC	SMITH & PEAY, 2014.

*The reverse primers have a barcode with 12 pb as described in CAPORASO et al., 2012.

Sequencing by Illumina MiSeq

The amplicons were quantified using the PicoGreen (Invitrogen, Carlsbad, CA, USA). Different volumes of each amplicon were combined so as to be represented equally. The purification of amplicons was done using the UltraClean® PCR Clean-Up Kit (Mobio Laboratories Inc., Carlsbad, CA, USA), and then quantification was carried out using the Qubit® (Invitrogen by Life Technologies). The molarity of the amplicon

pool was determined and diluted to 2 nM. The amplicons were denatured and then diluted to a final concentration of 6.75 pM for the sequencing by Illumina MiSeq.

Processing and analyses of sequencing data

The bioinformatic analyses were performed using the Quantitative Insights Into Microbial Ecology Software (Qiime v.1.8.0) (CAPORASO et al., 2010; BOKULICH et al., 2013), using the Brazilian Microbiome Project protocol (PYLRO et al., 2014a; 2014b).

For the analyses of 16S rRNA gene sequencing data, the following parameters were used: minimum quality score = 25, minimum length of the sequences = 200, maximum length of the sequence = 1000, no ambiguous base allowed, no mismatches allowed in the primer sequence. Truncated reads shorter than 240 bp were discarded. Sequences with more than 97% similarity were considered the same Operational Taxonomic Unit (OTU), and comparisons were made with the UPARSE v.7.1 (EDGAR, 2013). The chimeric sequences were identified and removed using UCHIME (EDGAR et al., 2011). The taxonomy of each OTU was assigned using the reference database GreenGenes (DESANTIS et al., 2006) by the uclust method.

For the analysis of fungal ITS region sequencing data, only the following parameters differed from the analysis cited for the 16S rRNA: truncated reads shoter than 140 bp were discarded and the taxonomy of each OTU was assigned using UNITE (KÕLJALG et al., 2013) as the reference database by the blast method.

Diversity analysis

Alpha diversity (whithin samples) and beta diversity (between samples) (LOZUPONE et al., 2007) were calculated using the Qiime software. For alpha diversity, three metrics were calculated: Chao, which estimates species richness; observed OTUs, which estimates the amount of OTUs found in each sample; and Shannon index, which estimates species diversity. Rarefaction curves were generated based on the number of observed OTUs in each treatment. For beta diversity, Principal Components Analyses (PCA) were carried out based on the relative abundance of OTU sampling and Principal Coordinates Analysis (PCoA) were used to check the relationship between treatments using the distance matrix UniFrac (unweighted) for 16S rRNA (LOZUPONE et al., 2011), and Bray Curtis metric for ITS. Venn diagrams were generated using a webtool (http://bioinformatics.psb.ugent.be/webtools/Venn/) and

clustered hierarchical heatmaps were made using GENESIS software (STURN et al., 2002).

Results

Sequencing results

The Illumina MiSeq sequencing for Zea mays rhizosphere soil in monoculture and in competition with weeds (Table 3) generated 200,408 sequences for bacteria, with an average of 13,360.53 sequences per sample For archaeal 16S rRNA, our results were as follows: 3,062 sequences were obtained with an average of 204.13 sequences per sample. For the fungal ITS region, 528,560 sequences were recorded, with an average of 35,237.33 per sample. For Glycine max, soil samples in monoculture and in competition with weeds (Table 4) showed the following results: a total of 210,376 sequences with an average of 14,025.07 per sample for the 16S rRNA gene of bacteria; 3,107 sequences for archaeal 16S rRNA gene with an average of 207.133 per sample; for fungal ITS, a total of 371,526 sequences were obtained with an average of 24,768.40 sequences per sample.

Table 3: Average of total reads, OTUs, indices of diversity and % coverage for each treatment of rhizosphere in soils cultivated with Zea mays in monoculture and in competition weeds. The results for each treatment comprises the three replicates.

Bacteria	**Total reads**	**OTUs[a]**	**Chao[a,b]**	**Shannon[a,c]**	**% Coverage[d]**
Zea mays	12,732.67	1,074.33	1,235.06	8.34	98.04
Z. mays vs G. max	17,474.50	1,145.00	1,238.40	8.42	98.95
Z. mays vs I. ramosissima	14,178.67	955.00	1,037.01	8.40	98.96
Z. mays vs A. conyzoides	14,838.33	1,188.67	1,336.08	8.39	98.15
Z. mays vs B. pilosa	15,875.00	1,071.67	1,239.00	8.27	98.18

Archaea	**Total reads**	**OTUs[a]**	**Chao[a,b]**	**Shannon[a,c]**	**% Coverage[d]**
Zea mays	186.67	51.00	61.61	4.73	91.79
Z. mays vs G. max	322.50	63.50	74.68	5.15	95.03
Z. mays vs I. ramosissima	199.00	39.00	45.95	4.40	96.50
Z. mays vs A. conyzoides	221.33	64.00	76.92	5.19	91.54
Z. mays vs B. pilosa	198.67	47.00	59.34	4.47	93.33

Fungi	**Total reads**	**OTUs[a]**	**Chao[a,b]**	**Shannon[a,c]**	**% Coverage[d]**
Zea mays	30,988.00	214.67	232.37	5.59	99.95
Z. mays vs G. max	17,092.00	80.00	84.67	4.23	99.95
Z. mays vs I. ramosissima	15,987.67	106.00	124.78	4.90	99.88
Z. mays vs A. conyzoides	45,614.67	252.33	270.63	5.82	99.96
Z. mays vs B. pilosa	72,151.33	302.00	323.25	5.54	99.97

[a] 97 % identity used to define operational taxonomic unit (OTU).
[b] Non-parametric estimator used to predict species richness (total number of OTUs presents), based in singletons and doubletons.
[c] Diversity index that indicates species richness, considering the abundance of individual taxa. A higher number indicate more diversity.
[d] Estimate of the proportion of the total diversity sampled (estimate phylotypes representation in the samples).

Table 4: Average of total reads, OTUs, indices of diversity and % coverage for each treatment of rhizosphere in soils cultivated with Glycine max in monoculture and in competition weeds. The results for each treatment comprises the three replicates.

Bacteria	**Total reads**	**OTUs[a]**	**Chao[a,b]**	**Shannon[a,c]**	**% Coverage[d]**
G. max	11,836.67	1,046.33	1,256.50	8.26	97.98
G. max vs Z. mays	17,485.50	1,142.00	1,235.85	8.43	98.97
G. max vs I. ramosissima	13,459.00	1,070.00	1,175.95	8.49	98.74
G. max vs A. conyzoides	15,401.67	1,002.67	1,099.75	8.26	98.79
G. max vs B. pilosa	17,770.00	1,151.33	1,263.64	8.35	98.90

Archaea	**Total reads**	**OTUs[a]**	**Chao[a,b]**	**Shannon[a,c]**	**% Coverage[d]**
G. max	156.00	43.00	53.74	4.34	89.35
G. max vs Z. mays	318.00	65.50	74.66	4.93	94.41
G. max vs I. ramosissima	171.67	42.67	48.04	4.18	90.22
G. max vs A. conyzoides	218.00	41.67	46.40	4.09	94.89
G. max vs B. pilosa	278.00	56.67	67.48	4.48	92.47

Fungi	**Total reads**	**OTUs[a]**	**Chao[a,b]**	**Shannon[a,c]**	**% Coverage[d]**
G. max	37,558.00	189.33	201.33	5.21	99.96
G. max vs Z. mays	17,028.00	76.00	87.25	4.16	99.90
G. max vs I. ramosissima	25,164.33	212.00	245.86	5.58	99.94
G. max vs A. conyzoides	19,142.67	142.33	151.89	5.38	99.96
G. max vs B. pilosa	30,575.00	211.00	228.82	5.25	99.94

[a] 97 % identity used to define operational taxonomic unit (OTU).
[b] Non-parametric estimator used to predict species richness (total number of OTUs presents), based in singletons and doubletons.
[c] Diversity index that indicates species richness, considering the abundance of individual taxa. A higher number indicate more diversity.
[d] Estimate of the proportion of the total diversity sampled (estimate phylotypes representation in the samples).

Richness and diversity analyses of OTUs

Archaea was the less abundant group in the rhizosphere of Zea mays and Glycine max in monoculture or in competition with weeds, followed by fungi (Table 3 and 4). In all treatments, the largest number of OTUs registered was those corresponding to bacteria.

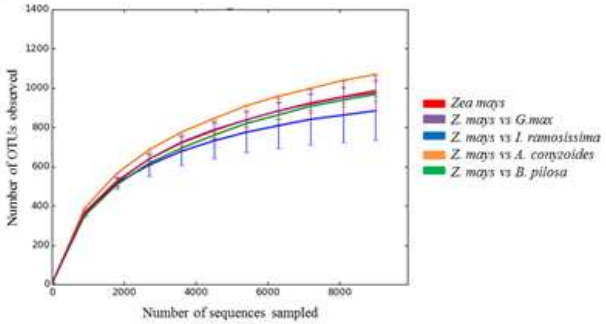
The coverage values showed that most phylotypes present in the soil were assessed during sampling, with values ranging from 89.35 % to 99.96 % (Table 3 and 4).

For bacteria and fungi in the rhizosphere of Zea mays and Glycine max, either in monoculture or in competition with weeds, the rarefaction curves reached a plateau, indicating that the sequencing was reasonable to reveal the total number of types of sequences in the samples (Fig. 1). For archaea, however, the rarefaction curves showed that more sequences would be necessary to retrieve all the sequence types present to better express diversity within the treatments (Fig. 1).

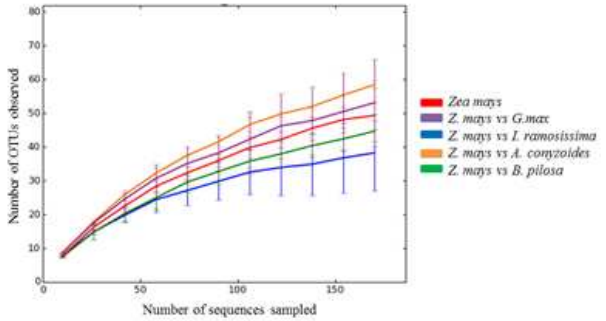
The largest number of bacterial and archaeal OTUs were observed in the rhizosphere of Z. mays in competition with A. conyzoides and the lowest was found in the soil cultivated with Z. mays vs I. ramosissima. The number of fungal OTUs (Fig. 1c) was highest in the Z. mays rhizosphere in competition with Bidens pilosa and lowest in soil from the Z. mays vs G. max. The OTU numbers for bacteria in Glycine max rhizosphere in monoculture or in competition with weeds was higher for G. max in competition with B. pilosa and lowest for G. max vs A. conyzoides (Fig. 1d). For archaea, the highest OTU numbers were found in the soil from G. max in competition with Z. mays and the smallest OTU number was observed for G. max vs A. conyzoides soil (Fig. 1e). The number of fungal OTUs (Fig. 1f) was higher in soil from G. max in competition with I. ramosissima and lowest for G. max vs Z. mays.

Fig. 1 Rarefaction curves for different treatments calculated based on OTUs defined as 97 % similarity level. **(a)** Rarefaction curves for Bacteria OTUs for soil treatments from Zea mays in monoculture and in competition with weeds **(b)** Archaea in Z. mays. **(c)** Fungi in Z. mays. **(d)** Bacteria in Glycine max. **(e)** Archaea in G. max. **(f)** Fungi in G. max.

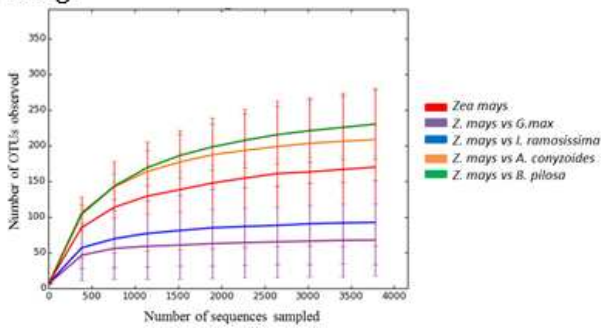
(a) Bacteria



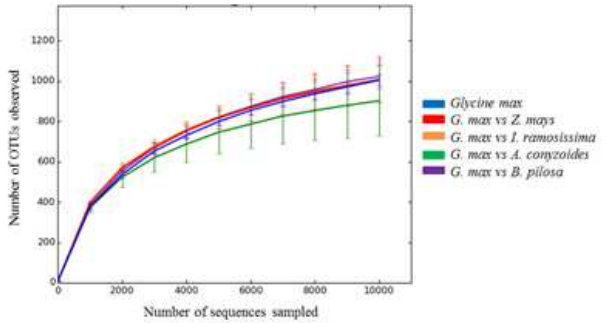
(b) Archaea



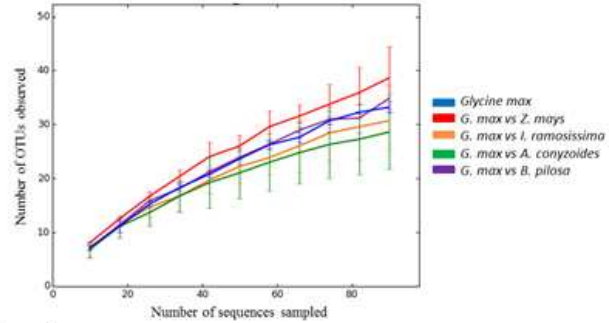
(c) Fungi



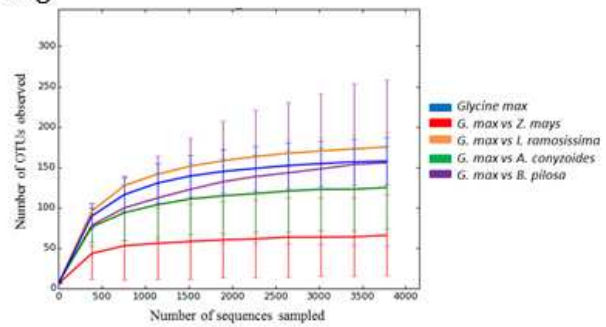
(d) Bacteria



(e) Archaea



(f) Fungi



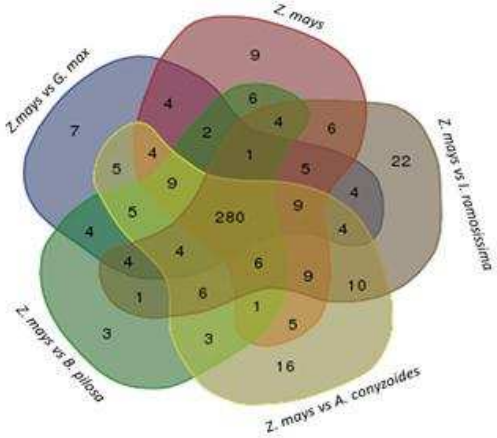
The Chao index, which represents the total number of species, or the total number of OTUs present in each treatment, based on singletons and doubletons (HUGHES et al., 2001), demonstrated, as expected, that all soil treatments that showed high OTU counts were also those which had high Chao indices (Table 3 and 4). Both for monocultures as well as for the competition treatments with weeds, the highest Chao indices were observed for bacteria, followed by fungi, and archaea. In the rhizosphere of Zea mays, the Chao index ranged from 45.95 to 1,336.08 (Table 3) while in the rhizosphere Glycine max, the values ranged from 46.40 to 1,263.64 (Table 4).

The Shannon diversity index, which indicates species richness, considering the number of taxa and the abundance of individual taxa (CHIARUCCI et al., 2011), is shown in Table 3 and Table 4. The Shannon diversity index range from 4.23 to 8.42 in the rhizosphere of Zea mays and from 4.09 to 8.49 in the rhizosphere Glycine max.

