LARISSA CASSEMIRO PACHECO MONTEIRO

RIZOSPHERIC MICROBIAL COMMUNITIES OF PLANTS IN COEXISTENCE UNDER DIFFERENT EDAPHIC CONDITIONS

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

Orientador: Maurício Dutra Costa

Coorientadores: Mateus Ferreira Santana
Tiago Antônio de O. Mendes

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Orientador

A Deus,

Aos meus pais, Neuza e Raimundo,

Aos meus irmãos, Marianne e Guilherme,

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Ao meu grande amigo e mestre, Professor Maurício,

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“E lembre-se sempre, na tristeza ou na felicidade, que isso também passa.”

Francisco Cândido Xavier

RESUMO

MONTEIRO, Larissa Cassemiro Pacheco, D.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Comunidades microbianas rizosféricas de plantas em coexistência sob diferentes condições edáficas.** Orientador: Maurício Dutra Costa. Coorientadores: Mateus Ferreira Santana e Tiago Antônio de Oliveira Mendes.

As plantas exercem forte influência sobre os microrganismos do solo através da exsudação radicular. Quando espécies de plantas crescem juntas, as comunidades microbianas rizosféricas resultantes diferem das observadas nas respectivas monoculturas. Esse novo rizobioma pode afetar diretamente a adaptabilidade e as interações ecológicas das espécies vegetais em coexistência. Além disso, condições edáficas do solo, como diferentes níveis de fertilidade e alterações na condutividade elétrica, também promovem alterações no rizobioma e, consequentemente, nas interações estabelecidas pelas plantas em coexistência. Assim, os objetivos deste trabalho foram: I) avaliar as relações ecológicas e os táxons microbianos presentes na rizosfera de plantas daninhas em monocultura e coexistência; II) avaliar como a fertilização do solo afeta as interações ecológicas entre Zea mays e plantas daninhas e as suas associações com os microrganismos da rizosfera, e III) avaliar os resultados das interações entre Zea mays e plantas daninhas em função da condutividade elétrica (CE) do solo associada a alterações na estrutura das comunidades microbianas da rizosfera. Para atingir o primeiro objetivo, um experimento foi conduzido em casa de vegetação, utilizando três espécies de plantas daninhas, Ageratum conyzoides, Ipomoea ramosissima e Bidens pilosa, cultivadas em monocultura e coexistência. O DNA total do solo foi extraído e sequenciado através da plataforma Illumina MiSeq, usando primers específicos para os genes que codificam o rRNA 16S de bactérias e arquéias e a região ITS de fungos. Foram observadas interações de competição entre todas as combinações de plantas daninhas avaliadas e diferenças na diversidade bacteriana rizosférica a depender das espécies em coexistência. Para alguns grupos bacterianos específicos, como os gêneros Bdellovibrio, Flexibacter e Domibacillus e o filo fúngico Ascomycota, foram observadas pequenas alterações na abundância. Para atingir o segundo objetivo, outro experimento foi conduzido em casa de vegetação com duas espécies de plantas daninhas, Amaranthus viridis e Bidens pilosa e a cultura Zea mays, crescidas em monocultivo e coexistência, e submetidas a dois diferentes manejos de fertilidade. O DNA total do solo também foi extraído e o sequenciamento foi realizado como no experimento anterior. O milho apresentou maior capacidade competitiva do que as plantas daninhas sob fertilidade natural do solo e não houve diferença de

competitividade entre as plantas testadas após a fertilização do solo. A diversidade bacteriana foi afetada negativamente pela fertilidade e táxons específicos foram afetados diferentemente pelos dois fatores avaliados, coexistência de plantas e fertilização do solo. A comunidade fúngica não apresentou alteração nas diversidades α e β em nenhuma das amostras analisadas. E para atingir o terceiro objetivo, um experimento semelhante ao anterior foi realizado em casa de vegetação, adotando-se dessa vez, níveis distintos de CE do solo. A interação entre milho e B. pilosa variou de competição para facilitação com o aumento da CE do solo. Após análise do sequenciamento, observou-se que os aumentos na CE do solo alteraram a diversidade bacteriana e a estrutura e composição dos táxons bacterianos e fúngicos, e essas alterações foram associadas com mudanças nas interações ecológicas entre milho e plantas daninhas.

Palavras-chave: Zea mays. Ageratum conizoydes. Amaranthus viridis. Ipomoea ramosissima. Bidens pilosa. Coexistência de Plantas. Diversidade Microbiana. Sequenciamento llumina MiSeq. rRNA 16S e ITS. Fertilidade. Condutividade Elétrica.

ABSTRACT

MONTEIRO, Larissa Cassemiro Pacheco, D.Sc., Universidade Federal de Viçosa, February, 2020. **Rhizospheric microbial communities of plants coexisting under different edaphic conditions.** Adviser: Maurício Dutra Costa. Co-adivisers: Mateus Ferreira Santana and Tiago Antônio de Oliveira Mendes.

Plants exert strong influence on soil microrganisms through root exudation. When two plant species are growing together, the resulting rhizospheric microbial communities differs from that observed in the respective monocultures. This new rhizobiome can directly affect the adaptability and the ecological interactions of the plant species living in coexistence. In addition, soil edaphic conditions such as different levels of fertility and changes in the electrical conductivity, also promote changes in the rhizobiome and, consequently, in the interactions established by the plants in coexistence. Thus, the aims of this work were: I) to evaluate the ecological relationships and the microbial taxons present in the rhizosphere of weeds in monoculture and coexistence; II) to evaluate how soil fertilization affects the ecological interactions between Zea mays and weeds and their associations with rhizosphere microroganisms and III) to evaluate the outcomes of Zea mays-weed interactions as a function of soil EC associated with changes in the structure of the rhizosphere microbial communities. To achieve the first objective, an experiment was conducted in a greenhouse using three weed species, Ageratum conyzoides, Ipomoea ramosissima, and Bidens pilosa that were grown in monoculture and in coexistence. The total soil DNA was extracted and sequenced via the Illumina MiSeq platform using primers specific for the 16S rRNA of bacteria and archaea and the ITS region of fungi. Competition interactions were observed between all tested weed combinations. Differences in the bacterial diversity of competing weeds were observed. For some specific bacterial groups, such as the genera Bdellovibrio, Flexibacter and Domibacillus, and the fungal phylum Ascomycota, smaller changes in abundance were recorded. To achieve the second objective, another experiment was conducted in a greenhouse with two weed species Amaranthus viridis and Bidens pilosa and Zea mays, in monoculture and in coexistence, submitted to two different fertility managements. The total DNA of the soil was also extracted and the sequencing carried out as in the previous experiment. Maize showed greater competitive ability than weeds under natural soil fertility, no difference in competiveness was observed among the plants tested after soil fertilization. Bacterial diversity was negatively affected by fertility and specific taxa were differently affected by the two factors evaluated, plant coexistence and soil fertilization. The fungal community did not

change at α and β diversity in any of the samples analyzed. To achieve the third objective, an experiment similar to the previous one was conducted in a greenhouse, adopting distinct levels of soil electrical conductivity (EC). For the interaction between maize and B. pilosa, the ecological relations between these plants changed from competition to facilitation as EC was increased. Increases in soil EC caused significant changes in bacterial diversity and in the structure and composition of bacterial and fungal taxa. These changes were associated to changes in the outcome of mayze-weed interactions.