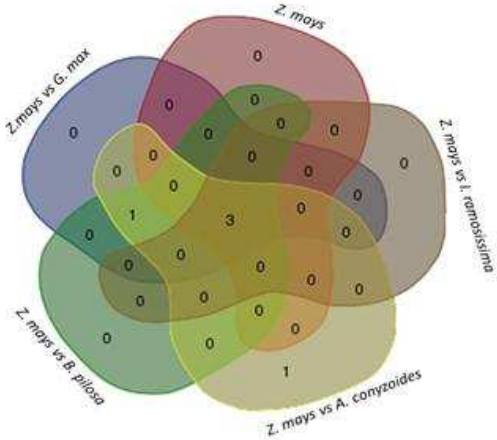
For soil treatments from Zea mays, 280 (Fig. 2a), 3 (Fig. 2b) and 44 (Fig. 2c) bacterial, archaeal and fungal OTUs, respectively, were shared among all tratments. The number of exclusive OTUs for rhizosphere soil varied according to the treatment. The largest number of unique OTUs was 22, observed in the competition treatments Z. mays vs Ipomoea ramosissima for bacteria and, for fungi, in the competition treatment between Z. mays vs Bidens pilosa (Fig. 2a, 2c). The soil treatments from Glycine max showed 252 bacterial OTUs shared among all treatments (Fig. 2d), 3 archaea OTUs shared among all the treatments (Fig. 2e), and 44 OTUs fungi present in all treatments (Fig. 2f). The taxonomy allocated for the unique OTUs for each rhizosphere soil treatment is shown in Table 5.

Fig. 2 Venn diagram of OTUs from Bacteria, Archaea and Fungi in the soil treatments from Zea mays and Glycine max in monoculture and in competition with weeds. **a)** Bacteria in Z. mays. **b)** Archaea in Z. mays. **(c)** Fungi in Z. mays. **(d)** Bacteria in G. max. **(e)** Archaea in G. max. **(f)** Fungi in G. max. Unique and shared OTUs among treatments are based on 97 % similarity. The numbers inside the diagram indicate the numbers of OTUs.

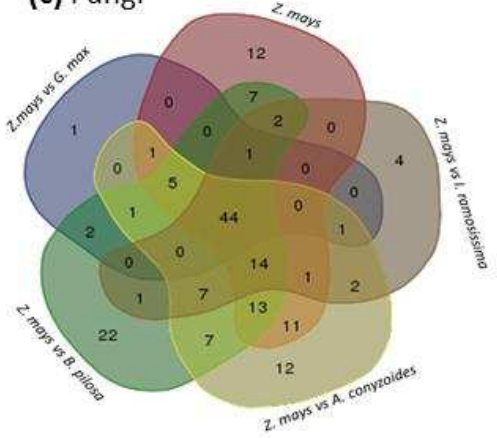
(a) Bacteria



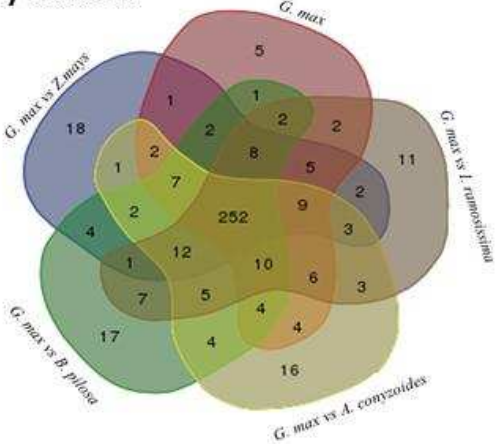
(b) Archaea



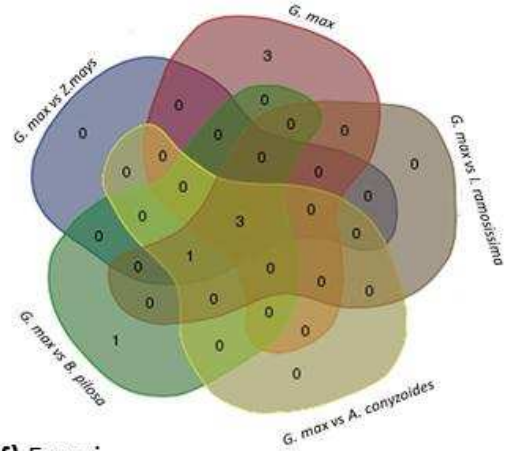
(c) Fungi



(d) Bacteria



(e) Archaea



(f) Fungi

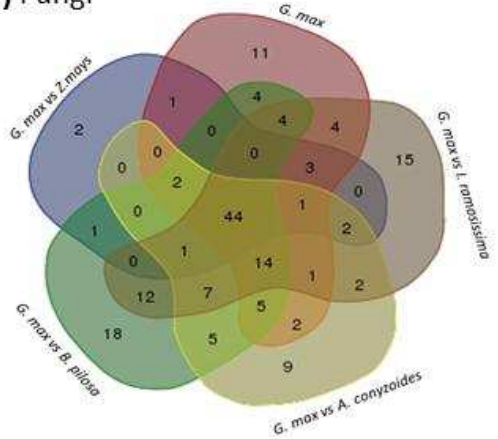


Table 5: Unique OTUs of Bacteria, Archaea and Fungi present in each soil treatments[a].

Bacteria

Zea mays
Acidobacteria; PAUC37f
Actinobacteria; Actinobacteria; Actinomycetales; Micrococcaceae; Rothia
Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; Sporocytophaga
Chloroflexi; Anaerolineae; SJA-15
Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Dorea
Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella
Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Planctomycetes
Proteobacteria; Alphaproteobacteria; Rickettsiales; Rickettsiaceae; Rickettsia

Z. mays vs Glycine max
Actinobacteria; Actinobacteria; Actinomycetales; Nocardioidaceae; Other
Actinobacteria; Rubrobacteria; Rubrobacterales; Rubrobacteraceae; Rubrobacter
Armatimonadetes; OPB50
Chlamydiae; Chlamydiia; Chlamydiales; Parachlamydiaceae
Gemmatimonadetes; Gemm-2
Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Azospira
Verrucomicrobia; [Pedosphaerae]; [Pedosphaerales]; Other; Other

Z. mays vs I. ramosíssima
Acidobacteria; Acidobacteria-6; iii1-15; mb2424
Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; Cytophaga
Bacteroidetes; Cytophagia; Cytophagales; [Amoebophilaceae]; Candidatus amoebophilus
Bacteroidetes; VC2_1_Bac22
Bacteroidetes; [Saprospirae]; [Saprospirales]; Chitinophagaceae; Niabella
Chlorobi; OPB56
Cyanobacteria; Nostocophycideae; Nostocales; Nostocaceae; Anabaena
Cyanobacteria; Nostocophycideae; Stigonematales; Rivulariaceae; Calothrix
Firmicutes; Clostridia; Clostridiales; Peptococcaceae
OP11; OP11-3
Proteobacteria; Alphaproteobacteria; Rhizobiales; Methylocystaceae; Pleomorphomonas
Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae; Aminobacter
Proteobacteria; Alphaproteobacteria; Rhodobacterales; Hyphomonadaceae
Proteobacteria; Alphaproteobacteria; Rhodospirillales
Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Other
Proteobacteria; Betaproteobacteria; A21b; UD5
Proteobacteria; Deltaproteobacteria; NB1-j; NB1-i
Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae;
Proteobacteria; Gammaproteobacteria; Xanthomonadales;Xanthomonadaceae; Lysobacter
TM6; SJA-4; S1198
Verrucomicrobia; [Methylacidiphilae]; S-BQ2-57

[Thermi]; Deinococci; Deinococcales; Trueperaceae; Truepera

Z. mays vs A. conyzoides

Acidobacteria; Acidobacteriia; Acidobacteriales; Acidobacteriaceae; Terriglobus
Acidobacteria; S035
Actinobacteria; Actinobacteria; Actinomycetales; Nocardiaceae; Nocardia
Bacteroidetes; Flavobacteriia; Flavobacteriales; Cryomorphaceae; Fluviicola
Chlamydiae; Chlamydiia; Chlamydiales; Parachlamydiaceae; Parachlamydia
Chloroflexi; Anaerolineae; WCHB1-50
Chloroflexi; TK17; mle1-48
Firmicutes; Clostridia; Clostridiales
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillospira
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus
Firmicutes; Clostridia; Clostridiales; Veillonellaceae
Nitrospirae; Nitrospira; Nitrospirales; Nitrospiraceae; JG37-AG-70
Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae
Proteobacteria; Deltaproteobacteria; FAC87
WS3; PRR-12; Sediment-1
WS3; PRR-12; Sediment-1; PRR-10

Z. mays vs B. pilosa

Actinobacteria; Actinobacteria; Actinomycetales; Streptosporangiaceae
Actinobacteria; Actinobacteria; Actinomycetales; Thermomonosporaceae; Other
Verrucomicrobia; [Spartobacteria]; [Chthoniobacterales]; [Chthoniobacteraceae]; OR-59

Archaea

Z. mays vs A. conyzoides

Euryarchaeota; Thermoplasmata; E2

Fungi

Z. mays

Ascomycota; Dothideomycetes; Botryosphaeriales; Aplosporellaceae; Aplosporella
Ascomycota; Dothideomycetes; Pleosporales; Other; Other
Ascomycota; Dothideomycetes; Pleosporales; Phaeosphaeriaceae; Other
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Edenia
Ascomycota; Dothideomycetes; Pleosporales; Tubeufiaceae; Helicosporium
Ascomycota; Eurotiomycetes; Chaetothyriales; Herpotrichiellaceae; Phialophora
Ascomycota; Sordariomycetes; Hypocreales; Incertae sedis; unidentified
Ascomycota; Sordariomycetes; Xylariales; Xylariaceae; Calceomyces
Basidiomycota; Agaricomycetes; Cantharellales; Botryobasidiaceae; unidentified
Basidiomycota; Agaricomycetes; Cantharellales; Ceratobasidiaceae; unidentified
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; Tomentella
Zygomycota; Incertae sedis; Mucorales; Umbelopsidaceae; Umbelopsis

Z. mays vs G. max

Ascomycota; Dothideomycetes; Pleosporales; Lophiostomataceae; Lophiostoma

Z. mays vs I. ramosíssima

Ascomycota; Sordariomycetes; Diaporthales; Schizoparmaceae; Coniella
Ascomycota; Sordariomycetes; Hypocreales; Clavicipitaceae; unidentified
Ascomycota; Sordariomycetes; Hypocreales; Incertae sedis; Acremonium
Basidiomycota; Agaricomycetes; Polyporales; Polyporaceae; Other

Z. mays vs A. conyzoides

Ascomycota; Dothideomycetes; Capnodiales; Teratosphaeriaceae; Devriesia
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Curvularia
Ascomycota; Orbiliomycetes; Orbiliales; Orbiliaceae; Arthrobotrys
Ascomycota; Sordariomycetes; Chaetosphaeriales; unidentified; unidentified
Ascomycota; Sordariomycetes; Diaporthales; Togniniaceae; Phaeoacremonium
Ascomycota; Sordariomycetes; Hypocreales; Other; Other
Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; Xenocylindrocladium
Ascomycota; Sordariomycetes; Incertae sedis; Incertae sedis; Myrmecridium
Basidiomycota; Agaricomycetes; Polyporales; Meruliaceae; Phlebia
Basidiomycota; Agaricomycetes; Polyporales; unidentified; unidentified
Basidiomycota; Agaricomycetes; unidentified; unidentified; unidentified
Glomeromycota; Glomeromycetes; Glomerales; Glomeraceae; unidentified

Z. mays vs B. pilosa

Ascomycota; Dothideomycetes; Capnodiales; unidentified; unidentified
Ascomycota; Dothideomycetes; Incertae sedis; Pseudeurotiaceae; Leuconeurospora
Ascomycota; Dothideomycetes; Patellariales; Patellariaceae; Rhytidhysteron
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Other
Ascomycota; Dothideomycetes; Pleosporales; Tetraplosphaeriaceae; Tetraplosphaeria
Ascomycota; Dothideomycetes; Pleosporales; Tubeufiaceae; Other
Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Other
Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Talaromyces
Ascomycota; Incertae sedis; Incertae sedis; Incertae sedis; Thelonectria
Ascomycota; Leotiomycetes; Helotiales; Other; Other
Ascomycota; Leotiomycetes; Helotiales; Incertae sedis; Scytalidium
Ascomycota; Sordariomycetes; Chaetosphaeriales; Chaetosphaeriaceae; Codinaeopsis
Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; Cosmospora
Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; unidentified
Ascomycota; Sordariomycetes; Incertae sedis; Annulatascaceae; Conlarium
Ascomycota; Sordariomycetes; Incertae sedis; Glomerellaceae; Colletotrichum
Ascomycota; Sordariomycetes; Microascales; Incertae sedis; Cephalotrichiella
Ascomycota; Sordariomycetes; Xylariales; unidentified; unidentified
Basidiomycota; Agaricomycetes; Boletales; Sclerodermataceae; Pisolithus

Basidiomycota; Agaricomycetes; Polyporales; Ganodermataceae; Amauroderma
Basidiomycota; Agaricomycetes; Polyporales; Ganodermataceae; Amauroderma
Basidiomycota; Tremellomycetes; Filobasidiales; Filobasidiaceae; Cryptococcus

Bacteria

G. max

Actinobacteria; Rubrobacteria; Rubrobacterales; Rubrobacteraceae; Rubrobacter
Armatimonadetes; SJA-176; RB046
Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; Adhaeribacter
Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Azospira
Proteobacteria; Deltaproteobacteria; Bdellovibrionales; Bacteriovoracaceae

G. max vs Z. mays

Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae; Actinomyces
Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae
Bacteroidetes; Bacteroidia; Bacteroidales; S24-7
Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; Flectobacillus
Chlorobi; BSV26; PK329
Chloroflexi; TK10
Firmicutes; Bacilli; Bacillales; Bacillaceae
Firmicutes; Clostridia; Clostridiales; Other; Other
Firmicutes; Clostridia; Clostridiales; Christensenellaceae
Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Pseudobutyrivibrio
Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Sporomusa
Fusobacteria; Fusobacteriia; Fusobacteriales
Proteobacteria; Alphaproteobacteria; Rhizobiales; Other; Other
Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Dechloromonas
Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Desulfuromonadaceae
Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophaceae; Desulfobacca
Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Moraxella

G. max vs I. ramisissima

Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; Emticicia
Bacteroidetes; Flavobacteriia; Flavobacteriales; Cryomorphaceae; Fluviicola
Cyanobacteria; Chloroplast; Chlorophyta; Trebouxiophyceae; Coccomyxa
Proteobacteria; Alphaproteobacteria; Rhizobiales; Bradyrhizobiaceae; Other
Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; Other
Proteobacteria; Alphaproteobacteria; Rickettsiales; mitochondria; Vermamoeba
Proteobac; Betaproteobac; Burkholderiales; Burkholderiaceae; Candidatus glomeribacter
Proteobacteria; Deltaproteobacteria; NB1-j; NB1-i
Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae; Coxiella
Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae
Verrucomic; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae; Luteolibacter

G. max vs A. conyzoides

Actinobacteria; Thermoleophilia; Gaiellales; AK1AB1_02E
Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; Rudanella
Bacteroidetes; Cytophagia; Cytophagales; [Amoebophilaceae]; Candidatus amoebophilus
Chlamydiae; Chlamydia; Chlamydiales; Rhabdochlamydiaceae; C. rhabdochlamydia
Chloroflexi; Anaerolineae; Anaerolineales; Anaerolinaceae; Anaerolinea
Chloroflexi; Anaerolineae; SBR1031; A4b
Chloroflexi; TK17; mle1-48
Cyanobacteria; Synechococcophycideae; Pseudanabaenales
Elusimicrobia; Endomicrobia
Firmicutes; Bacilli; Lactobacillales; Leuconostocaceae
Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Sporotomaculum
Gemmatimonadetes; Gemm-3
Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhodobiaceae; Afifella
Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Other
Proteobacteria; TA18; CV90
Verrucomicrobia; Opitutae; [Cerasicoccales]; [Cerasicoccaceae];

G. max vs B. pilosa

Acidobacteria; EC1113
Acidobacteria; S035
Actinobacteria; Actinobacteria; Actinomycetales; Microbacteriaceae; Agromyces
Chloroflexi; Anaerolineae; SBR1031; oc28
Elusimicrobia; Elusimicrobia; MVP-88
Firmicutes; Bacilli; Turicibacterales; Turicibacteraceae; Turicibacter
Firmicutes; Clostridia; Clostridiales; Clostridiaceae
Firmicutes; Clostridia; Clostridiales; Veillonellaceae; BSV43
MVP-21
OP11
Planctomycetes; Phycisphaerae; Phycisphaerales; Phycisphaeraceae
Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Other
Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae; Other
Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Skermanella
Proteobacteria; Betaproteobacteria; A21b; UD5
Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Enhydrobacter
[Thermi]; Deinococci; Deinococcales; Deinococcaceae; Deinococcus

Archaea

G. max

Crenarchaeota; MCG; pGrfC26
Euryarchaeota; Methanomicrobia; Methanomicrobiales; Methanospirillaceae; Methanospirillum
Euryarchaeota; Methanomicrobia; Methanosarcinales; Methanosarcinaceae; Methanolobus

G. max vs B. pilosa

Euryarchaeota; Thermoplasmata; E2

Fungi

G. max

Ascomycota; Dothideomycetes; Capnodiales; Mycosphaerellaceae; unidentified
Ascomycota; Dothideomycetes; Capnodiales; unidentified; unidentified
Ascomycota; Dothideomycetes; Pleosporales; Massarinaceae; Massarina
Ascomycota; Dothideomycetes; Pleosporales; Phaeosphaeriaceae; Ophiosphaerella
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Cochliobolus
Ascomycota; Sordariomycetes; Chaetosphaeriales; Chaetosphaeriaceae; Sporoschisma
Ascomycota; Sordariomycetes; Incertae sedis; Annulatascaceae; Vertexicola
Ascomycota; Sordariomycetes; Microascales; Microascaceae; Scedosporium
Ascomycota; Sordariomycetes; Ophiostomatales; Ophiostomataceae; Sporothrix
Ascomycota; Sordariomycetes; Xylariales; Diatrypaceae; Other
Basidiomycota; Agaricomycetes; Polyporales; Polyporaceae; Grammothele

G. max vs Z. mays

Ascomycota; Eurotiomycetes; Chaetothyriales; Chaetothyriaceae; Cyphellophora
Ascomycota; Sordariomycetes; Hypocreales; Clavicipitaceae; unidentified

G. max vs I. ramisissima

Ascomycota; Dothideomycetes; Other; Other; Other
Ascomycota; Dothideomycetes; Capnodiales; Davidiellaceae; Other
Ascomycota; Dothideomycetes; Capnodiales; Incertae sedis; Capnobotryella
Ascomycota; Dothideomycetes; Capnodiales; Mycosphaerellaceae; Other
Ascomycota; Dothideomycetes; Incertae sedis; Pseudeurotiaceae; Leuconeurospora
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Curvularia
Ascomycota; Eurotiomycetes; Chaetothyriales; Incertae sedis; Coniosporium
Ascomycota; Orbiliomycetes; Orbiliales; Orbiliaceae; Other
Ascomycota; Sordariomycetes; Diaporthales; Diaporthaceae; Other
Ascomycota; Sordariomycetes; Diaporthales; Togniniaceae; Phaeoacremonium
Ascomycota; Sordariomycetes; Sordariales; Lasiosphaeriaceae; Other
Ascomycota; Sordariomycetes; Xylariales; Other; Other
Ascomycota; Sordariomycetes; Xylariales; Diatrypaceae; unidentified
Basidiomycota; Agaricomycetes; Auriculariales; Incertae sedis; Exidia
Basidiomycota; Microbotryomycetes; Sporidiobolales; Incertae sedis; Sporobolomyces

G. max vs A. conyzoides

Ascomycota; Dothideomycetes; Botryosphaeriales; Botryosphaeriaceae; Sphaeropsis
Ascomycota; Dothideomycetes; Capnodiales; Teratosphaeriaceae; Other
Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae; Edenia
Ascomycota; Dothideomycetes; Pleosporales; Sporormiaceae; unidentified
Ascomycota; Leotiomycetes; Helotiales; Incertae sedis; Other
Ascomycota; Sordariomycetes; Diaporthales; Diaporthaceae; unidentified

Basidiomycota; Agaricomycetes; Agaricales; Schizophyllaceae; Schizophyllum
Basidiomycota; Agaricomycetes; Polyporales; Polyporaceae; Coriolopsis
Basidiomycota; Cystobasidiomycetes; Erythrobasidiales; Incertae sedis; Erythrobasidium

G. max vs B. pilosa

Ascomycota; Dothideomycetes; Botryosphaeriales; Botryosphaeriaceae; Neodeightonia
Ascomycota; Dothideomycetes; Capnodiales; Other; Other
Ascomycota; Dothideomycetes; Pleosporales; Phaeosphaeriaceae; Phaeosphaeria
Ascomycota; Dothideomycetes; Pleosporales; Tetraplosphaeriaceae; Tetraplosphaeria
Ascomycota; Incertae sedis; Incertae sedis; Incertae sedis; Phaeoisaria
Ascomycota; Saccharomycetes; Saccharomycetales; Incertae sedis; unidentified
Ascomycota; Sordariomycetes; Chaetosphaeriales; unidentified; unidentified
Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; Xenocylindrocladium
Ascomycota; Sordariomycetes; Sordariales; Cephalothecaceae; unidentified
Ascomycota; Sordariomycetes; Xylariales; Xylariaceae; Other
Basidiomycota; Agaricomycetes; Auriculariales; Incertae sedis; Heterochaete
Basidiomycota; Agaricomycetes; Boletales; Sclerodermataceae; Scleroderma
Basidiomycota; Agaricomycetes; Cantharellales; Botryobasidiaceae; unidentified
Basidiomycota; Agaricomycetes; Polyporales; Meruliaceae; Bjerkandera
Basidiomycota; Agaricomycetes; Polyporales; Polyporaceae; Panus
Basidiomycota; Agaricomycetes; Trechisporales; unidentified; unidentified
Rozellomycota; unidentified; unidentified; unidentified; unidentified
Zygomycota; Incertae sedis; Mortierellales; unidentified; unidentified

[a] OTU identification was generated in the QIIME (CAPORASO et al., 2010; BOKULICH et al., 2013) program by comparing OTUs sequences against the GreenGenes (DESANTIS et al., 2006) (for Bacteria and Archaea) and UNITE (KÕLJALG et al, 2013) (for Fungi) databases.

Taxonomic composition of the microbial community in Zea mays and Glycine max rhizosphere

The OTUs of bacteria corresponding to less than 1 % of the total OTUs were grouped and named as 'Others'. For the taxonomy of Archaea, the term 'Others' represent the OTUs whose taxonomy was not assigned. For fungal OTUs, the term 'Others' included the OTUs that were present in less than 1 % of the total OTUs and OTUs whose taxonomy was not assigned.

The OTUs of bacteria found in the rhizosphere of Zea mays in monoculture and in competition with weeds were classified in 31 different phyla, 181 orders and 430 genera. The dominant phyla (> 1 % of total OTUs) were Proteobacteria (34.7 % of all sequences), Actinobacteria (24.7 %), Acidobacteria (16.3 %), Verrucomicrobia (6.8 %), Cloroflexi (4.1 %), Planctomycetes (2.3 %), AD3 (2.1 %), Firmicutes (1.9 %), Gemmatimonadetes (1.9 %), Bacteroidetes (1.6 %), and Cyanobacteria (1.1 %), making up 97.5% of the abundance of bacterial OTUS (Fig. 3a). The competition treatment between Z. mays vs I. ramosissima presented a more differentiated profile for bacterial OTUs, evidenced by the greater abundance of the phyla Proteobacteria (36.2 %), Cyanobacteria (2.7 %) and Bacteroidetes (3.2 %), and a lower abundance of Acidobacteria (14.0 %) compared to the abundances of the same phyla in the other treatments. Within the phylum Proteobacteria, the most abundant in all treatments, 40 orders were detected. Of these, 6 were present at a frequency higher than 1 %: Rhizobiales (12.2 %), Sphingomonadales (5.3 %), Rhodospirillales (5.1 %), Myxococcales (3.1 %), Xanthomonadales (2.5 %) and Burkholderiales (2.1 %) (Fig. S1, Supplementary data). For the second most abundant bacterial phylum, Actinobacteria, 4 orders were present at a frequency higher than 1 %: Actinomycetales (14.7 %), Gaiellales (4.3 %), Solirubrobacterales (3.8 %), and Acidimicrobiales (1.7 %) (Fig. S2, Supplementary data).

The archeal OTUs present in the treatments of Z. mays in monoculture and in competition with weeds were classified into 3 different phyla. Of these, only the Crenarchaeota phylum (35.7 %) was dominant. The other two phyla were identified Parvarchaeota (0.6 %) and Euryarchaeota (0.1 %) (Fig. 3b). The competition treatment between Z. mays vs A. conyzoides was the only one in which the phylum Euryarchaeota could be detected in the Z. mays rhizosphere. For Z. mays in monoculture and Z. mays vs I. ramosissima treatments the phylum Parvarchaeota could not be found. The OTUs were classified into four different orders: Nitrososphaerales (35.0 %), NRP-J (0.6 %),

YLA114 (0.6 %) and E2 (0.1 %). For most of them (63.7 %), taxonomy could not be assigned (Fig. S3, Supplementary data).

The OTUs of fungi found in the rhizosphere soil from Z. mays in monoculture and in competition with weeds were classified into 6 phyla, 50 orders, and 170 different genera. The dominant phyla were: Ascomycota (64.3 %), Zygomycota (3.5 %), and Glomeromycota (1.1 %), accounting for 68.9 % of the abundance of fungi found (Fig. 3c). The treatment of competition between Z. mays vs B. pilosa showed the highest abundance of OTUs belonging to the Ascomycota phylum (63.3 %), and the competition between Z. mays vs A. conyzoides showed the highest abundance of Glomeromycota (2.2 %) compared to the abundances of these same phyla in the other treatments. Within Ascomycota, the most abundant in all treatments, 31 orders were found. Of these, eight were present in more than 1 %: Hypocreales (33.1 %), Eurotiales (7.4 %), Pleosorales (4.6 %), Sordariales (2.8 %), Trichosphaeriales (2.6 %), Chaetothrytiales (2.2 %), Xylariales (2.2 %) and Incertae sedis (1.2 %) (Fig. S4, Supplementary data). Within the second most abundant fungal phylum, Zygomycota, only one order was found, Mortierellales (3.5 %) (data not shown).

The OTUs of bacteria found in the rhizosphere soil from Glycine max in monoculture and in competition with weeds were classified in 32 different phyla, 177 orders, and 424 genera. The dominant phyla were present at more than 1 % and were: Proteobacteria (34.2 %), Actinobacteria (24.4 %) Acidobacteria (17.5 %), Verrucomicrobia (7.3 %), Cloroflexi (4.1 %), Planctomycetes (2.4 %), AD3 (2.3 %), Gemmatimonadetes (1.8 %), Firmicutes (1.6 %), Bacteriodetes (1.2 %), and Nitrospirae (1.0 %), making up 97.8% of the total abundance of bacteria found (Fig. 3d). Within the Proteobacteria phylum, the most abundant in all treatments, 39 orders were found. The most abundant were: Rhizobiales (12.6 %), Rhodospirillales (5.6 %), Sphingomonadales (4.5 %), Myxococcales (2.9 %), Xanthomonadales (2.3 %) Burkholderiales (1.6 %) and Syntrophobacterales (1.3 %) (Fig. S5, Supplementary data). For the second most abundant phylum of bacteria, Actinobacteria, 4 orders were most abundant: Actinomycetales (14.0 %), Gaiellales (4.6 %), Solirubrobacterales (3.8 %) and Acidimicrobiales (1.8 %) (Fig. S6 Supplementary data).

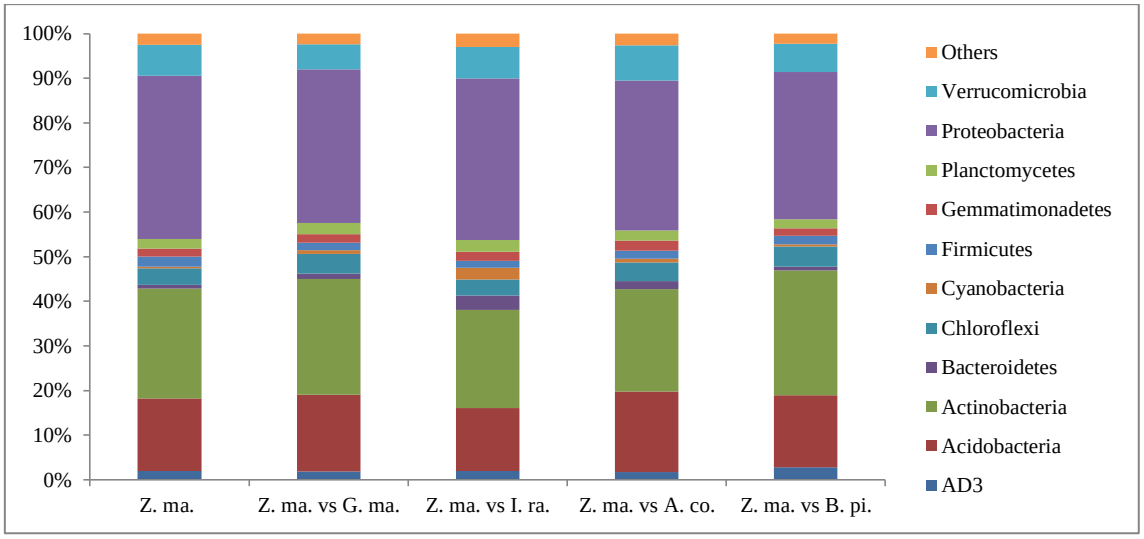
The OTUs of archaea present in the soil treatments of G. max in monoculture and in competition with weeds were classified into three different phyla. Of these, only the Crenarchaeota phylum (40.6 %) was dominant. The other two identified phyla were Euryarchaeota (0.7 %) and Parvarchaeota (0.5 %) (Fig. 3e). The competition treatment

between G. max vs A. conyzoides was the one in which the Crenarchaeota phylum was in greater abundance. In G. max monoculture, the Euryarchaeota phylum was the most abundant and the Parvarchaeota phylum was absent. The OTUs of archaea were classified into 7 different orders: Nitrososphaerales (38.8 %), NRP-J (1.8 %), YLA114 (0.5 %), Methanomicrobiales (0.4 %), Methanosarcinales (0. 2 %), E2 (0.1 %) and pGrfC26 (0.1 %). For most of them (58.3 %), the taxonomy could not assigned (Fig. S7, Supplementary data).

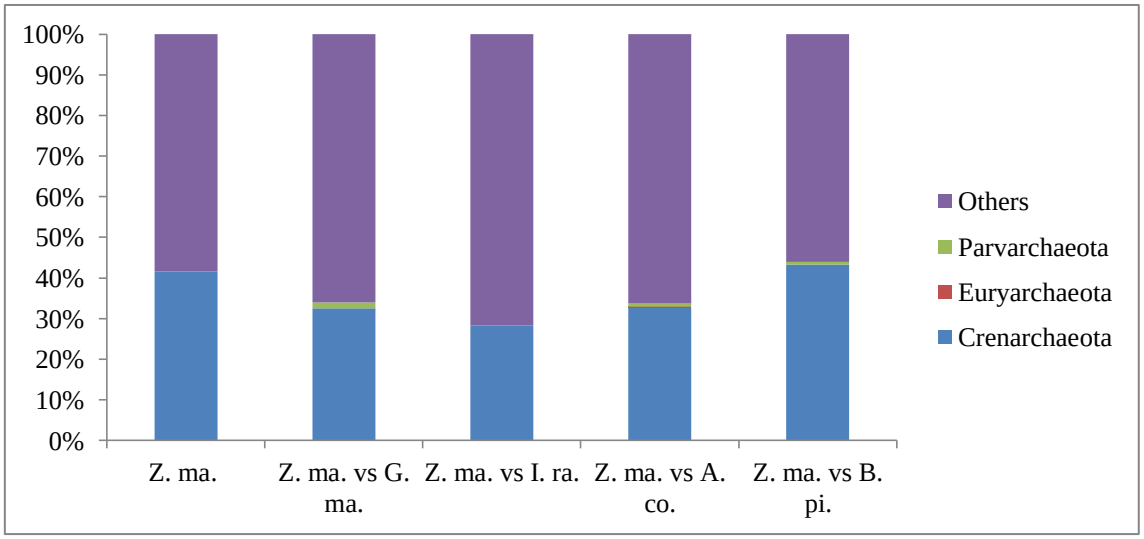
The fungal OTUs were found in the rhizosphere of G. max in monoculture and in competition with weeds and were classified into five phyla, 53 orders, and 168 different genera. Ascomycota (58.5 %), Zygomycota (5.8 %), Glomeromycota (1.5 %), and Basidiomycota (1.2 %), were dominant phyla, making up to 67,0 % of the abundance of fungi found (Fig. 3f). The competition treatment G. max vs Z. mays showed the highest abundance of OTUs belonging to the Ascomycota phylum (63.6 %), and the competition between G. max vs B. pilosa presented the highest abundance of the phylum Zygomycota (8.3 %), compared to the abundances of the same phylum in the other treatments. Within the Ascomycota phylum, the most abundant in all treatments, 31 orders were found. The most abundant orders were: Hypocreales (31.1 %), Eurotiales (5.5 %), Pleosorales (5.1 %), Sordariales (2.7 %), Xylariales (2.4 %), Chaetothrytiales (2.1 %), Incertae sedis (1.3 %), and Trichosphaeriales (1.2 %) (Fig. S8, Supplementary data). For the second most abundant fungal phylum, Zygomycota, only one order was found, Mortierellales (5.8 %) (data not shown).