Keywords: Zea mays. Ageratum conizoydes. Amaranthus viridis. Ipomoea ramosissima. Bidens pilosa. Plant Coexistence. Microbial Diversity. llumina MiSeq sequencing. 16S rRNA and ITS. Fertility. Electrical Conductivity.

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INTRODUÇÃO GERAL

RIZOBIOMA DE *Zea mays* EM COEXISTÊNCIA COM PLANTAS DANINHAS

1. Introdução

A produção de grãos no Brasil ocupa área de 63,2 milhões de hectares, sendo que 17,5 milhões foram destinados à cultura do milho na safra de 2018/19. O milho é cultivado em duas safras por ano no país, em diferentes sistemas produtivos, e está presente em todos os estado brasileiros (CONAB: Companhia Navional de Abastecimento 2019), tendo como principal destino, as indústrias de rações para animais. O Brasil é o terceiro maior produtor mundial de milho, tendo produzido cerca 99,9 milhões de toneladas na última safra (Carvalho et al. 2014; CONAB: Companhia Nacional de Abastecimento 2019). No entanto, a cultura do milho é amplamente afetada pela coexistência com plantas daninhas.

As plantas daninhas competem com as culturas por espaço, luz, água e nutrientes, interferindo também diretamente na colheita mecânica (Karam & Melhorança 2007; Faria et al. 2014), e os resultados dessa interação irão depender da espécie da planta daninha e da sua densidade nas áreas cultivadas. Para culturas economicamente importantes, a interferência de plantas daninhas pode ocasionar perdas significativas na produção. No caso do milho, essas perdas podem variar de 13,1 a 85 % em situações que não são utilizados nenhum método de controle (Karam & Melhorança 2007).

A rizosfera é definida como a região do solo que recebe a influência direta das raízes, possibilitando o crescimento microbiano. Por meio da exsudação radicular de diversos compostos, as plantas interferem diretamente na comunidade microbiana a ela associada, possuindo capacidade de moldar a estrutura dessa comunidade (Bais et al. 2006; Massenssini 2014; Sasse et al. 2018).

A identificação das possíveis interações entre plantas e os microrganismos do solo que podem beneficiar ou prejudicar a convivência entre plantas daninhas e culturas, bem como quais os táxons microbianos que são mantidos ou recrutados em cada situação de interação, permitirão o desenvolvimento de novas técnicas de manejo, garantindo 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 integrado de plantas daninhas na cultura do milho.

O objetivo desse estudo é o de determinar a composição e a diversidade microbiana rizosférica de plantas daninhas e Zea mays em coexistência com plantas daninhas e em monocultuvio, além de conhecer os possíveis efeitos das caractetísticas edáficas do solo nas

interações planta-planta microrganismos do solo, a partir de experimentos conduzidos em casa de vegetação.

2. Coexistência entre plantas daninhas e culturas

Em uma comunidade de plantas podem ser observadas complexas combinações de interações positivas e negativas que irão determinar a estrutura final dessa comunidade. Vários fatores afetam o balanço entre competição e facilitação de plantas, como estresses abióticos, espécie, porte, estágio de vida e densidade das plantas envolvidas (Callaway & Walker 1997).

A competição pode ser definida como uma interação negativa que ocorre quando os indivíduos ou plantas competem por recursos do ambiente, resultando na redução do crescimento ou da sobrevivência das espécies menos adaptadas (Rizzardi & Wandscheer 2014). A habilidade competitiva de uma dada espécie vegetal está relacionada com a utilização eficiente dos recursos do meio no qual se encontra e a capacidade de manter a produtividade ou suprimir o crescimento das plantas concorrentes, monopolizando recursos e espaço (Rizzardi 2001; Carvalho 2011; Rizzardi & Wandscheer 2014; Aschehoug 2016).

As plantas competem por espaço e, ou recursos, tais como luz, água e nutrientes (Casper & Jackson 1997; Karam & Melhorança 2007; Aschehoug 2016; Mommer et al. 2016). Na competição por espaço acima do solo, a planta pode adquirir uma conformação diferente daquela apresentada quando cresce sem a interferência de outras plantas, mudando o posicionamento de suas folhas, o que a depender da cultura, pode acarretar em sérios prejuízos econômicos para a produção (Karam & Melhorança 2007). Por outro lado, esse tipo de competição pode impulsionar o crescimento, a densidade e o tamanho da copa das plantas, o que interfere diretamente na dinâmica de fluxo de carbono, no armazenamento de carbono e na produtividade (Purves et al. 2007). Uma estratégia importante na competição abaixo do solo é o rápido crescimento para ocupar o espaço disponível e, assim, conseguir captar mais recursos, o que vai depender da taxa de crescimento das raízes, da biomassa, da densidade de raízes finas, da área de superfície total e da profundidade (Casper & Jackson 1997; Mommer et al. 2016).

Fatores importantes na competição das plantas por luz são a altura e a taxa de crescimento vegetal, o arranjo foliar e capacidade das folhas em interceptar a luz (Niklas & Hammond 2013; Aschehoug 2016). Já em situações de competição por água, as plantas geralmente apresentam duas estratégias principais: investem em características que permitam

rápida absorção como o aumento de raízes profundas ou aumentam o uso eficiente da água, por mecanismos de osmorregulação celular, por exemplo, aumentando sua capacidade competitiva (Casper & Jackson 1997; Aschehoug 2016).

A competição por nutrientes pode ocorrer por cada um dos 15 nutrientes minerais essenciais, que diferem entre si quanto ao peso molecular, o estado de oxidação, a valência e a mobilidade dentro no solo, sendo diretamente afetada pela distribuição espacial e temporal do elemento químico (Casper & Jackson 1997; Mommer et al. 2016). O aumento da densidade das raízes confere às plantas maior interface de contato com o solo, podendo conferir vantagens adaptativas importantes em situações de competição (Aschehoug 2016). Os nutrientes mais limitantes nos solos são o N e o P e as plantas que apresentam alta eficiência no uso desses nutrientes podem se sobressair perante às outras (Mommer et al. 2016).

As plantas daninhas são fortes competidoras com as culturas, disputando espaço, luz e recursos, além de interferir no processo de colheita (Karam & Melhorança 2007). Em um conceito abrangente, plantas daninhas são todas as espécies de plantas que, em algum momento, estão direta ou indiretamente prejudicando uma determinada atividade humana, como por exemplo, as plantas que interferem na produção de culturas comerciais, plantas que são tóxicas em pastagens, plantas estranhas em jardins, dentre outras (Silva & Silva 2007; Brighenti & Oliveira 2011). A competição entre culturas e plantas daninhas é responsável por perdas significativas na produção agrícola, e vai depender das espécies de plantas daninhas envolvidas, da cultura, da época e duração da coexistência, da densidade das plantas, das condições do ambiente, bem como das práticas de manejo agrícola aplicadas (Galon et al. 2009; Rizzardi & Wandscheer 2014; Ramesh et al. 2017).