Fig. 3 Taxonomic distribution of OTUs at the phylum level in soil treatments. **(a)** Bacteria in Zea mays in monoculture and in competition with weeds. **(b)** Archaea in Z. mays. **(c)** Fungi in Z. mays. **(d)** Bacteria in Glycine max. **(e)** Archaea in G. max. **(f)** Fungi in G. max. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Competition treatments are indicated by "vs" between the name of two species.

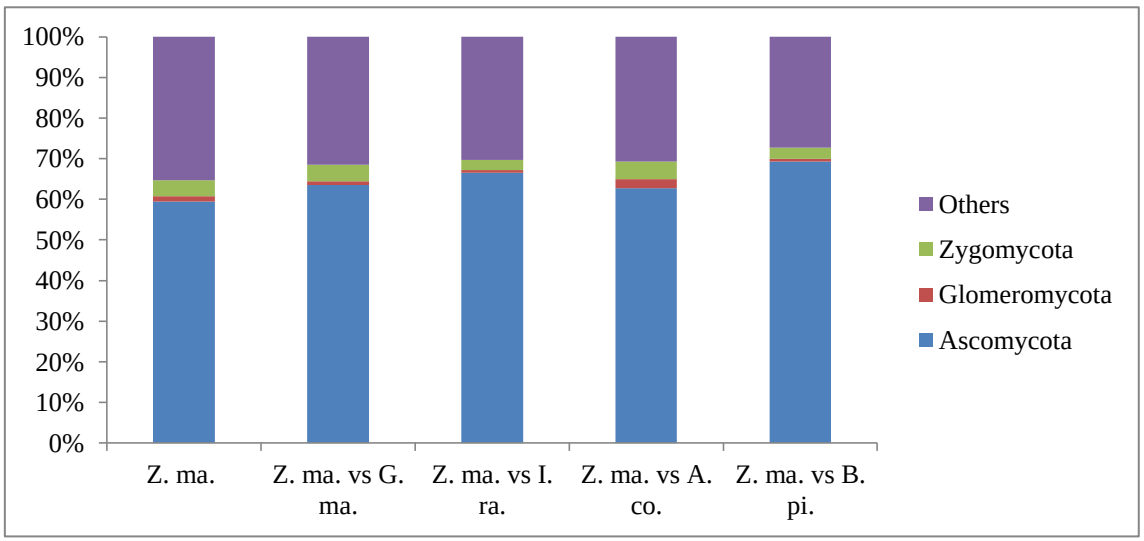
(a) Bacteria



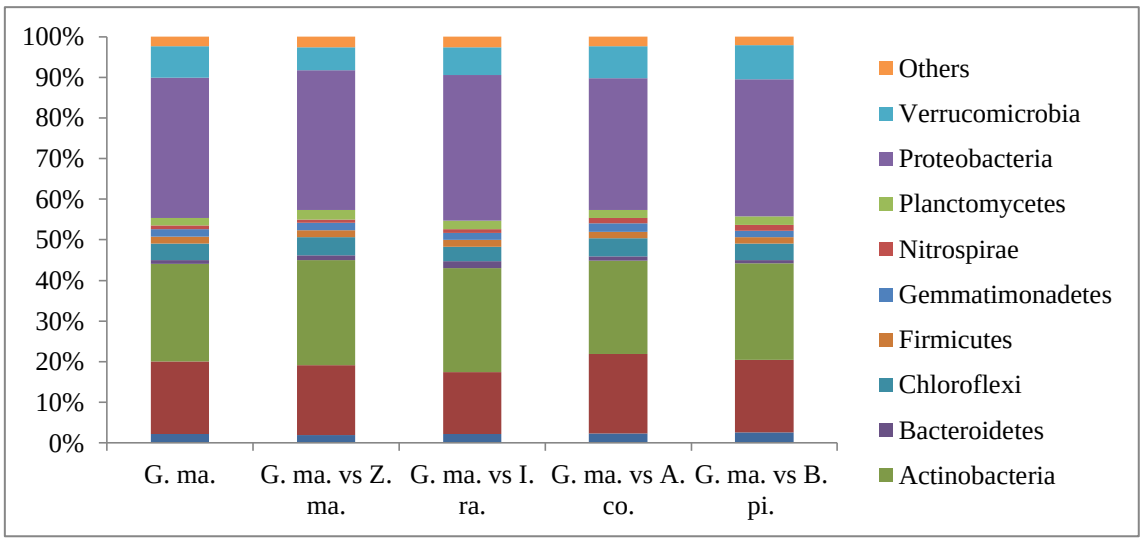
(b) Archaea



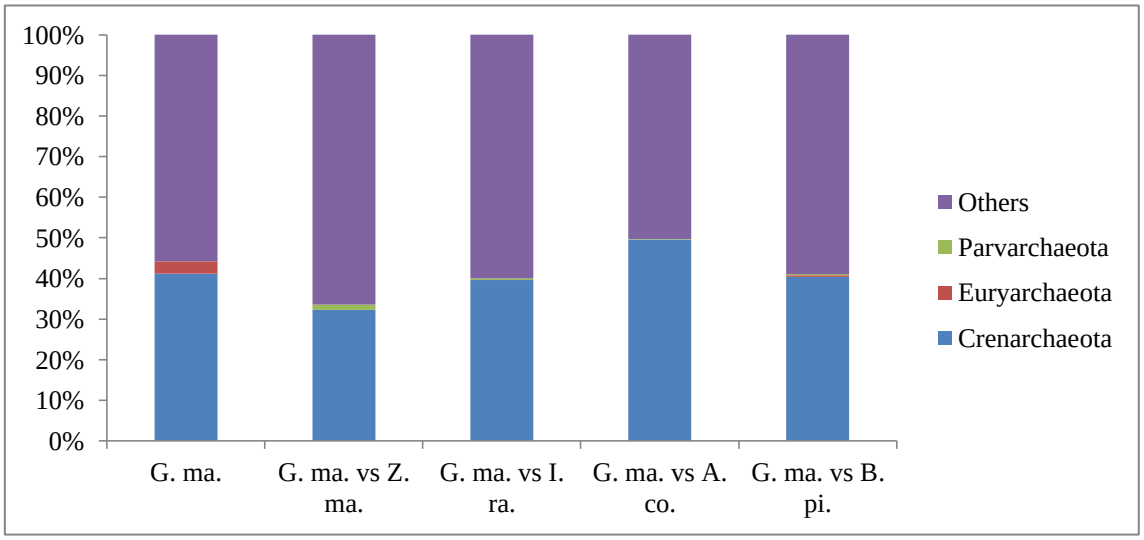
(c) Fungi



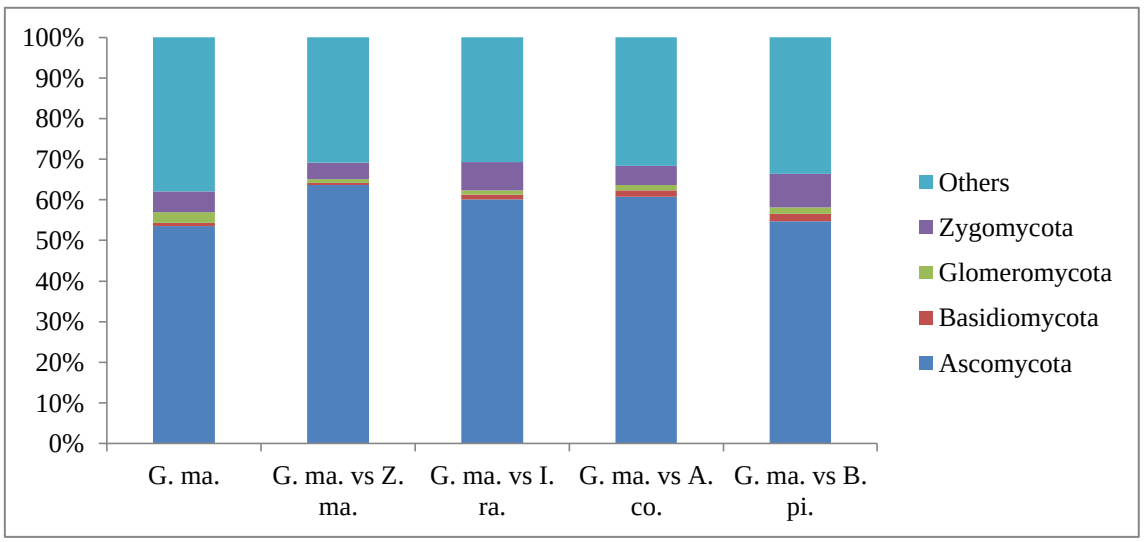
(d) Bacteria



(e) Archaea



(f) Fungi



The microbial distribution of the most abundant genera, for rhizosphere soil treatments of Zea mays (Fig. 4a and 4b) and Glycine max (Fig. 4c and 4d) in monoculture and in competition with weeds, were represented by hierarchical clustering heat map. The values of the relative abundances of each OTU were indicated by color intensity and those that were significantly different between treatments were marked with an asterisk (ANOVA, $p < 0.05$).

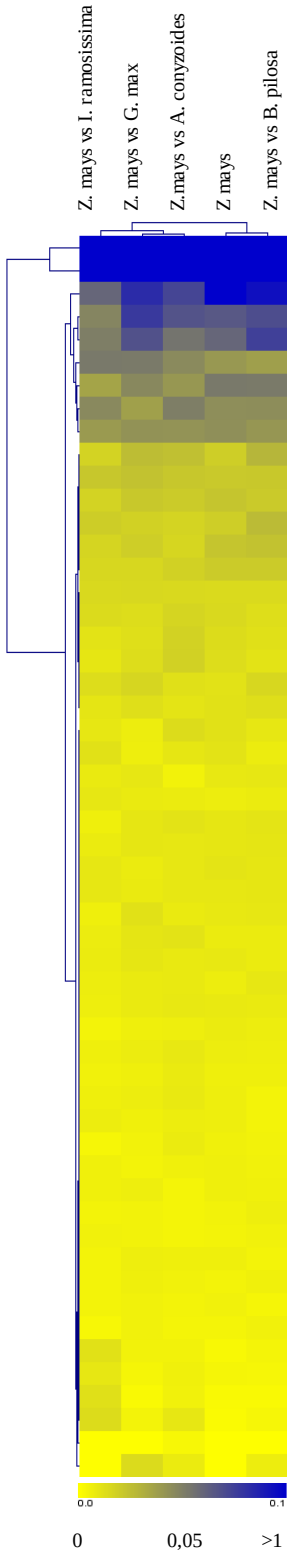
Comparison of the rhizospheric microbial community structure of different plants

The Principal Components Analyses (PCA) was performed to assess differences in the microbial community structure of the rhizosphere of the different tratments tested (Fig. 5). The represented phyla were found in the rhizosphere of all plants, however, the relative abundances of each group varied according to the treatments and in Fig. 5 it can be observed that the phyla that interfere with the distribution of each rhizosphere soil treatments along the principal components.

The Principal Coordinates Analysis (PCoA), based in distance matrix UniFrac (unweighted) for bacteria e archaea, and based in Bray Curtis metrics for fungi, was used to analyze the relationship among soil tratments. It can be noticed that some soil samples from the same treatment did not grouped together. This demonstrates differences in microbial communities between replications, what can be considered normal, since natural variation between samples are expected to occur (Fig. S9, Supplementary data).

Fig. 4 Distribuition of most abundant microbial genera among soil treatments. **(a)** Bacterial and Archaeal distribution in soil treatments of Zea mays in monoculture and competition with weeds. **(b)** Fungal in Z. mays. **(c)** Bacterial and Archaeal in Glycine max**. (d)** Fungal in G. max. For Bacteria and Fungi are showed only 50 most abundant genera. For Achaea all genera are showed. The values of the relative abundances of each OTU were indicated by color intensity and those that were significantly different between treatments were marked with an asterisk (ANOVA, $p < 0.05$). The taxonomic assignment was done with

(a)



Unassigned (Archaea)
Archaea; Crenarchaeota; Thaumarchaeota; Nitrososphaerales; Nitrososp.; Candidatus nitrososphaera
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae; Rhodoplanes
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; Koribacteraceae
Bacteria; Actinobacteria; Actinobacteria; Actinomycetales
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Kaistobacter
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae *
Bacteria; Verrucomicrobia; [Spartobacteria]; [Chthoniobacterales]; [Chthoniobacteraceae]; DA101
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; Gaiellaceae *
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Conexibacteraceae
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales *
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Bradyrhizobiaceae
Bacteria; AD3; ABS-6
Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Sinobacteraceae *
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; Koribacteraceae; Candidatus koribacter
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; Acidobacteriaceae *
Bacteria; Acidobacteria; Acidobacteria-6; iii1-15 *
Bacteria; Acidobacteria; Solibacteres; Solibacterales; Solibacteraceae; Candidatus solibacter
Bacteria; Chloroflexi; TK10; B07_WMSP1
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales
Bacteria; Verrucomicrobia; [Spartobacteria]; [Chthoniobacterales];; Candidatus xiphinematobacter
Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Burkholderia
Bacteria; Actinobacteria; Acidimicrobiia; Acidimicrobiales; EB1017
Bacteria; WPS-2
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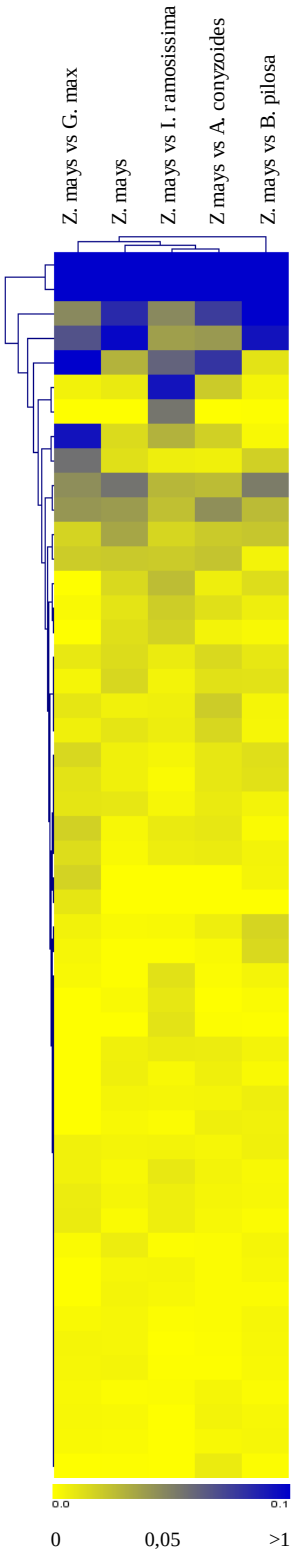
(b)



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Fungi
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Fungi
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Fungi; Ascomycota; Leotiomycetes; Helotiales; Incertae sedis; Xylogone
Fungi; Ascomycota; Sordariomycetes; Xylariales; Amphisphaeriaceae; Pestalotiopsis
Fungi; Basidiomycota; Agaricomycetes; Polyporales; Ganodermataceae; Ganoderma
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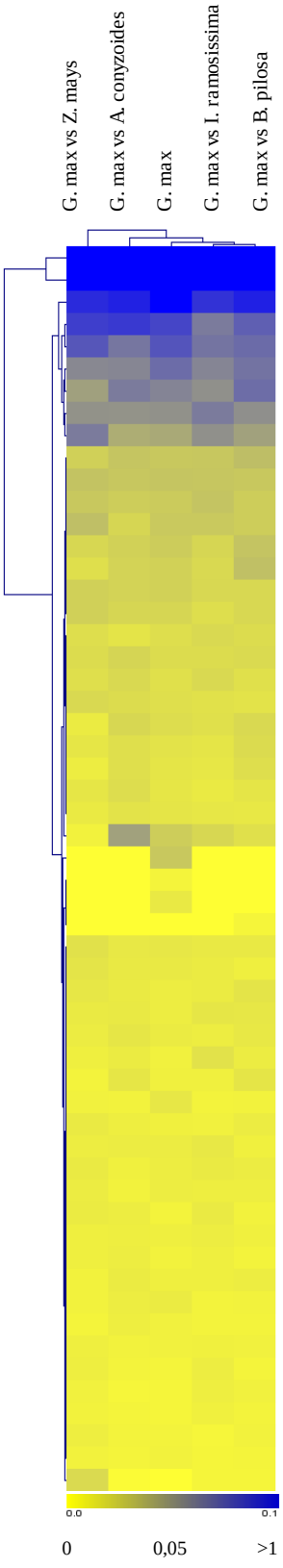
Fungi; Ascomycota; Sordariomycetes; Sordariales

(c)



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Bacteria; Actinobacteria; Actinobacteria; Actinomycetales
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae *
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales; Chthoniobacteraceae; DA101 *
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; Gaiellaceae
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Bacteria; AD3; ABS-6
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales *
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Bradyrhizobiaceae
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Archaea; Euryarchaeota; Methanomicrobia; Methanomicrobiales; Methanosp; Methanospirillum
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Archaea; Euryarchaeota; Thermoplasmata; E2
Bacteria; Planctomycetes; Planctomycetia; Gemmatales; Gemmataceae *
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Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Burkholderia
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Bacteria; Verrucomicrobia; [Pedosphaerae]; [Pedosphaerales]; Ellin515
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Bacteria; Proteobacteria; Alphaproteobacteria; Ellin329
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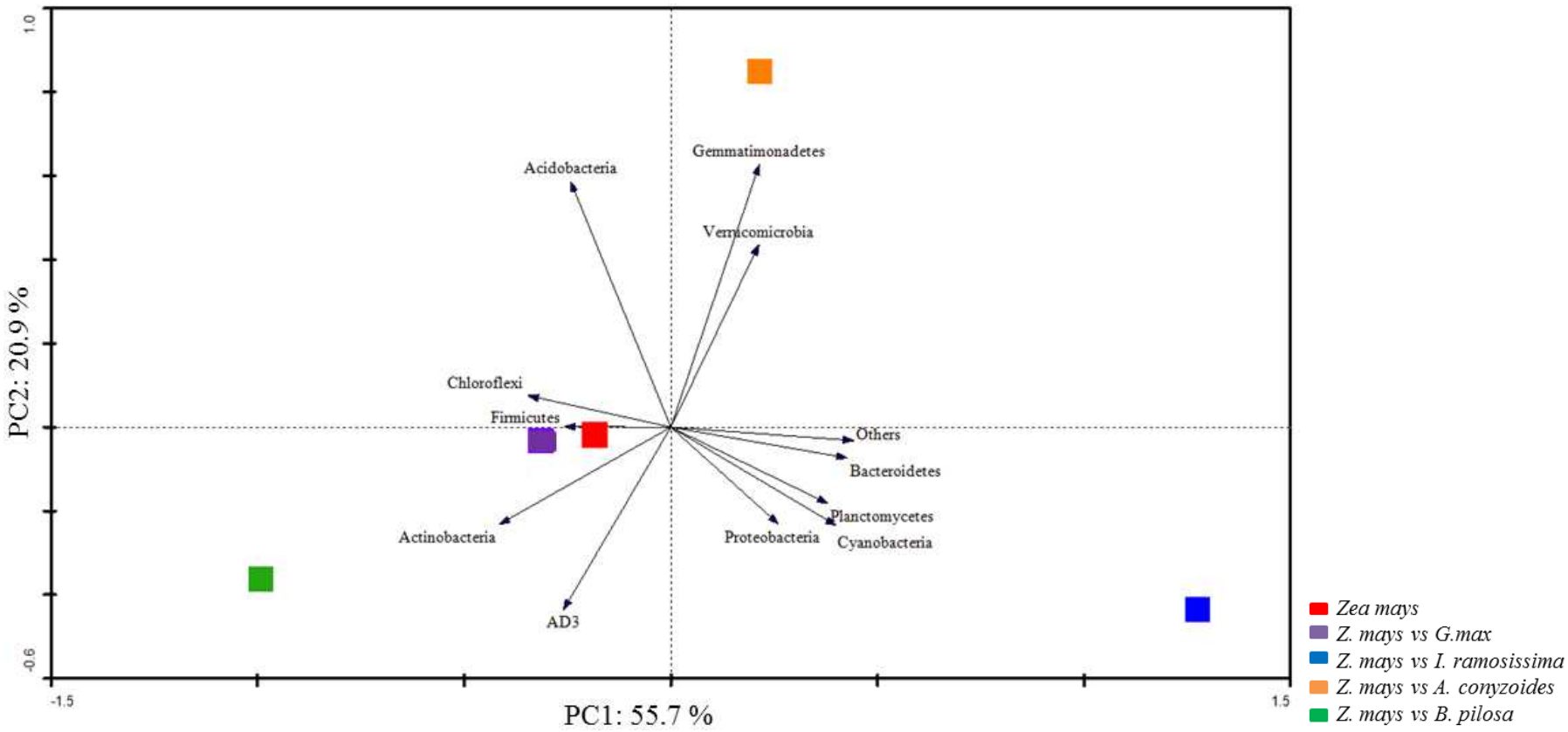
Bacteria; Verrucomicrobia; [Pedosphaerae]; [Pedosphaerales] *
Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Streptomycetaceae; Streptomyces
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae *
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobact.; Phenylobacterium *
Bacteria; Planctomycetes; Planctomycetia; Gemmatales; Isosphaeraceae
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae *
Bacteria; Chloroflexi; Ktedonobacteria; JG30-KF-AS9
Archaea; [Parvarchaeota]; [Parvarchaea]; YLA114

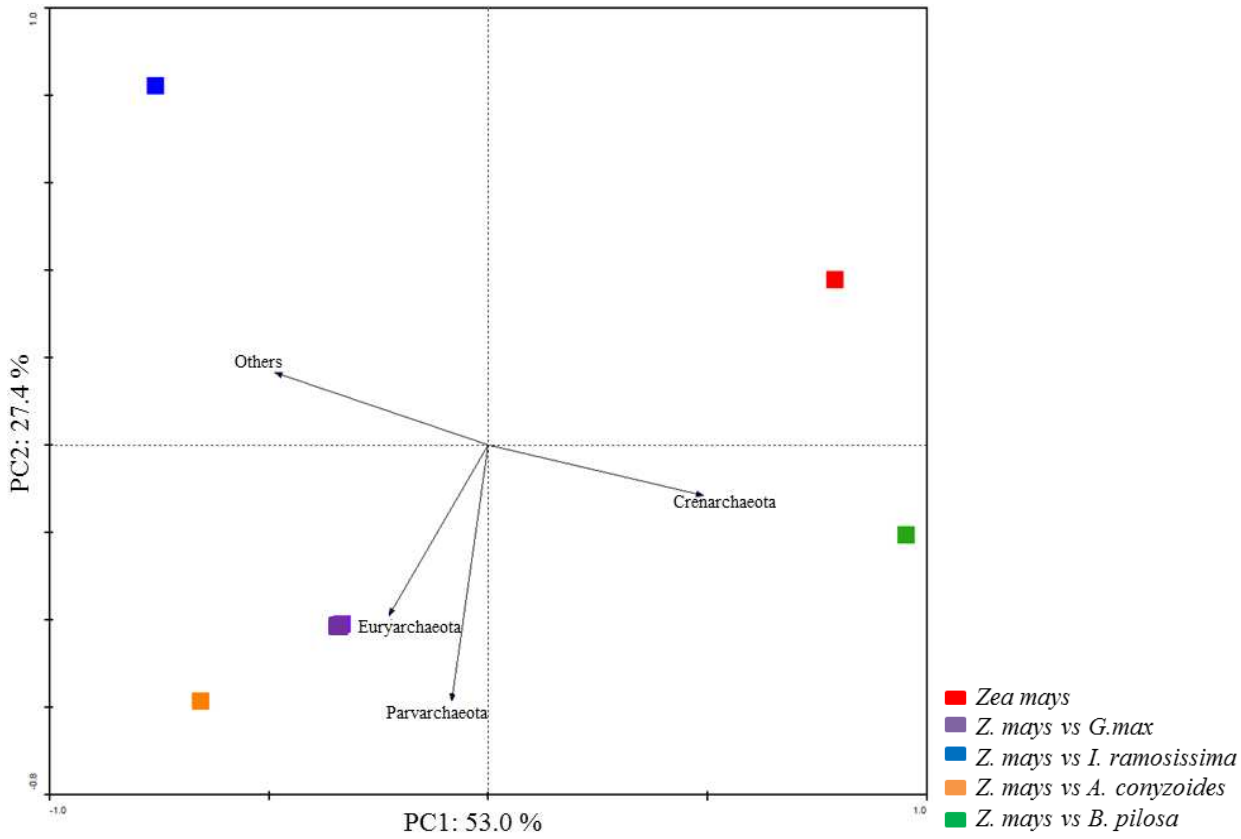
(d)

Fig. 5 Principal Components Analyses (PCA) for soil treatment in monoculture and competition with weeds. **(a)** Bacteria in Zea mays. **(b)** Archaea in Z. mays. **(c)** Fungi in Z. mays. **(d)** Bacteria in Glycine max**. (e)** Archaea in G. max. **(f)** Fungi in G. max.

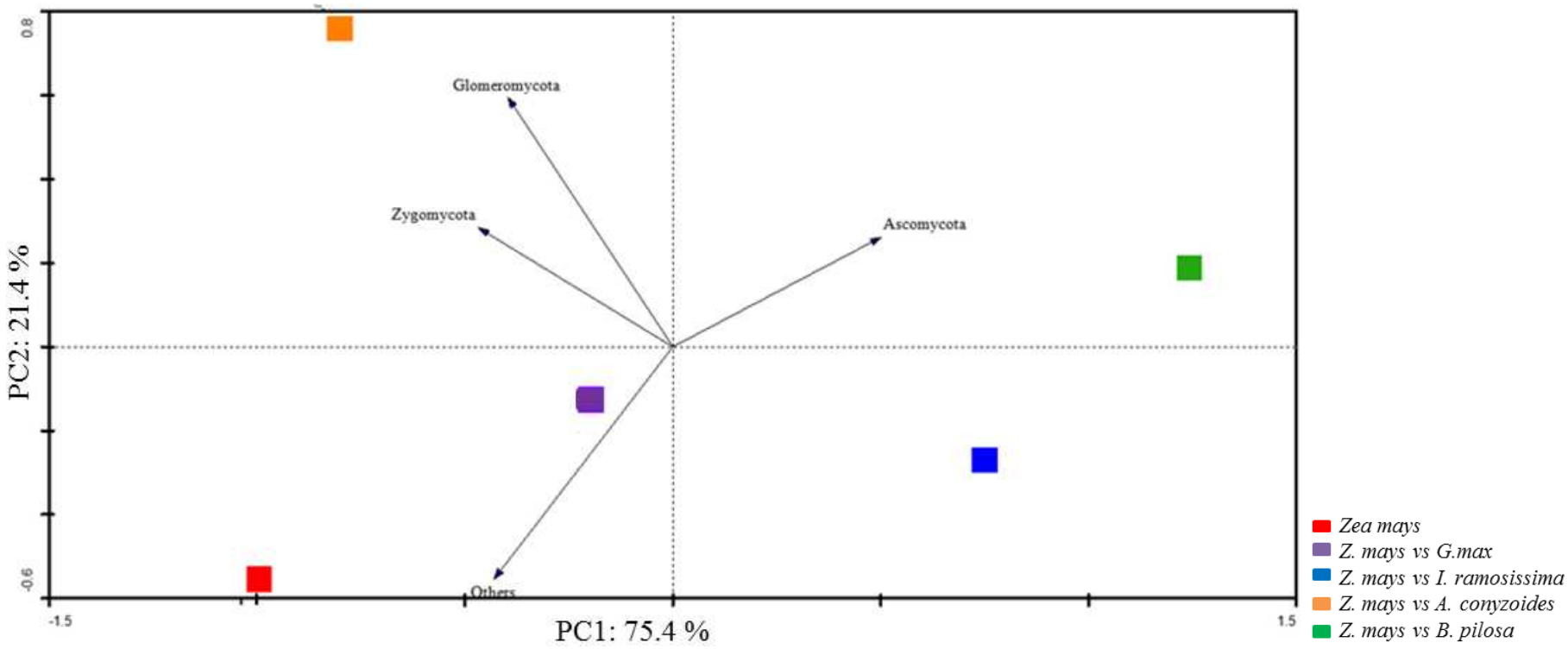
(a) Bacteria


1.0
-0.6
-1.5
1.5
PC2: 20.9 %
PC1: 55.7 %
Acidobacteria
Gemmatimonadetes
Verrucomicrobia
Chloroflexi
Firmicutes
Others
Bacteroidetes
Planctomycetes
Actinobacteria
Proteobacteria
Cyanobacteria
AD3
Zea mays
Z. mays vs G.max
Z. mays vs I. ramosissima
Z. mays vs A. conyzoides
Z. mays vs B. pilosa

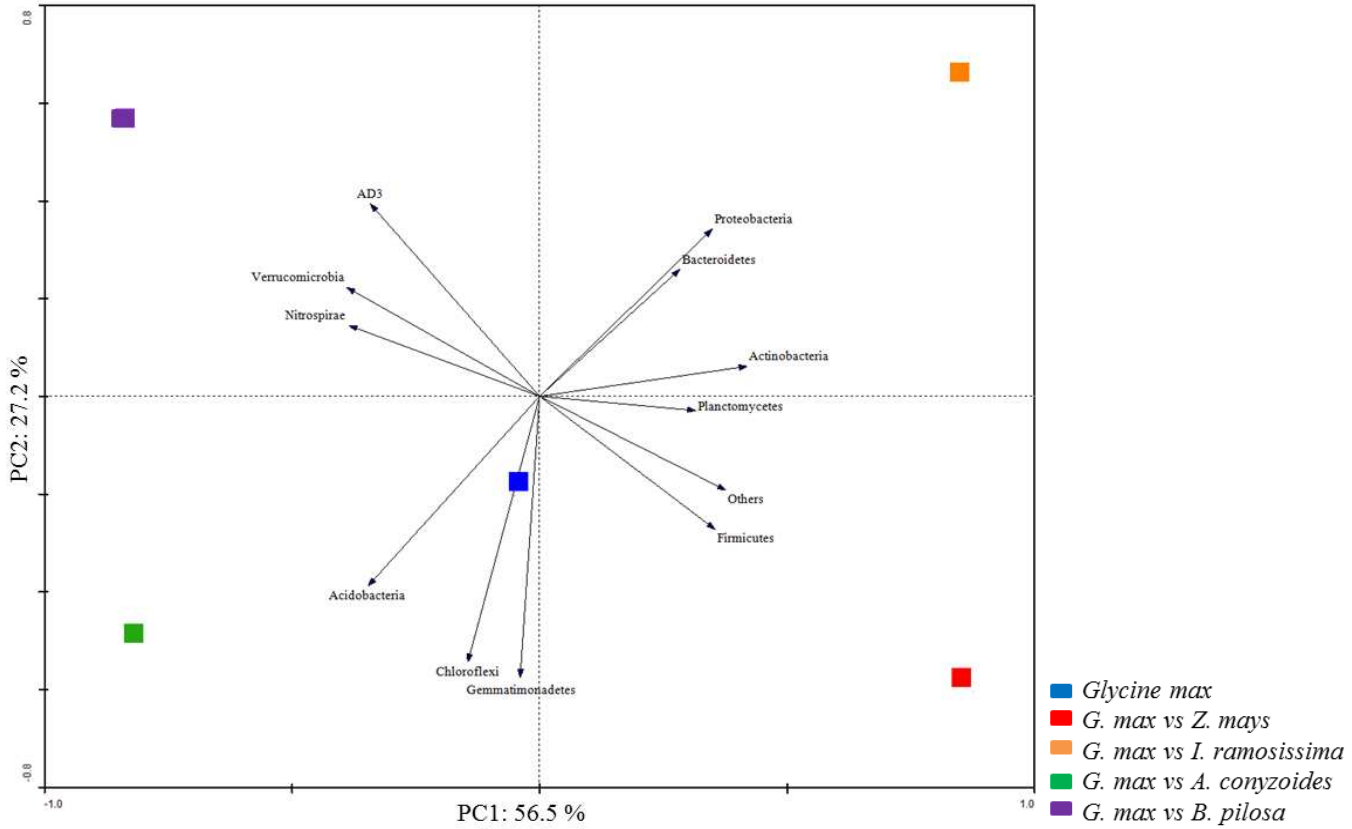
(b) Archaea


PC2: 27.4 %
PC1: 53.0 %
Others
Crenarchaeota
Euryarchaeota
Parvarchaeota
Zea mays
Z. mays vs G.max
Z. mays vs I. ramosissima
Z. mays vs A. conyzoides
Z. mays vs B. pilosa