Características específicas que permitem a utilização dos recursos do solo de forma mais eficiente podem ser atribuídas às plantas daninhas, fazendo com que estas se sobressaiam às culturas. Algumas dessas características são, a elevada capacidade de produção de sementes ou outros dissemínulos, o rápido desenvolvimento inicial, a capacidade diferenciada de germinação, a manutenção da viabilidade das sementes no solo por longos períodos, a produção e liberação de substâncias alelopáticas e a adaptação às mais variadas condições ambientais (Silva & Silva 2007; Brighenti & Oliveira 2011).

Em contrapartida, acredita-se que as culturas comerciais apresentam baixa capacidade competitiva devido aos constantes processos de melhoramento que culminaram com mudanças expressivas não apenas na produtividade das principais culturas, mas permitiram também maior eficácia de resposta à adubação, maior adaptabilidade ao estresse hídrico e

maior resistência às pragas e doenças. Essas mudanças podem ter permitido a seleção de cultivares menos dependentes de processos ecológicos e altamente adaptadas às condições artificiais impostas nos plantios, resultando em baixa capacidade competitiva quando as condições de cultivo não são ideais para as culturas (Melo 2012; Massenssini 2014).

O resultado da competição entre plantas daninhas e o milho é determinado pelas espécies que ocorrem na área bem como pela distribuição da comunidade infestante (Karam & Melhorança 2007). Em estudo realizado para avaliar a interferência de seis espécies de plantas daninhas em duas densidades (15 ou 30 plantas) sobre o crescimento de milho transgênico, verificou-se que as plantas daninhas Sorghum arundinaceum e Brachiaria brizantha foram as que mais interferiram na produção de matéria seca do milho. O acúmulo de matéria seca do caule e das folhas foram maiores nas plantas de milho que estavam em competição com 15 plantas daninhas do que naquelas que estavam em competição com 30 plantas daninhas, indicando que a densidade de plantas daninhas interfere diretamente no resultado da competição (Faria et al. 2014). Mais estudos são necessários para entender melhor a competição entre milho e plantas daninhas, bem como quais são os fatores que afetam a competição e em qual direção, favorecendo o desenvolvimento da cultura ou da planta daninha.

Apesar de a competição ser a interação planta-planta mais abordada na literatura, as plantas também podem estabelecer relações positivas e promover o crescimento de outras plantas em coexistência, em um processo chamado de facilitação (Callaway & Walker 1997; Brooker et al. 2008; Michalet & Pugnaire 2016; Matos et al. 2019). Inicialmente, definiu-se que essas interações positivas eram mais comuns em ambientes severos, a chamada Hipótese do Gradiente de Estresse, nos quais as plantas que experimentavam altos níveis de estresse fisiológico se beneficiavam da coexistência com certas espécies e melhoravam o desempenho das plantas residentes, além de aumentar a diversidade biológica nas comunidades de plantas (Valiente-Banuet & Verdú 2007; Brooker et al. 2008; Wright et al. 2014; Michalet & Pugnaire 2016). Nos primeiros estudos sobre facilitação de plantas, foram avaliadas interações planta-planta específicas, em processos de sucessão em comunidades de plantas (Brooket et al. 2008).

Recentemente, os estudos sobre facilitação têm abordado a importância desse tipo de interação na regulação do sucesso das plantas e na composição das comunidades. As interações positivas que podem ser estabelecidas por essas plantas são, por exemplo, a atração de polinizadores, o compartilhamento de recursos através de redes micorrízicas comuns, o

impacto de certas espécies de plantas sobre a disponibilidade de nitrogênio no solo, na diminuição da irradiação direta, na diminuição da secagem do solo, na redução das temperaturas do ar e do solo, no aumento da umidade relativa e o efeito clássico das plantas enfermeiras (Brooker et al. 2008; Wright et al. 2014; Michalet & Pugnaire 2016).

A facilitação pode ser mais comum do que originalmente sugerido e uma vez que competição e facilitação podem ocorrer simultaneamente em quase todas as comunidades vegetais, a força de um tipo de interação pode mascarar a força do outro, sendo o resultado das interações entre as plantas a soma da competição por recursos e a facilitação na melhoria dos fatores abióticos locais. Além disso, as interações positivas e negativas podem mudar de uma para outra em uma única estação de crescimento (Wright et al. 2014). Estudos realizados em casa de vegetação, avaliando a coexistência entre milho e plantas daninhas, tem demonstrado a linha tênue entre as interações positivas e negativas. Monteiro (2016) avaliou Z. mays em coexistência com três espécies de plantas daninhas, Ageratum conyzoides, Ipomoea ramosissima e Bidens pilosa, e observou que as interações variam de acordo com as plantas envolvidas na coexistência, sendo que o milho facilitou o crescimento da daninha I. ramosissima, já nas outras situações de coexistência foi observada competição entre as plantas. A autora ainda demonstrou, que a depender da combinação de plantas, a composição do rizobioma é alterada significativamente. Já em outro estudo realizado com milho em coexistência com Amaranthus viridis e B. pilosa, o milho teve seu crescimento facilitado pelas plantas daninhas, e os autores concluiram que a microbiota do solo estava envolvida no processo de facilitação observado (Matos et al. 2019). Esses resultados revelam a importância do estudo do rizobioma de plantas em coexistência na tentativa de entender como as relações entre plantas podem variar de negativas a positivas.

3. Rizobioma de Z. mays em coexistência com plantas daninhas

Uma parte importante da competição entre plantas ocorre abaixo da superfície do solo (Casper & Jackson 1997), sendo que as raízes exercem papel decisivo no processo competitivo e na determinação do resultado das interações entre plantas (Casper & Jackson 1997; Aschehoug 2016; Mommer et al. 2016). Por meio da exsudação radicular, as plantas liberam no solo diversos compostos orgânicos, e a qualidade e a quantidade de compostos liberados na rizosfera vai depender da espécie da planta, do tamanho, das condições do solo e da atividade fotossintética, sendo determinantes para a estruturação da comunidade microbiana rizosférica ou rizobioma (Bais et al. 2006; Mommer et al. 2016; Sasse et al. 2018).

Os microrganismos do rizobioma possuem efeitos diretos e indiretos no crescimento das plantas, apresentando papel importante na habilidade competitiva de culturas e plantas daninhas, podendo alterar os resultados da interação (Massessini 2014; Massenssini et al. 2014). O resultado da competição entre plantas pode ser afetado também pela densidade de relações mutualísticas que as plantas envolvidas estabelecem com fungos micorrízicos, por exemplo. Espécies de plantas responsivas possuem habilidade competitiva aumentada quando esses microrganismos são abundantes no solo (Abbott et al. 2015).