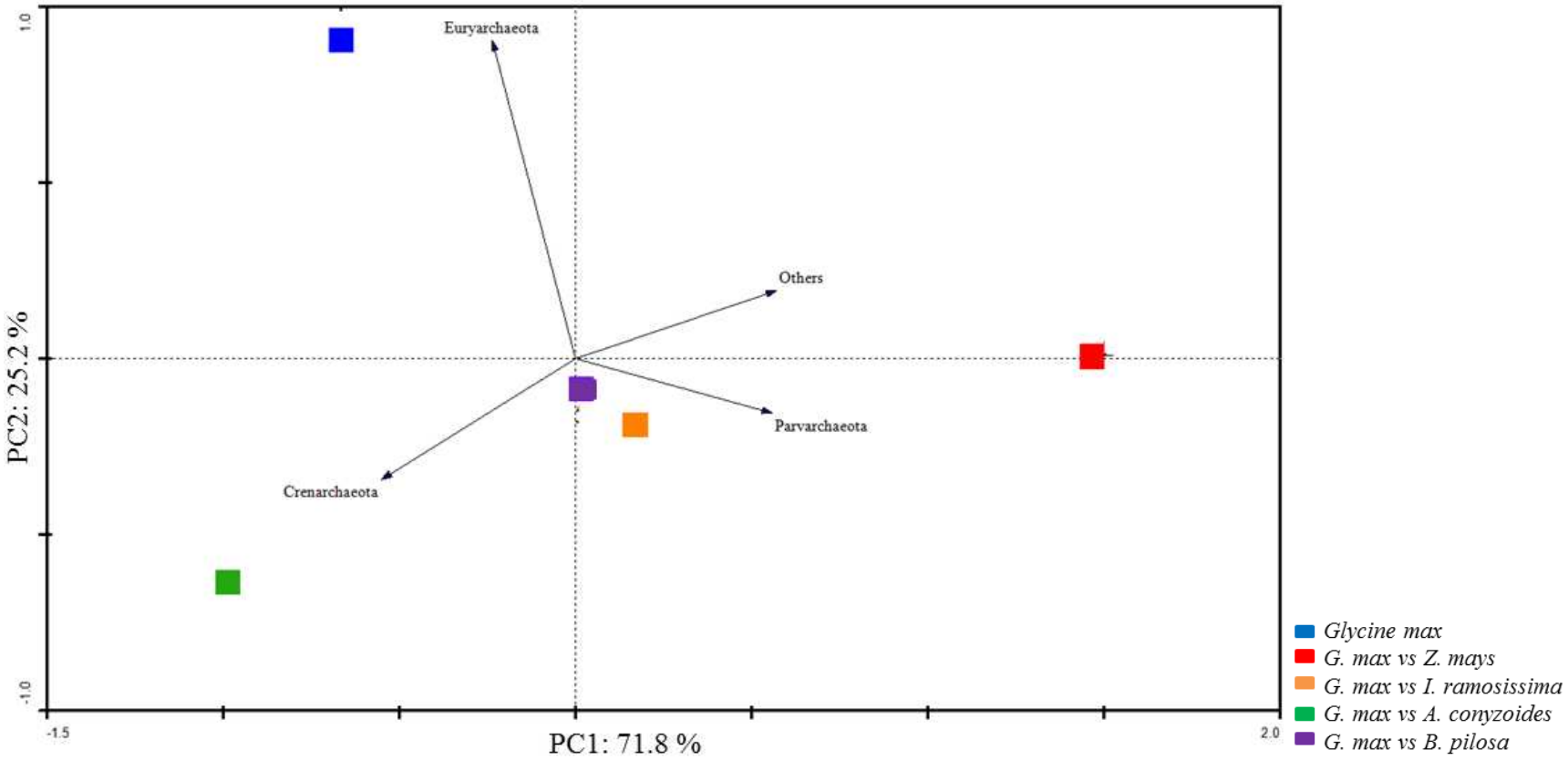
(c) Fungi


Glomeromycota
Zygomycota
Ascomycota
Others
PC2: 21.4 %
PC1: 75.4 %
0.8
-0.6
-1.5
1.5
Zea mays
Z. mays vs G.max
Z. mays vs I. ramosissima
Z. mays vs A. conyzoides
Z. mays vs B. pilosa

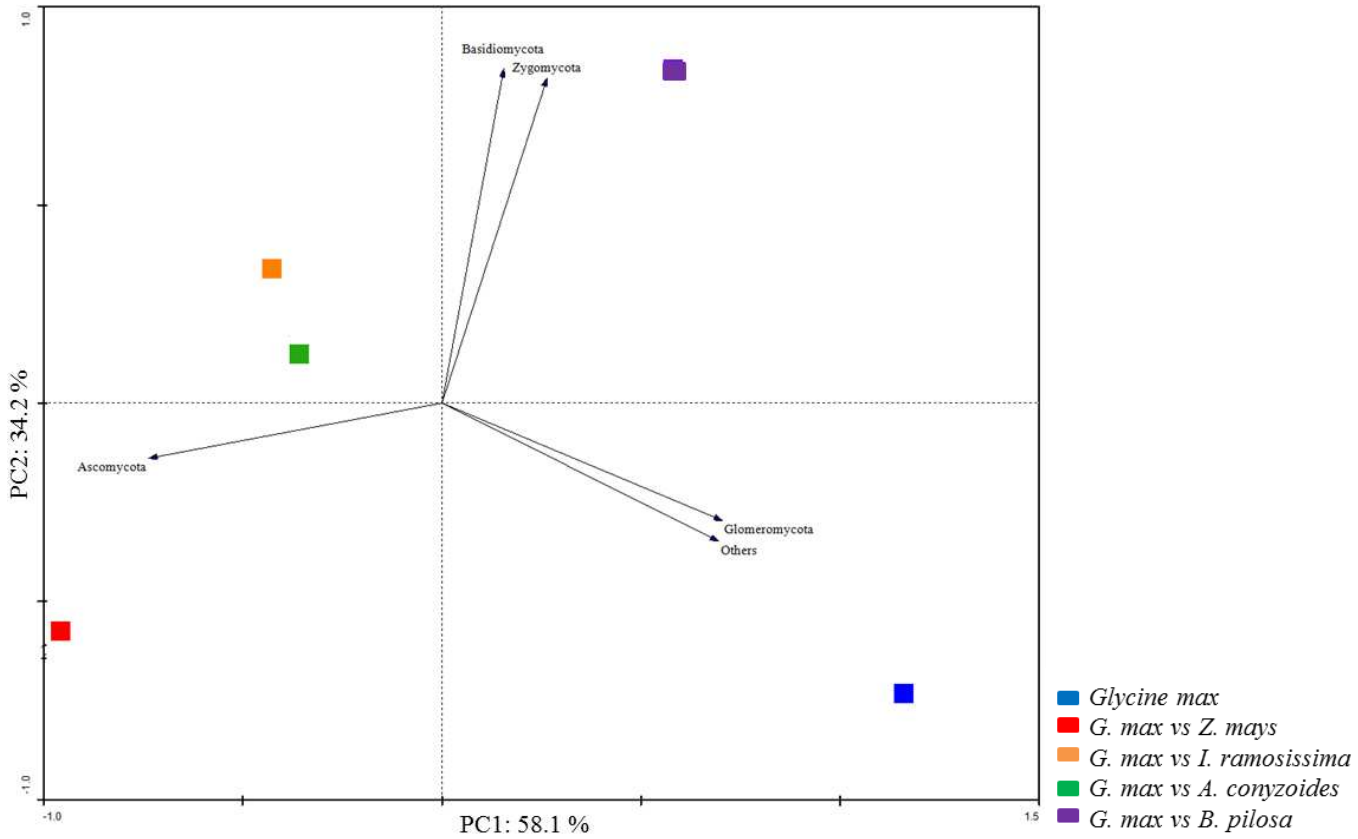
(d) Bacteria


AD3
Proteobacteria
Bacteroidetes
Verrucomicrobia
Nitrospirae
Actinobacteria
Planctomycetes
Others
Firmicutes
Acidobacteria
Chloroflexi
Gemmatimonadetes
PC2: 27.2 %
PC1: 56.5 %
0.8
-0.8
-1.0
1.0
Glycine max
G. max vs Z. mays
G. max vs I. ramosissima
G. max vs A. conyzoides
G. max vs B. pilosa

(e) Archaea


Euryarchaeota
Others
Parvarchaeota
Crenarchaeota
PC2: 25.2 %
PC1: 71.8 %
1.0
-1.0
-1.5
2.0
Glycine max
G. max vs Z. mays
G. max vs I. ramosissima
G. max vs A. conyzoides
G. max vs B. pilosa

(f) Fungi


Basidiomycota
Zygomycota
Ascomycota
Glomeromycota
Others
PC2: 34.2 %
PC1: 58.1 %
1.0
-1.0
-1.0
1.5
Glycine max
G. max vs Z. mays
G. max vs I. ramosissima
G. max vs A. conyzoides
G. max vs B. pilosa

Discussion

Recent studies from our group have shown that the competition between crops and weeds are able to change the structure of the soil microbial community (MASSENSSINI, 2014), highlighting the importance of rhizosphere microorganisms for the establishment of these interactions and, thus, for the competitive ability of plants (MASSENSSINI et al., 2014). In this study, we have used the high throughput analyses by Illumina MiSeq sequencing to evaluate the diversity and characterize taxonomically Bacteria, Archaea, and Fungi associated with the rhizosphere of plants in competition. The results of our sequencing indicate that the approach used was sufficient to detect differences in the microbial community in the rhizosphere of plants depending on the presence or absence of different competing species. The present study is the first report on the use of this approach to study plant-weed interactions.

The competitive ability of the plants was previously assessed by calculating the Competition Balance Index (Cb) (MASSENSSINI, 2014). The plants tested showed similar or disctint competitive ability, depending on the plant combinations. In the competition treatments Zea mays vs Ipomoea ramosissima, Zea mays vs Ageratum conyzoides and Glycine max vs Ageratum conyzoides, the crops showed greater competitive ability. Massenssini (2014) proposed that corn is a strong competitor and this capacity would probably be explained by the fact that Zea mays is not dependent on high microbial biodiversity in the soil, while the weeds would require that for their optimal growth. Our results corroborate this idea. The competition between Z. mays vs I. ramosissima resulted in a Shannon diversity index for archaea and fungi lower than those measured for maize monoculture (Table 3), indicating that the decrease in the diversity of these groups during competition may be associated with less competitive ability of weeds. The comparison between Zea mays in monoculture and in the competition Zea mays vs Ageratum conyzoides demonstrates that the microbial profiles found in the soil of the rhizosphere from these treatments were similar. A total of 323 bacterial (Fig. 2a) and 89 fungal OTUs (Fig. 2c) were shared between these treatments. These values are higher than those shared between other competition treatments and in the monoculture rhizosphere and may be an indication that Zea mays, even under competition, is able to influence rhizospheric microbial community structure in such a way that it becomes similar to that found in monoculture, highlighting its characteristic as strong competitor. In the treatment of Glycine max vs Ageratum conyzoides, the crop showed greater competitive ability. In this treatment, smaller numbers of bacterial and

archaeal OTUs and lower Shannon diversity index for bacteria were found, which is consistent with the idea that the weed A. conyzoides is highly dependent on a high diversity of microorganisms in the soil, becoming a weak competitor when microbial diversity decreases.

Comparing the OTUs present in the rhizospheric soil of the treatments, it was observed that most of the OTUs were shared between all treatments (Fig. 2). However, we have detected exclusive OTUs for each treatment (Table 5). The supply of different organic compounds in the exudates released by plants may promote the recruitment of specific microbiota in the rhizosphere, being representing a determinant for the structure of the microbial community of the rhizosphere (MARSCHNER et al., 2004; BAIS et al., 2006; HAICHAR et al., 2014). When two plants are competing, the composition of the exudates will be different from that observed from plants in monoculture and it is believed that this new combination of carbon sources for microorganisms can be responsible for the differential microbial profiles observed in the rhizosphere of plants in the different treatments tested. This demonstrates, that depending on the competitive situation, the plant recruits specific microorganisms that can benefit them or reduce the competitive ability of competing plants.

The taxa of ammonia-oxidizer bacteria Nitrospirae (Phylum) and Nitrospira (Class) were found only in the rhizosphere of competition between Z. mays vs A. conyzoides (Table 5). Other groups involved in nitrogen cycling were also found, such as nitrogen-fixing bacteria belonging to the order Rhodobacterales, classified in the family Hyphomonadaceae (in the treatment of competition between Z. mays vs I. ramosissima) and Rhodobacteraceae (in the rhizospheric soil of competition between G . max vs A. conyzoides) (Table 5). In a study conducted to determine the changes in microbial communities associated with the establishment of invasive plants it was shown that the abundance of bacteria responsible for nitrogen cycling in the soil increases in the presence of invasive plants (RODRIGUES et al., 2015), and this change may affect the outcome of competition between plants in the environment.

For Zea mays and Glycine max in monoculture or in competition with weeds, the dominant bacterial phyla were Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia, and Cloroflexi (Fig. 3a and 3d). These findings confirm those reported for maize rhizosphere in monoculture obtained by pyrosequencing (LI et al., 2014) and those reported for soybean monoculture by shotgun analyses (MENDES et al., 2014). The Proteobateria and Actinobacteria phyla make up together, 59.4 % and

58.6 % of the total relative abundance of bacteria in the rhizosphere of Z. mays and G. max, respectively. Because of root exudation, the rhizosphere represents an environment rich in readily assimilable organic carbon. The β-Proteobacteria display copiotrofic characteristics, and are abundant in soils with highe availability of organic carbon (FIERER et al., 2007). The Actinobacteria are capable of using a wide variety of carbon sources, including complex polysaccharides (BARKA et al., 2015), a trait that may be responsible for the high abundance observed. The ability of Actinobacteria to catabolize recalcitrant compounds may reduce competition with other microorganisms of rhizosphere, favoring their presence in this location. Besides, this phylum includes bacteria which exert antagonism and biological control of plants pathogens and are capable of promoting plant growth by nitrogen fixation, synthesis of siderophore and phytohormone and, solubilization of minerals (BARKA et al., 2015). The most abundant bacterial orders were Rhizobiales (Phylum Proteobacteria) (Fig. S1 and S5, Supplementary data) and Actinomycetales (Phylum Actinobacteria) (Fig. S2 and S6, Supplementary data). The Rhizobiales order includes nitrogen-fixing bacteria (SOUZA et al., 2015), especially within the Bradyrhizobiaceae family and genus Rhodoplanes, included among the most abundant OTUs (Fig 4). The Actinomycetales order includes biotechnologically important microorganisms for the production of antibiotics (SOUZA et al., 2013), such as those belonging to the genus Streptomyces (Fig. 4) and symbiotic nitrogen-fixing (SOUZA et al., 2013), such as those belonging to the family Frankiacea (Fig. 4).

Crenarchaeota, the most abundant archaeal phylum found in all treatments (Fig. 3b and 3e), is usually found in environments rich in organic matter (SOUZA et al., 2013) and are also reported to colonize plant roots (SIMON et al., 2000). Within this phylum, the Thaumarchaeota group could also be found, comprising archaeae involved in the oxidation of ammonium to nitrite that are found in large numbers in aerobic terrestrial environments (OFFRE et al., 2013).

In our work, Ascomycota was the most abundant fungal phylum found in the soil from all the treatments (Fig. 3c and 3f). Studies to assess the composition of the community of rhizospheric fungi in soybean subjected to continuous cropping by pyrosequencing also revealed that Ascomycota was the most abundant fungal phylum (BAI et al., 2015). The Glomeromycota represented only 1.1 % and 1.5 % of the total fungal relative abundance in the rhizosphere of corn and soybean, respectively. This phylum includes all arbuscular mycorrhizal fungi, which have important ecological

roles, such as improving plant nutrition, protecting their hosts from pathogens, facilitating mineral nutrient uptake, among other functions that promote plant growth (BORRIELO et al., 2012). The presence of arbuscular mycorrhizal fungi in weed roots has been demonstrated (MASSENSSINI et al., 2014a), and these associations can provide competitive advantages to these plants over crops in agricultural ecossystems. The most abundant order of fungi was Hypocreales (Phylum Ascomycota) (Fig. S4 and S8, Supplementary date), which includes the genus Trichoderma (Fig. 4). Fungi belonging to this genus are known biological control agents against plant pathogens in the soil, especially for the production of antibiotics. In addition these microorganisms exert plant growth promotion functions both in soybean (JOHN et al., 2010) and in maize (FERRIGO et al., 2014).

The hierarchical clustering heat maps were used to evaluate which treatments microbial communities present in the rhizosphere are more similar to each other. The rhizosphere of Z. mays and Z. mays vs B. pilosa showed a profile of bacterial and archaeal community more alike, as well the rhizosphere between Z. mays vs G. max and Z. mays vs A. conyzoides (Fig. 4a), and this was evidenced by their grouping together. The Z. mays vs I. ramosissima and Z. mays vs A. conyzoides treatments showed a profile for the fungal community more alike (Fig. 4b). The G. max vs I. ramosissima and G. max vs B. pilosa treatments showed a profile of bacterial, archaeal (Fig. 4c) and fungal (Fig. 4d) communities more similar. For Zea mays rhizosphere in monoculture and in competition with weeds, two genera of fungi were significantly different between all treatments and were present among the 50 most abundant genera (Fig. 4b). Fusarium showed higher abundance in the competition between Z. mays vs A. conyzoides and Penicillium showed higher abundance in monoculture. Fusarium species are responsable for the contamination of corn grain by mycotoxins (DEGRAEVE et al., 2016), which causes risks to human health and economic losses of production resulting from the decline in the quality of the final product. However, it has been shown that certain species within this genus may be potentially useful as biological control agents of the weed Striga hermonthica, which affects the corn crop in Africa (VENNE et al., 2009). This reiterates the need for more studies focusing the role of microorganisms in the outcome of the competition between crops and weeds. The Penicillium genus is considered a plant growth promoting agent because of its ability to solubilize inorganic phosphate, making it available to plants and increasing the yield of maize (LEGGETT et al., 2015). In the rihospheric soil from treatments with Glycine max, the abundance of

the genus Penicillium was significantly different between all treatments and showed higher abundance in the competition between G. max vs Z. mays (Fig. 4d), evidencing that maize may have favored this group of fungi for growth promotion.

The Principal Components Analyses (PCA) (Fig. 5) revealed that the abundance of microbial phyla vary between treatments, demonstrating that even the ubiquitous groups of microrganisms of the rhizosphere of plants respond to the combination of plants in the competition. These changes can be decisive for the outcome of plant-plant interactions, highlighting the ability of the plants to mold the structure of the microbial community associated with them.

In conclusion, the Illumina MiSeq sequencing revealed, in detail, the taxonomic composition of microbial communities present in the rhizosphere of plants in competition. The data presented demonstrate that certain microbial groups are favored in the rhizosphere of plants depending on the competitive situation and that there are unique OTUs associated whith specific plant-plant combinations, emphasizing the role of plants in the recruitment of specific microrganisms which play important roles in the rhizosphere. The presence of these microbial groups may directly affect the competitive ability of plants. However, more studies are needed to better assess the functional roles of microrganisms associated with the roots of plants unde the competition.

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Suplementary data

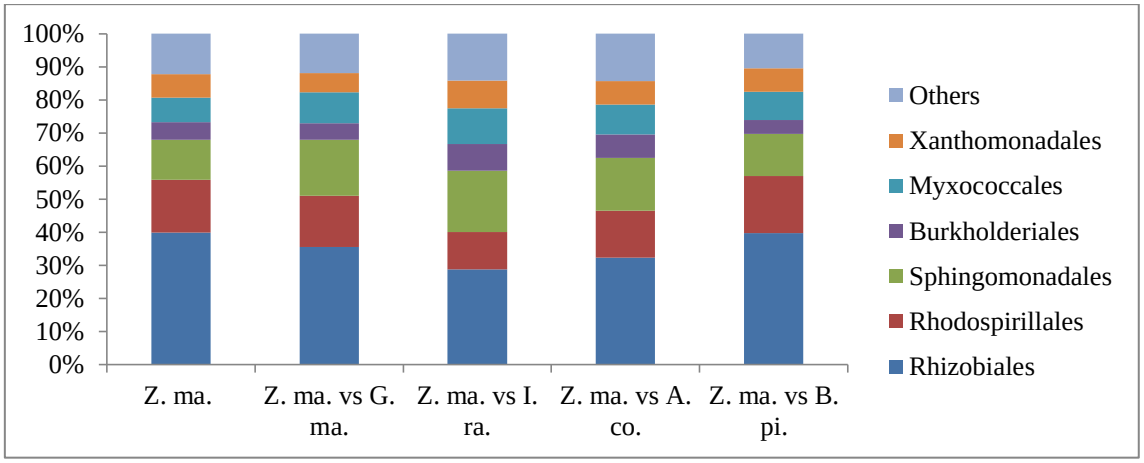


Fig. S1: Taxonomic distribution of Bacteria at order level within Proteobacteria phylum for soil treatment of Zea mays in monoculture and in competition with weeds. Only orders with abundance > 1 % are shown. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Two species separated by 'vs' indicate a competition trateament.

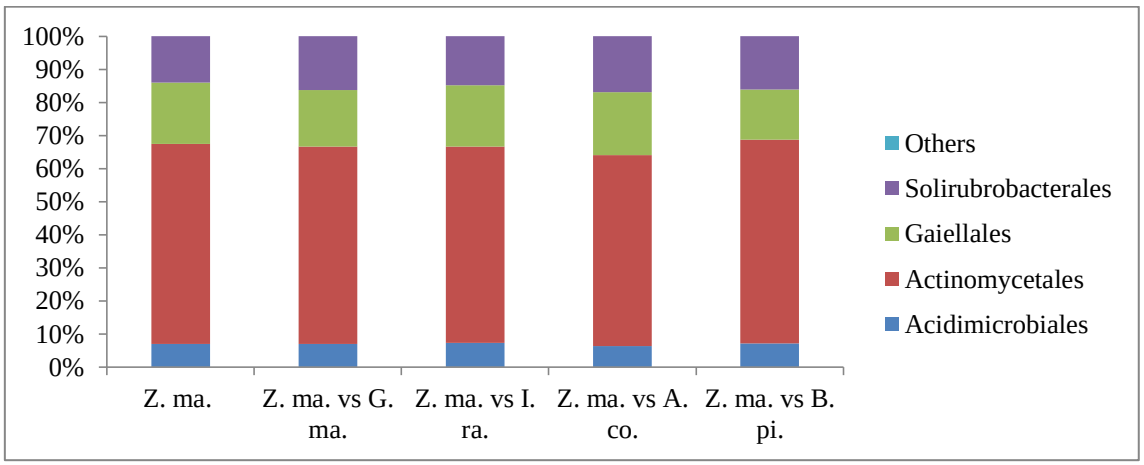


Fig. S2: Taxonomic distribution of Bacteria at order level within Actinobacteria phylum for soil treatment of Zea mays in monoculture and in competition with weeds. Only orders with abundance > 1 % are shown. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Two species separated by 'vs' indicate a competition trateament.

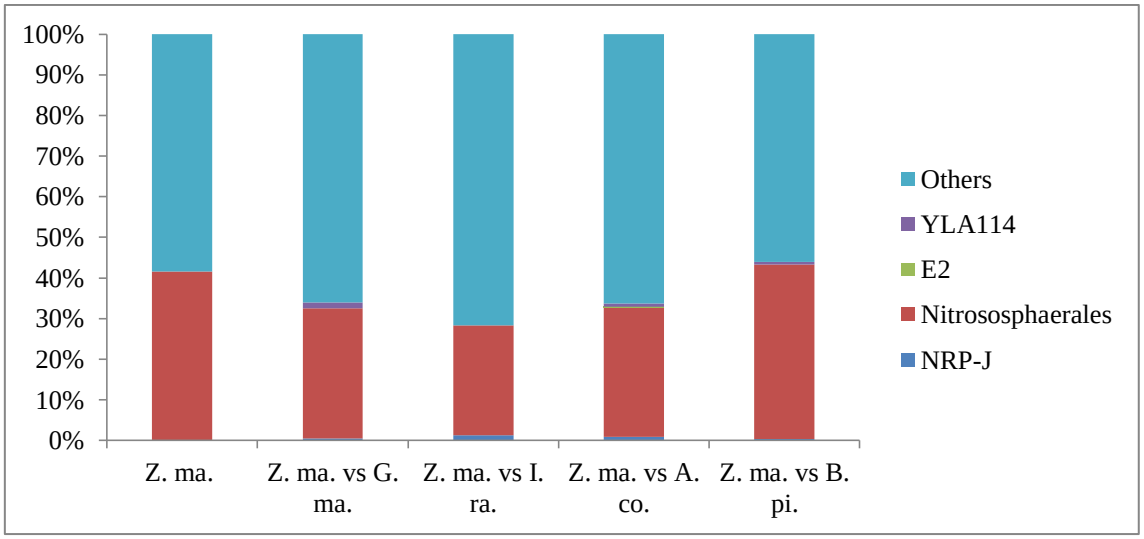


Fig. S3: Taxonomic distribution of Archaea at order level for soil treatments of Zea mays in monoculture and in competition with weeds. Only orders with abundance > 1 % are shown. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Two species separated by 'vs' indicate a competition trateament.

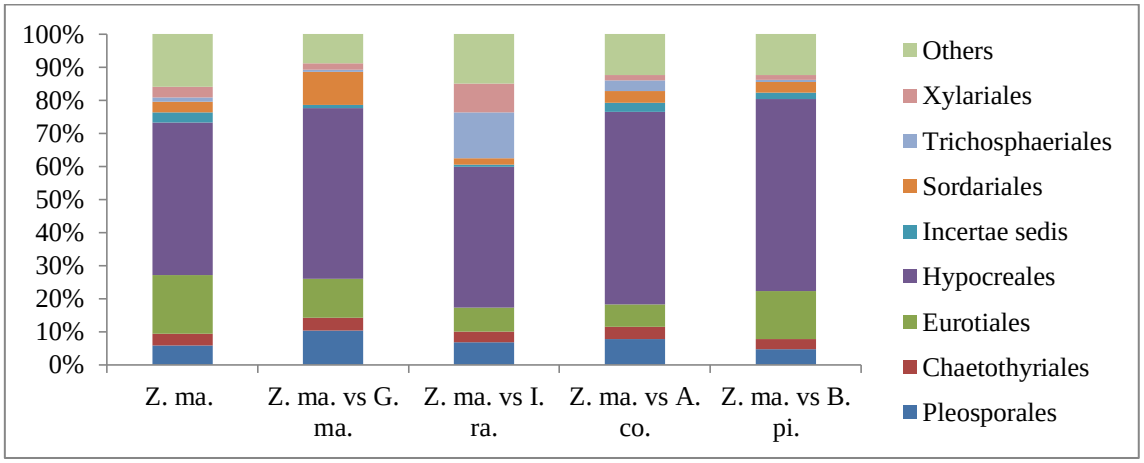


Fig. S4: Taxonomic distribution of Fungi at order level within Ascomycota phylum for soil treatment of Zea mays in monoculture and in competition with weeds. Only orders with abundance > 1 % are shown. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Two species separated by 'vs' indicate a competition trateament.

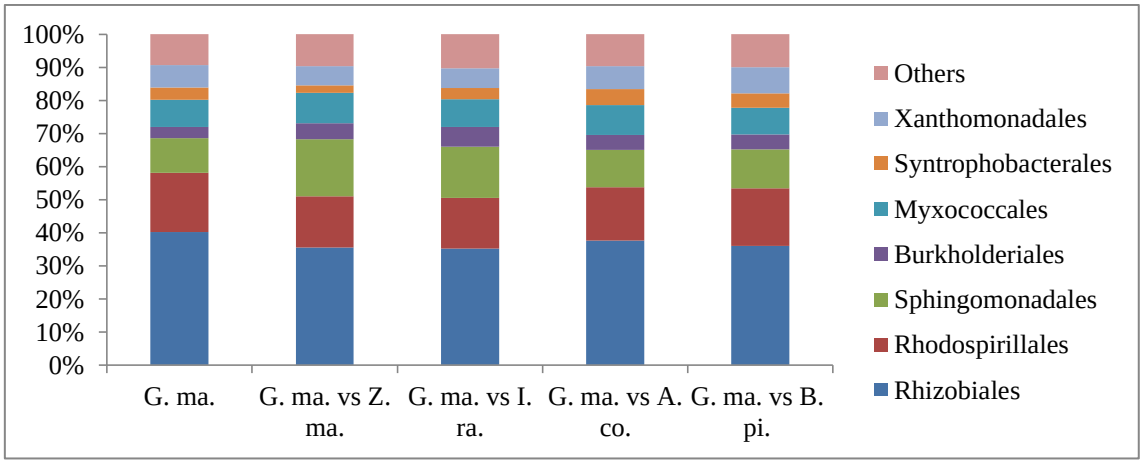


Fig. S5: Taxonomic distribution of Bacteria at order level within Proteobacteria phylum for soil treatment of Glycine max in monoculture and in competition with weeds. Only orders with abundance > 1 % are shown. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Two species separated by 'vs' indicate a competition trateament.

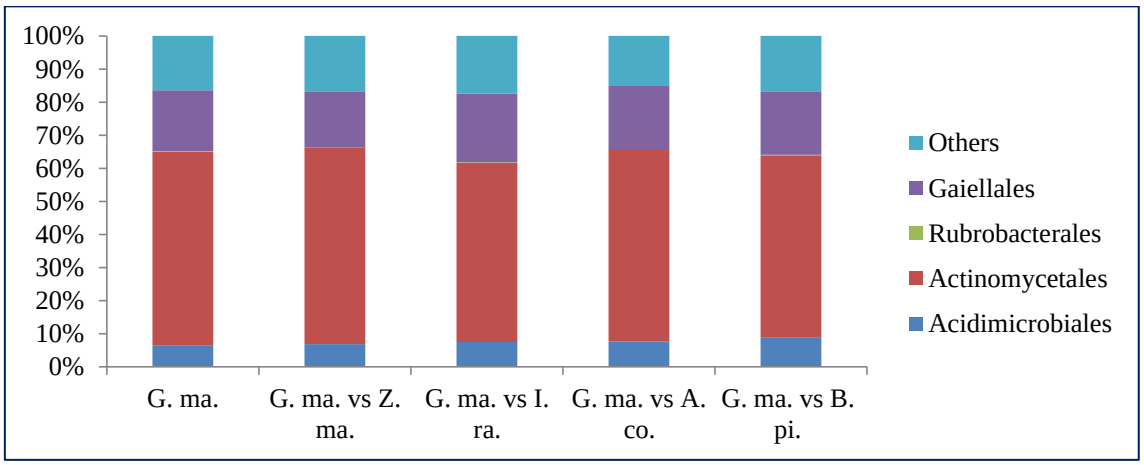


Fig. S6: Taxonomic distribution of Bacteria at order level within Actinobacteria phylum for soil treatment of Glycine max in monoculture and in competition with weeds. Only orders with abundance > 1 % are shown. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Two species separated by 'vs' indicate a competition trateament.