As plantas de milho possuem rizobioma que difere do conjunto de microrganismos presentes no solo não rizosférico, o que demonstra a habilidade da planta em determinar quais os microrganismos estarão associados a sua rizosfera (Sasse et al. 2018). Além disso, características físico-químicas do solo, a espécie da planta e as situações de competição, são exemplos de condições que interferem diretamente na estrutura do rizobioma das plantas (Massenssini 2014; Massenssini et al. 2015). Santos et al. (2012) ao avaliar o crescimento e o acúmulo de macro e micronutrientes em oitos 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 comparados aos efeitos causados nas plantas daninhas. Acredita-se que os processos de melhoramento genético das culturas contribuíram com esse resultado, demonstrando que estas são menos dependentes das associações com os microrganismos edáficos do solo se comparadas às plantas daninhas.

Massenssini (2014) estudou o impacto da competição entre duas culturas (Zea mays L. e Glycine max (L.)) e três espécies de plantas daninhas (Ageratum conyzoides L., Ipomoea ramosissima (Poir.) Choisy e Bidens pilosa L.) na estrutura da comunidade microbiana rizosférica. O autor concluiu, após a determinação dos perfis de T-RFLP da comunidade microbiana, que a estrutura do rizobioma observado nos tratamentos de competição diferem significativamente daquele observado quando as plantas estão em monocultivo. Além disso, o autor observou que a planta daninha A. conyzoides é um competidor fraco, dependente de alta diversidade microbiana para crescimento ótimo, enquanto o milho foi um competidor forte, uma vez que provavelmente apresenta baixa dependência da diversidade microbiana do solo.

Em continuação ao estudo anterior, Monteiro (2016) realizou o sequenciamento de amplicons 16S de bactérias e arquéias e ITS de fungos do solo rizosférico do milho em monocultivo e em coexistência com as plantas daninhas, para avaliar quais táxons são mantidos ou recrutados na comunidade microbiana rizosférica das plantas em tais situações.

Foi possível concluir que mais do que a diversidade ou a riqueza de Unidades Taxonômicas Operacionais (UTOs), a composição dos microrganismos na rizosfera é mais importante no processo e no resultado das interações cultura-daninhas. Para Bactérias e Fungos, alguns táxons demonstraram diferenças significativas na abundância entre os tratamentos e UTOs são exclusivas de combinações específicas de plantas. A capacidade das plantas em moldar seu rizobioma e a presença dessas UTOs exclusivas podem conferir vantagens adaptativas às plantas em situações de competição. No entanto, estudos são necessários para avaliar melhor as funções desses grupos específicos de microrganismos e também entender a dinâmica da determinação do rizobioma em função de alterações físico-químicas no solo, por exemplo, na interação de plantas daninhas e culturas.

Dentre os microrganismos alterados no rizobioma de plantas em competição, encontram-se aqueles reconhecidos como promotores de crescimento de plantas, que nessa situação de estresse, podem conferir habilidades competitivas às plantas envolvidas, como é o caso dos Fungos Micorrízicos Arbusculares (FMA), pertencentes ao filo Glomeromycota (Monteiro 2016). Os FMAs são reconhecidos por estabelecer associações mutualísticas com mais de 80 % das plantas terrestres, formando uma rede micorrízica no solo que permite o aumento da captação de nutrientes, principalmente nitrogênio e fósforo, e água do solo (Wagg et al. 2011; Klabi et al. 2014; Mommer et al. 2016; Weremijewicz et al. 2016; Shi et al. 2017). A presença de FMAs pode alterar significativamente a habilidade competitiva de plantas em competição por potencialmente aumentar o pool de recursos disponíveis abaixo do solo, tornando-os mais acessíveis (Wagg et al. 2011; Klabi et al. 2014; Lin et al. 2015; Aschehoug 2016; Shi et al. 2017).

Evidências sugerem que a presença de interações de facilitação promove comunidades microbianas do solo maiores, mais diversificadas, com alta atividade e redes micorrízicas maiores, em comparação com solos adjacentes sem processos de facilitação (Michalet & Pugnaire 2016). Essas comunidades microbianas possuem efeitos positivos no estabelecimento e no crescimento das espécies de plantas envolvidas, no entanto mais pesquisas são necessárias para entender a função desses microrganismos do solo e os mecanismos envolvidos nos processos de facilitação entre as plantas. Em um experimento conduzido com a alteração intencional da microbiota do solo por esterilização por autoclavagem e reconstituição da microbiota por meio de uma suspensão de solo, os autores observaram que o milho em coexistência com Bidens pilosa demonstrou maior incremento de massa seca no solo esterilizado do que no solo com microbiota reconstituída. A intensidade

desse processo de facilitação vai depender de como cultura-daninha modelam a estrutura do rizobioma e das espécies de plantas daninhas envolvidas. Além disso, os autores concluem que a comunidade microbiana rizosférica associada às plantas em coexistência é dependente do conjunto inicial de microrganismos do solo e das alterações que acontecem durante o decorrer dessa coexistência (Matos et al. 2019).

As plantas daninhas podem estabelecer interações positivas com diversos grupos microbianos no solo, incluindo os FMAs (Fialho 2013; Massenssini et al. 2014b). Um estudo da competição de Zea mays com Bidens pilosa e Eulesine indica (L.) Gaerth., demonstrou que as plantas daninhas possuíam maior colonização por fungos micorrízicos do que a cultura (Fialho 2013). Já foi observada também, a presença de FMAs em 41 espécies de plantas daninhas em estudo realizado com o total de 50 espécies (Massenssini et al. 2014b). Em um estudo mais recente, 47 espécies de plantas daninhas, pertencentes a 25 famílias botânicas foram avaliadas quanto a presença de FMAs e de Dark septate endophytes (DSE) e foi constatado que todas as espécies estavam colonizadas com FMA e cerca de 30 %, associadas com DSE, tendo sido esse trabalho o primeiro relato da ocorrência de micorrizas arbusculares em 21 espécies de plantas daninhas (Candido 2018). Esses resultados ressaltam a importância das interações entre plantas daninhas e FMAs, demonstrando que a presença de fungos micorrízicos pode conferir vantagens competitivas às plantas daninhas sobre as culturas nos sistemas agrícolas.

Compreender quais táxons estão presentes no rizobioma de culturas economicamente importantes em coexistência com plantas daninhas, torna-se fundamental para o entendimento das relações ecológicas estabelecidas por essas plantas e para o estabelecimento de práticas de manejo que sejam mais integrativas. Nesse aspecto, faz-se fundamental também, entender quais os fatores que mais afetam a coexistência cultura-daninha buscando maneiras mais adequadas para o controle de daninhas nos agrossistemas.

4. Fatores edáficos que afetam a coexistência entre plantas daninhas e culturas

As características físico-químicas do solo podem interferir na intensidade e no resultado das interações entre as plantas. Os fatores abióticos, além de exercerem papel decisivo na composição das comunidades vegetais, possuem papel indireto na modulação das interações entre as plantas (Massenssini et al. 2015). 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 & Silva 2007). O tipo de solo, a

intensidade luminosa, os regimes hídricos, os níveis de fertilidade e a salinidade do solo também podem afetar o desenvolvimento das espécies vegetais e, consequentemente, afetar a coexistência das plantas (Melo et al. 2014; Laurent et al. 2017; Florentine et al. 2018).