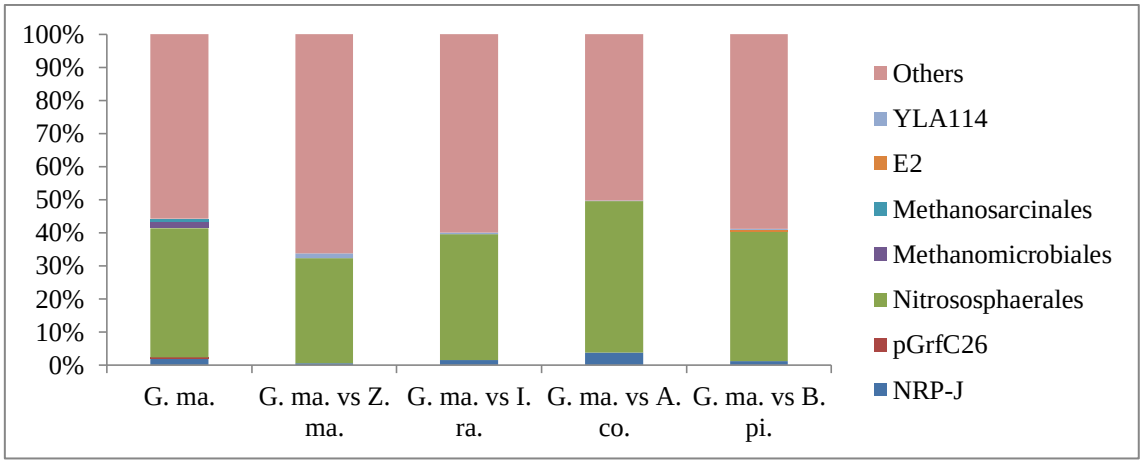


Fig. S7: Taxonomic distribution of Archaea at order level for soil treatment of Glycine max in monoculture and in competition with weeds. Only orders with abundance > 1 % are shown. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Two species separated by 'vs' indicate a competition trateament.

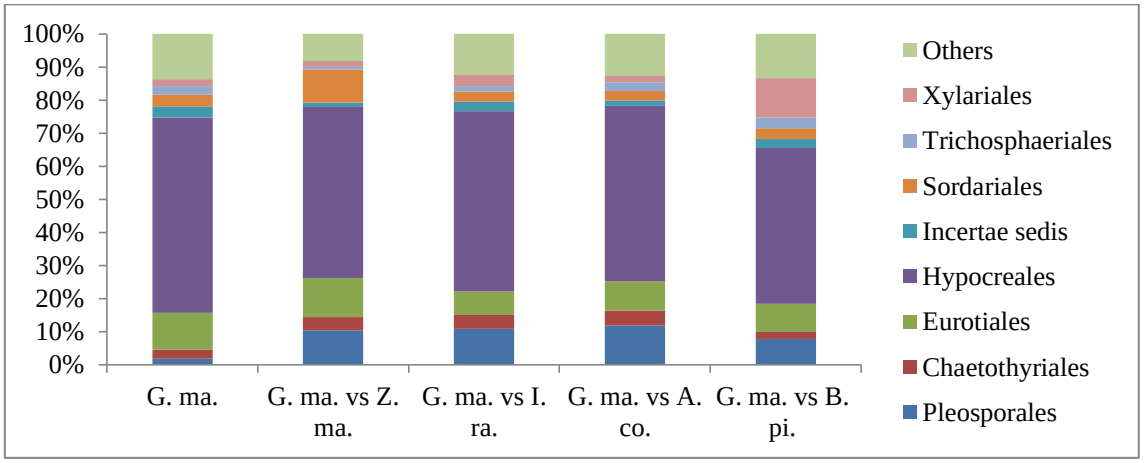
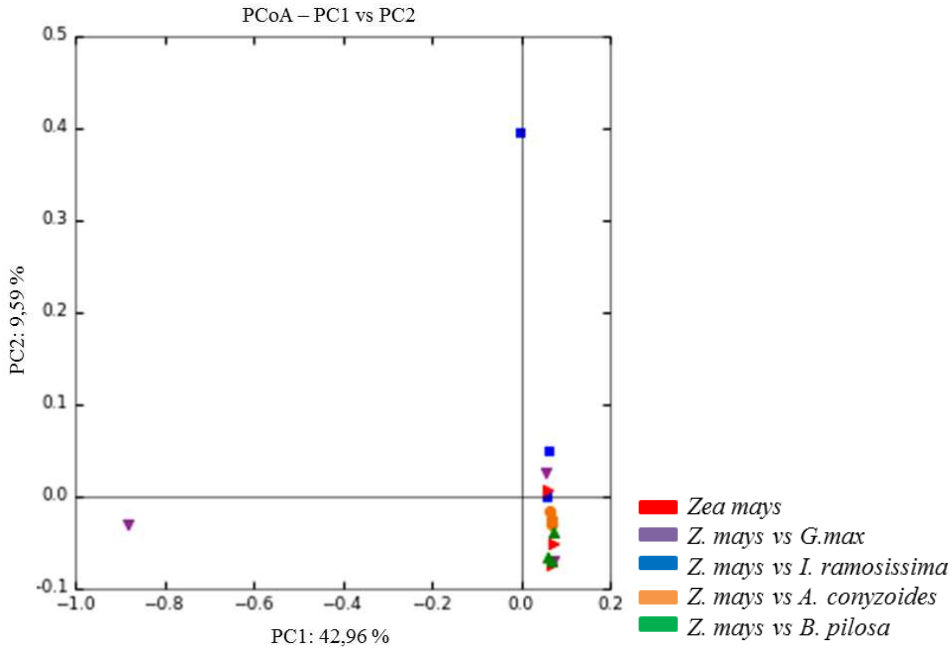


Fig. S8: Taxonomic distribution of Fungi at order level within Ascomycota phylum for soil treatment of Glycine max in monoculture and in competition with weeds. Only orders with abundance > 1 % are shown. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa; I.ra – Ipomoea ramosissima; G.ma – Glycine max and Z.ma – Zea mays. Two species separated by 'vs' indicate a competition trateament.

(a)



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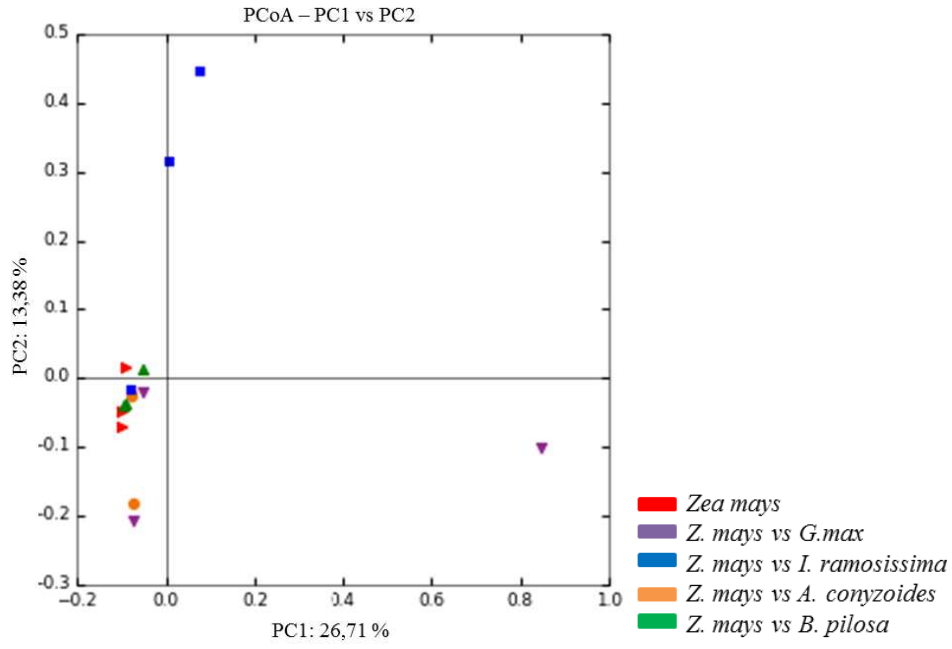
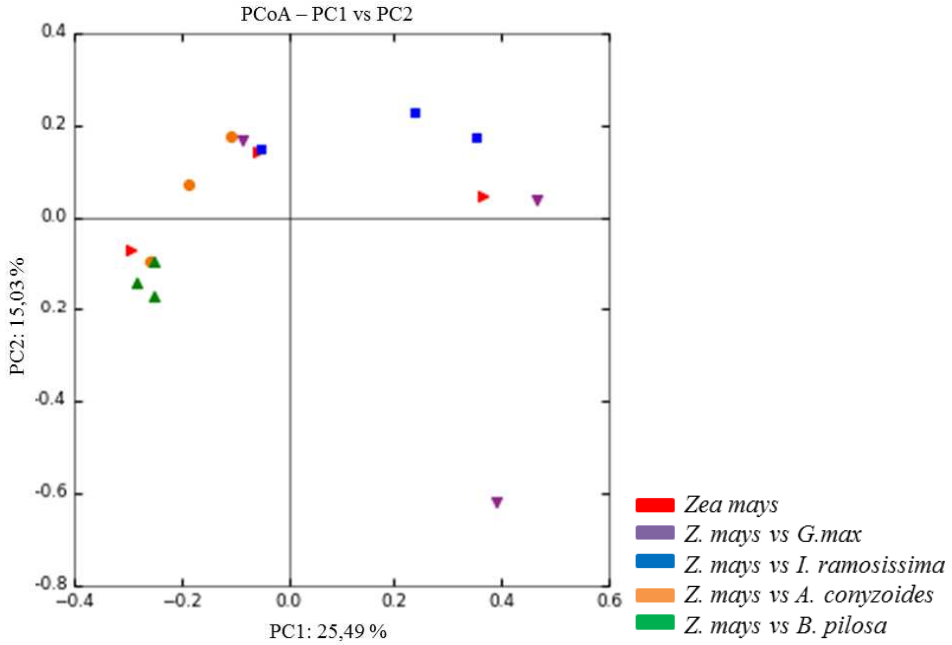


Fig. S9 Principal Coordinate Analysis (PCoA) for different soil treatments in monoculture and in competition with weeds. **(a)** PCoA of Bacteria from Zea mays. **(b)** Archaea in Z. mays. **(c)** Fungi in Z. mays. **(d)** Bacteria in Glycine max. **(e)** Archaea in G. max. **(f)** Fungi in G. max. For Bacteria and Archaea, the PCoA was based in distance matrix UniFrac (unweighted). For Fungi was used to the metric Bray Curtis.

(c)



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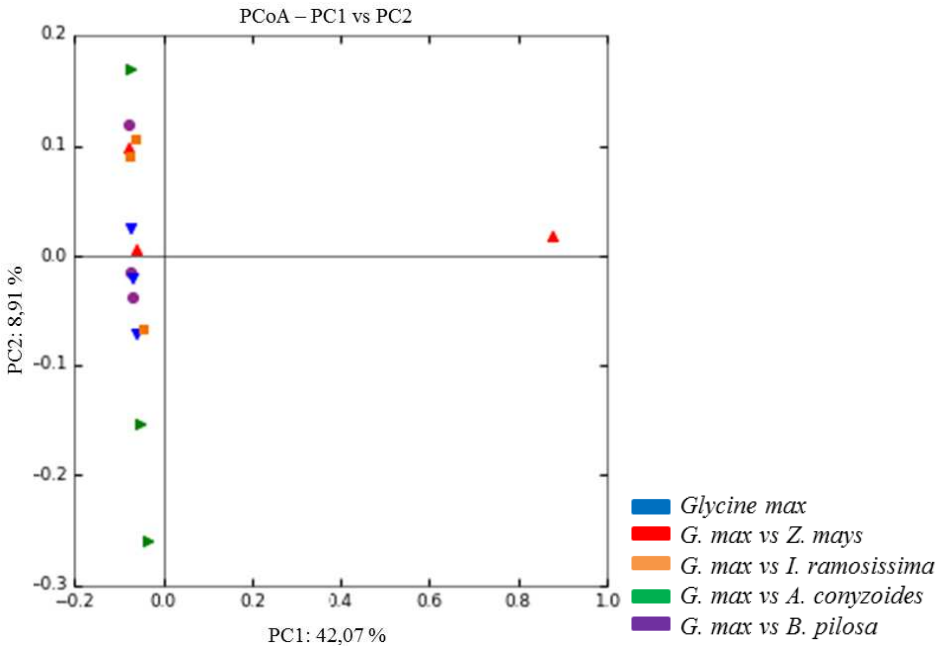

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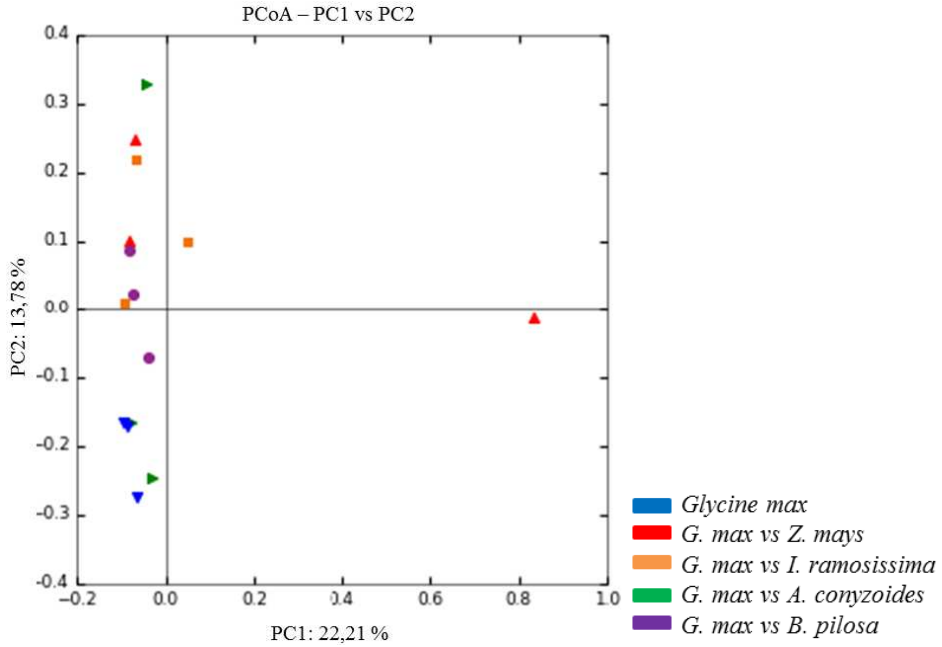
(e)



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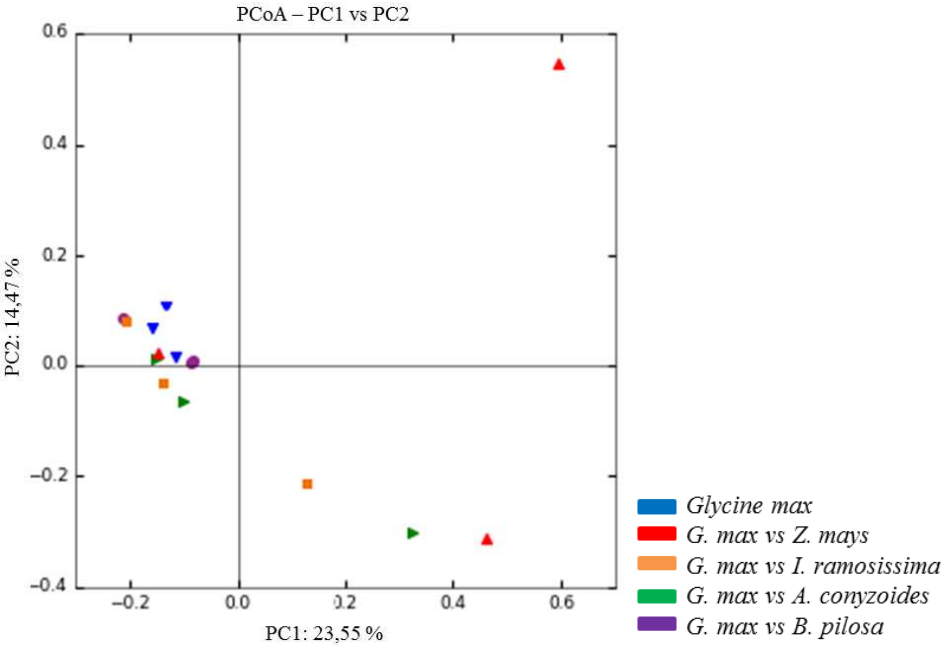

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