Mais de 800 milhões de hectares de terra em todo o mundo são afetadas pela salinidade. O estresse salino gera nas plantas estresse osmótico elevado, toxicidade por íons de sódio ou cloreto, além de prejudicar a nutrição da planta, uma vez que a alta salinidade diminui a capacidade das plantas de captar água e nutrientes (Medeiros et al. 2013; Farooq et al. 2015). Em experimento realizado com Echium plantagineum, uma planta daninha que foi introduzida como planta ornamental na Austrália e que atualmente gera grandes prejuízos na produção agrícola, observou-se que a disponibilidade de umidade e a elevada salinidade podem interferir na germinação das sementes dessa planta (Florentine et al. 2018).

Estudos têm sido realizados para entender como a salinidade afeta a competição de plantas halofílicas em ambientes salinos, como os pântanos (Greiner La Peyre et al. 2001; Greenwood & MacFarlane 2009; Medeiros et al. 2013). Um experimento em casa de vegetação foi realizado para avaliar o efeito da salinidade sob o crescimento e as interações de duas espécies de plantas, Juncus kraussii; uma espécie nativa da Autrália, e Juncus acutus, uma espécie exótica, ambas as espécies típicas de ambientes costeiros. Os autores observaram que as plantas apresentaram diminuição da altura e biomassa total em função do aumento da salinidade e os resultados demonstram que a competição varia com a salinidade do solo, a depender da tolerância relativa de cada espécie à salinidade (Greenwood & MacFarlane 2009). No entanto, o efeito direto da salinidade na coexistência das plantas daninhas com culturas comerciais ainda não é conhecido, nem como sutis alterações na condutividade elétrica do solo, que acontecem durante o início do processo de salinização pode afetar as interações entre cultura-daninha e o rizobioma do solo.

A colonização das raízes das plantas por fungos micorrízicos arbusculares (FMA) é diminuída em situações de estresse salino. No entanto, plantas micorrizadas apresentam maior crescimento e desempenho em situações de salinidade (Farooq et al. 2015). Bactérias endofíticas promotoras de crescimento de plantas ou bactérias que colonizam as raízes, também possuem a capacidade de aumentar o crescimento ou promover resistência em situações de estresse abiótico (Akhtar et al. 2015; Farooq et al. 2015). Já foi demonstrado que a inoculação de duas espécies de bactérias promotoras de crescimento, Burkholderia phytofirmans e Enterobacter sp., associadas com o uso de biochar, possuem a capacidade de diminuir os efeitos negativos da salinidade sobre plantas de milho possivelmente por diminuir

a concentração de Na^+ no xilema das plantas (Akhtar et al. 2015). Porém, mais estudos são necessários para investigar quais são os táxons de microrganismos rizosféricos que permanecem associados às plantas daninhas e às culturas em situações de coexistência sob alteração na condutividade elétrica do solo e sob estresse salino.

A aplicação de fertilizantes nos solos pode beneficiar as plantas daninhas, uma vez que algumas espécies possuem a capacidade de absorver nutrientes mais rapidamente do que as culturas e ainda acumulá-los em maiores níveis em seus tecidos (Zimdahl 2007; Kaur et al. 2018). No entanto, o manejo adequado de nutrientes tem papel fundamental na redução de plantas daninhas em sistemas agrícolas. O aumento da fertilização do solo pode, em alguns casos, aumentar o efeito dos herbicidas e exercer pressões seletivas, reduzindo a abundância de algumas espécies (Kaur et al. 2018). Ali et al. (2016) demonstraram que a adição de NPK aumentou significativamente a biomassa de plantas daninhas associadas à cultura do milho.

Melo et al. (2014) avaliaram a biomassa microbiana, a taxa de respiração e o quociente metabólico do solo cultivado com milho em competição com seis espécies de plantas daninhas, sob diferentes níveis de fertilidade. Os autores concluíram que a biomassa e a atividade microbiana são alteradas pelas espécies das plantas, pela competição e pelo manejo de fertilidade do solo e sugeriram que as alterações na atividade microbiana pode ser uma estratégia utilizada pelas espécies de plantas para minimizar os efeitos da competição.

Embora existam evidências de que a comunidade microbiana da rizosfera de plantas em coexistência é alterada mediante diferentes níveis de fertilizante e salinidade do solo, não se sabe sobre quais táxons são perdidos ou recrutados nessas situações e como tais alterações podem interferir nos resultados das interações entre plantas daninhas e culturas. Avaliar o efeito do aumento da condutividade elétrica dos solos e da fertilidade na coexistência de culturas com plantas daninhas e na diversidade de microrganismos rizosféricos é fundamental para entender melhor tais interações.

Conhecer quais os microrganismos estão presentes na rizosfera de plantas em coexistência e como esses são afetados por alterações nas características físico-químicas do solo é fundamental para entender em quais situações a cultura pode ser favorecida sobre a planta daninha. Consequentemente, é importante para a manipulação e a implementação de programas de manejo que sejam mais integrativos, levando em consideração a importância e as funções dos microrganismos do solo na manutenção das lavouras de milho, frente à existência e prejuízos causados por plantas daninhas.

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CAPÍTULO 1

MICROBIAL DIVERSITY AND COMPOSITION IN THE RHIZOSPHERE OF WEEDS IN COEXISTENCE

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Abstract

Aims Plants exert strong influence on soil microrganisms through root exudation. When two plant species are growing together, the resulting rhizospheric microbial communities differs from that observed in the respective monocultures. This new rhizobiome can directly affect the adaptability and the ecological interactions of the plant species living in coexistence. Here, we have evaluated the ecological relationships and the microbial taxons present in the rhizosphere of weeds in monoculture and coexistence.

Methods The experiments were carried out under greenhouse conditions. Three weed species Ageratum conyzoides, Ipomoea ramosissima, and Bidens pilosa, were grown in monoculture and in coexistence pairs. Total DNA from microbial populations in the rhizosphere soil was extracted, followed by PCR amplification and sequencing using the Illumina MiSeq platform.

Results The coexistence treatments led to competive interactions among the weed species tested. A. conyzoides was the most benefited with significant increments in dry matter grown in coexistence with the other species. Differences in the bacterial diversity of competing weeds were observed. For some specific bacterial groups, such as the genera Bdellovibrio, Flexibacter and Domibacillus, and the fungal phylum Ascomycota, small but significant changes in abundance were recorded when comparing all treatments. Our results show that plant dependency on soil microorganimsms is more related to the specific composition of the rhizobiome and on the speficific functional roles that certain microbes play in the rhizosphere rather than on microbial diversity per si.

Conclusions Coexistence of the weeds tested changes the rhizosphere microbial communities. These were associated with the establishment of distinct ecological interactions between the plant species studied and their competitive ability.

Keywords: Ageratum conizoydes, Bidens pilosa, Ipomoea ramosissima, Plant Coexistence, llumina MiSeq sequencing, 16S rRNA and ITS.

Abbreviations

OTUs - Operational Taxonomic Units

RII - Relative Interaction Intensity Index

A.co or A.conizoydes - Ageratum conyzoides L.

I.ra or I.ramosissima - Ipomoea ramosissima (Poir.) Choisy

B.pi or B.pilosa - Bidens pilosa L.

x - Indicate coexistence treatments

1. Introduction

There is no universal definition of weeds, but in general, the term refers to plants of undesired occurrence that can cause negative impacts on human activities (Pitelli 1987; Beltrão and Melhorança 1998; Pitelli and Durigan 2001; Silva and Silva 2007; Brighenti and Oliveira 2011; Silva and Silva Lima 2012; Stewart 2017). The main species of plants considered weeds in plantations in Brazil are highly aggressive, presenting rapid adaptability, establishment, and perpetuation (Santos et al. 2012). When in coexistence with economically important crops, these plants reduce agricultural efficiency, increasing production costs (Silva and Silva 2007; Brighenti and Oliveira 2011).

Weeds often occur in disturbed environments and their interactions with the soil microbiome, especially the associations with mutualistic microorganisms and plant growth-promoting, can directly affect their adaptability and competitive ability (Trognitz et al. 2016). Recent studies have demonstrated the importance of weed interactions with soil microorganisms (Santos et al. 2012, 2013; Massenssini et al. 2014a, b; Melo et al. 2014; Matos et al. 2019). Weeds have already been reported in association with growth promoting microorganisms such as mycorrhizal and dark sepatate endophytic fungi, and with microorganisms with potential for phosphate solubilization in the rhizosphere. Weeds have also been shown to establish positive feedback interactions with the soil microorganisms, suggesting a high dependence on host-specific root microbiota (Santos et al. 2013; Massenssini et al. 2014a, b; Trognitz et al. 2016). Weed-microbiota associations can contribute to important weed traits such as growth, abiotic stress tolerance, disease resistance, nutrient acquisition, and biomass production (Trognitz et al. 2016). Despite the great strides that have been made in the area, a better understanding of the association of microbial taxa to specific weed species and their functions in plant adaptability and competitiveness is still needed.

In fact, plants select a specific rhizospheric microbial community which varies in composition, abundance, and activity depending on the plant species (Caper and Jackson 1997; Hodge and Fitter 2013; Aschehoug 2016; Mommer et al. 2016; Hortal et al. 2017).

Weeds can be more dependent on associations with soil microorganisms than crops (Santos et al. 2012; Massenssini 2014; Melo et al. 2014). Weeds, such as Bidens pilosa, have already been shown to harbor higher microbial biomasses in the rhizosphere, being more affected by soil fumigation than crops. On the other hand, Ageratum conyzoides is more dependent on high soil microbial diversity than other weeds and crop species (Santos et al. 2012; Massenssini 2014; Melo et al. 2014). Additionally, a determining factor for plant performance during competition is the ability of the best competitor to be more determinant for the structure of rhizospheric microbial communities than the weakers ones (Hortal et al. 2017). When in competition with soybean, B. pilosa has a greater ability to shape the structure of the soil rhizobiome making it closer to that of the weed itself in monoculture (Massenssini 2014). Differently from B. pilosa, A. conyzoides has shown a lesser influence on the structure of the soil microbiome when in competition with B. pilosa or Ipomoea ramosissima (Massenssini 2014).

The structure of the soil rhizobiome is fundamental for the determination of the ecological relations developed by plants in coexistence. In an experiment conducted with the intentional alteration of soil microbiota by soil autoclaving and microbiota reconstitution, the authors observed that maize competing with B. pilosa showed greater dry matter increment in sterile soil than in the soil with reconstituted microbiota. The intensity of this facilitation process was dependent on how the weeds shaped rhizobiome structure and on the weed species involved (Matos et al. 2019).

The interactions between plants and their rhizobiome are so close that together they can be considered as a whole entity (Hortal et al. 2017). The study of weed ecology in agroecosystems still requires important answers that will allow a clear understanding of which microbial taxa are associated with coexisting plant roots and their functions. This will allow the development of effective microbial-based weed management techniques. Therefore, the aim of this work was to evaluate the ecological relationships and the microbial taxons present in the rhizosphere of weeds in monoculture and coexistence.

2. Materials and Methods

2.1. Assembly of the experiment

The experiments were conducted under greenhouse conditions at Departamento de Microbiologia, Universidade Federal de Viçosa, Minas Gerais, Brazil. Three weed species, Ageratum conyzoides L., Ipomoea ramosissima (Poir.) Choisy, and Bidens pilosa L., 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 collected at an experimental area belonging to Universidade Federal de Viçosa.

The seeds 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 November/2012 to January/2013. The experiment was conducted in a randomized block design with six treatments corresponding to three monocultures and three competition treatments, with three replicates. In the monoculture treatments, the plant species were grown separately, with two individuals per pot. In the coexistence treatments, two plants of a species were combined with two plants of the coexisting one. 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. After the growth period, the plants were cut at the 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 coexistence treatments, the soil was collected in the regions where the roots were in contact with each other. In other words, where there was a rhizosphere common to both species.

2.2. Plant interaction analysis

The Relative Interaction Intensity Index (RII) was used to determine the intensity of interactions between weeds. This index was calculated as described by Armas et al. (2004): RII = (total dry matter of plant growing with competitor - total dry matter of plant growing in monoculture)/(total dry matter of plant growing with competitor + total dry matter of plant growing in monoculture). The RII is a measure of the strength of interaction between species and ranges from −1 to 1, and is symmetrical around zero. Negative values indicate suppressive effects due to resource competition or other antagonistic effects; positive values indicate facilitation effects.

2.3. Extraction of DNA total from soil and sequencing by Illumina MiSeq

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. The DNA samples were quantified using Qubit® 2.0 Fluorometer (Invitrogen by Life Technologies) and lyophilized.

The samples were sent to Argonne National Laboratory, USA; where the steps of amplification, library preparation, and sequencing were done. The V4 region of the bacterial and archaeal 16S rDNA gene was amplified using the 515f/806r primer set (Caporaso et al. 2012), and the fungal ITS region from rDNA was amplified with primers ITS2 and ITS1f (Smith and Peay 2014). The sequencing was realized using the Illumina MiSeq, with reads paired-end 150 x150 bp and the 16S and ITS amplicons were sequenced in separate runs.

2.4. Processing and analyses of sequencing data

The raw data were processed as described by the Brazilian Microbiome Project protocol (Pylro et al. 2014a, b) using the BMP Operating System (BMPOS) (Pylro et al. 2016) and the results were analyzed by the Quantitative Insights Into Microbial Ecology Software (Qiime v.1.9.1) (Caporaso et al. 2010). For the analyses of 16S rRNA gene sequencing data, briefly, the quality filtering of the sequences were performed using VSEARCH v.2.3.4 (Rognes et al. 2016). The truncated reads shorter than 240 bp and the reads with number of expected errors higher than 1.0 were discarded. The filtered reads were dereplicated and unique sequences (singletons) were also removed using VSEARCH. Sequences with more than 97 % similarity were considered the same Operational Taxonomic Unit (OTU), and comparisons were made with the UPARSE v.7.1b (Edgar 2013). The chimeric sequences were identified and removed using UCHIME (Edgar et al. 2011). After clustering, the sequences were aligned and the taxonomy of each OTU was assigned using the reference database SILVA release 132 by the uclust method.

For the analysis of fungal ITS region sequencing data, only the forward file (R1.fastq) was used. The quality filtering of the sequences were performed using USEARCH 8.1 (Edgar 2010) discarding reads with number of expected errors higher than 0.5. After the dereplication, the ITSx software was used to extract only ITS1 region from fungi. The truncated reads shorter than 140 bp was removed and the chimeric sequences were removed using UCHIME (Edgar et al. 2011) and USEARCH. Sequences with similarity greater than 97 % were grouped in the same OTU and the taxonomy of each was assigned using UNITE (Kõljalg et al. 2013) as the reference database by the uclust method.

2.5. Diversity and statistical analysis

Alpha diversity was calculated using the Qiime software and three metrics were calculated: Chao, Simpson and Shannon index, at genera level. The rarefaction curves were

generated based on the number of observed OTUs in each treatment. For beta diversity, Multidimensional Scaling (MDS) were carried out based on the presence and absence of OTU sampling using the Jaccard's distance. For the Venn diagram, the OTU that was present in at least two of the three replicates and with read number greater than or equal to 2 were considered as present in a sample, and the diagrams was created with the online tool jvenn (Bardou et al. 2014). The clustered hierarchical heatmaps were made only with OTUs shared among all samples. The construction of the graphs, multivariate analyzes, and statistics were done using the R software, package ggplot2 (Wickham and Chang 2009), GraphPad Prism 7, and PAST. To compare the RII index between the samples a one-way ANOVAs was used followed by the Tukey's test, to compare the dry matter of weeds in monoculture and coexistence a one-way ANOVAs was used followed by correction with the Dunnett´s test, always using one of the monocultures as a reference. To compare the diversity indexes and abundance of OTUs among the samples a one-way ANOVAs was used followed by correction by Bonferroni´s test.

3. Results

3.1. Competiton between weeds

The Relative Interaction Intensity Index (RII) varied according to the combination of plants in coexistence (Fig. 1). Negative values indicate competition interaction between plants and the more negative the values, the greater the suppressive effect among coexisting plants. Less negative values indicate that plants have greater capacity to withstand competition (Liao et al. 2015). Thus, our results demonstrate that the competition between A. conizoydes and I. ramosissima and between A. conizoydes and B. pilosa were the most intense (Fig. 1), and the dry matter of A. conizoydes was the most significantly affected in all competition combinations when compared to the dry matter obtained in monoculture ($p<0.01$) (Fig. S1). The weeds I. ramossima and B. pilosa were the ones that best supported the interference of the coexisting plant (Fig. 1). The dry matter of B. pilosa showed a significant decrease only when in competition with A. conizoydes ($p<0.05$) and I. ramosissima did not have its dry matter significantly affected in the combinations tested ($p>0.05$), when compared to the dry matter recorded in the monocultures (Fig. S1).

3.2. Sequencing summary

The sequencing using the Illumina MiSeq platform of weed rhizosphere microbial community in the treatments with monoculture and those with competition generated 329,836 sequences for the 16S rRNA gene. After quality filtering and removal of singletons and chimera by UCHIME, 295,269 (89.52 %) reads remained, with an average of 16,403.833 reads per sample. These reads, 290,946 (98.54 %) were classified as OTUs belonging to phyla of Bacteria, 1,834 (0.62 %) reads were classified as phyla of Archaea, and 2,489 (0.84 %) reads were attributed as Unassigned (data not shown).

For the ITS region from rDNA, sequencing generated 2,065,107 sequences. After quality filtering, singletons removal, extraction by ITSx, and chimera removal by UCHIME, 746,736 (36.11 %) reads remained, with an average of 44,110 reads for sample. These reads, 565,378 (75.34 %), were classified as OTUs of fungal phyla by UNITE database, 8,776 (1.17 %) reads were classified as Unidentified Fungi, and 176,245 (23.49 %) reads were attributed as Unassigned.

The rarefaction curve tended to reach a plateau for Bacteria and Archaea (Fig. 2a). The Good's coverage index values showed that more sequences would be necessary to retrieve all the sequence types present to better express the diversity within the treatments analyzed, mainly for Archaea, since this index was lower than that observed for Bacteria (Table S1). The rarefaction curves reached a plateau for Fungi, indicating that the sequencing was reasonable to reveal the total number of types of sequences in the samples (Fig. 2b), and the Good's coverage index values were higher than 99 % for all samples (Table S1).

3.3. Comparison of the rhizospheric microbial community structure between different weed plants

For analyzing the relationship between soil treatments, Multidimensional Scaling (MDS) based on a presence and absence matrix of OTUs with the Jaccard's distance was used (Fig. 2c and 2d). A hierarchical clustering heat map was buit to determine whether single or shared OTUs for all treatments interfere with the distribution of samples for which the values of relative abundances for each shared OTU were indicated by color intensity (Fig. 2e and 2f).

For the bacterial and archaeal communities, the MDS showed that the samples were separated into two groups by the Dimension 2, demonstrating that the monoculture of I. ramosissima is the most dissimilar among all the samples (Fig. 2c). The heat map based only on the shared OTUs allowed to observe that the shared microbiota between the samples does

not define their distributions, these being more influenced by the exclusive OTUs of each sample (Fig 2e).

For the fungal communities, it can be noted that soil samples from the same treatment did not grouped together. So neither the MDS analysis nor the heat map were sufficient to reveal dissimilarities or similarities for the rhizosphere soil samples for the fungal community (Fig. 2d and 2f).

3.4. Richness and diversity analyses of OTUs

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), showed that there was no significant difference among treatments by the ANOVA test ($p>0.05$) for the bacterial and archaeal communities (Fig. 3a and Table S2). However, it is possible to observe that the rhizospheric soil of the competition between I. ramosissima and A. conizoydes presented the highest number of OTUs among all treatments, 782 in total, with 96 exclusive OTUs. The treatment that presented the lowest number of OTUs was the monoculture of A. conizoydes, with the total of 496 OTUs, with 29 exclusive ones. In addition, 336 OTUs of bacteria and archaea were shared among all treatments (Fig. 2g). For the fungal community, the Chao index did not present significant differences between samples (Fig. 3b and Table S2). However, interestingly, the treatment with the greatest number of OTUs for fungi was the monoculture of A. conizoydes with 118 OTUs, with 20 exclusive OTUs (Fig. 2h). The competition between I. ramosissima and B. pilosa presented the lowest number of OTUs, 83, with 11 exclusive OTUs (Fig. 2h). Thirty three fungal OTUs were shared among all treatments (Fig. 2h).

The Simpson diversity index did not show a significant difference between treatments (Table S2) for bacterial, archaeal (Fig. 3c), and fungal (Fig. 3d) communities The Shannon diversity index, based on species richness, considering the number and the abundance of individual taxa (Chiarucci et al. 2011), presented a significant difference between the rhizosphere soils analyzed for the bacterial and archaeal communities (Fig. 3e and Table S2). The highest diversity was found in the monoculture treatments of I. ramosissima and competition between I. ramosissima and A.conozoydes, and the lower diversity in A. conizoydes monoculture (Fig. 3e), which corroborates the values of OTUs presented previously, since this index takes into account the richness of species. As for the fungal

community, no significant difference was observed for the Shannon diversity (Fig. 3f and Table S2).

The Figure S2 presents the exclusive and shared OTUs for the treatments that presented significant changes in the structure of the microbial community. To better understand these changes, we have compared the monocultures and their respective competition, for the weeds A. conizoydes and B. pilosa (Fig. S2a and S2b) and for A. conizoydes and I. ramosissima (Fig. S2c and S2d), since A. conizoydes was the plant with less competitive ability and with a lower increment of dry matter among the three evaluated plants.

3.5. Taxonomic composition of the microbial community

The OTUs of Bacteria were classified in 27 different phyla, 223 orders of which 136 (61 %) were assigned to known taxa, and 720 genera, of which 311 (43.2 %) received taxonomic attribution (Figure 4). Eight-seven orders (39 %) and the 409 genera (56.8 %) that did not receive taxonomic attribution are those taxa classified as “uncultured bacterium”, “metagenome”, “unassigned” and “other”. Regarding Archaea, OTUs were classified in 3 phyla and 7 orders of which only 3 received taxonomic attribution, and 10 genera which only one being assigned to Candidatus nitrososphaera belonging to the Thaumarchaeota phylum (data not shown).

The bacterial profile at the phylum level was very similar among all the samples of rhizospheric soil analyzed (Fig. 4a). The most abundant bacterial phyla, with more than 1 % of relative abundance, corresponded to Proteobacteria (33.7 %), Actinobacteria (23.3 %), Acidobacteria (16.9 %), Verrucomicrobia (7.6 %), Chloroflexi (6.6 %), Planctomycetes (2.2 %), Gemmatimonadetes (1.8 %), Firmicutes (1.8 %) and Bacteroidetes (1.3 %) (Fig. 4a). This represented 95.2 % of the total abundance of bacterial OTUs (Fig. 4a). Achaea corresponded only to 0.62 % of the reads classified at the phylum level (Fig. 4a). “Unassigned” reads correspondend to 0.84 %, while 3.32 % were grouped into “Others (Fig. 4a). These groups did not show significant differences in the abundances between treatments ($p > 0.05$) (data not shown).

At the order level, reads with more than 1 % of relative abundance were also analyzed (Fig. 4b). While at the genus level, the 20 most abundant reads were selected (Fig. 4c). Among these, no significant differences in the abundances ($p > 0.05$) were observed. All other OTUs classified at genus level were compared and figure S3 shows those that demonstrated

significant differences in abundance between samples. For Archaea, no OTU at any taxonomic classification level showed a significant difference in relative abundance between the rhizospheric soil samples.

For fungi, the OTUs were classified in 6 different phyla, 67 orders, of which 54 (80.6 %) received taxonomic attribution, and 208 genera, of which 97 (46.6 %) were assigned to specific taxa. The orders and genera that did not receive taxonomic attribution were classified as “Unassigned”. The average relative abundance of the phyla with more than 1 % of relative abundance, were Ascomycota (62.6 %), Mortierellomycota (3.4 %), Basidiomycota (1.2 %), Glomeromycota (1.1 %), making up 68.3 % of the abundance of fungal OTUs. The reads that were not taxonomically classified were said to be “Unassigned” and the others reads that were classified within the Fungi domain but had no taxonomic attribution at the phylum level were grouped into “Others” (Fig. 5a). From these phyla, Ascomycota presented a significant difference in the abundances between treatments ($p<0.05$) and showed lower abundance in I. ramosissima monoculture (Fig. S4).

At the order level, reads that presented relative abundance greater than 1 % (Fig. 5b) were analyzed and the 20 most abundant genera were selected (Fig. 5c). No significant difference in the abundance between the rhizospheric soil samples were observed at the genus level ($p>0.05$). All other OTUs classified at the genus level were also compared and did not reveal significant differences in abundance between samples ($p>0.05$) (data not shown).

4. Discussion

In this study, we have used the high throughput analyses by Illumina MiSeq sequencing to evaluate the richness, diversity, and to taxonomically characterize Bacteria, Archaea, and Fungi associated with the rhizosphere of weeds in monoculture and in competition. The results of our sequencing indicate that the approach used was sufficient to detect differences in microbial community in the rhizosphere of plants depending on the different competing species. This study is the first report on the use of this approach to evaluate weed interactions.

4.1. Competiton between weeds and alteration of diversity bacterial index

The RII was used to evaluate the ecologic interations of the plants and, in all combinations tested, negative interactions of competition were observed. Competition interactions with A. conizydes were the most intense (Fig. 1) and this plant was the one most

affected in dry matter production ($p<0.01$), when compared to its growth in monoculture (Fig. S1). Interestingly, among all treatments, A. conizoydes monoculture presented the lowest OTU number for bacteria and archaea and the highest for fungi (Fig. 2g and 2h). The rhizobiome change during competition, associated with increased OUT numbers of bacterial and archaea, ranged from 496 (44 exclusive) OTUs, in A. conizoydes monoculture to 759 (164 exclusive) OTUs in the competition between A. conizoydes and B. pilosa. The number of fugal OTUs decresed from 118 (31 exclusive) in monoculture to 101 (23 exclusive) in the same competition treatment (Fig. S2). This decrease in the number of fungal OTUs was associated with decreased dry matter production and lower competitive ability of A. conizoydes. We hypothesize that the observed changes in the soil microbial communities were associated with the lowest performance presented by A. conizoydes when compared to other plants tested. In fact, changes in the structure of bacterial soil and fungal communities have been reported to alter the intensity of plant interactions (Hodge and Fitter 2013; Hortal et al. 2017; Matos et al. 2019), as already shown for maize coexisting with Amaranthus viridis and B. pilosa (Matos et al. 2019).

Through root exudation, plants release organic compounds of low and high molecular weight into the soil, and the quality and quantity of these compounds are dependent on the plant species, cultivar, size, and photosynthetic activity, and on the soil conditions, being determinant for the rhizobiome structure (Bais et al. 2006; Bakker et al. 2012; Hodge and Fitter 2013; Zahar Haichar et al. 2014; Huang et al. 2014; Mommer et al. 2016; Sasse et al. 2018). Competition between plants directly affects the quality and quantity of compounds released into the rhizosphere and these compounds, besides serving as a growth substrate for the microorganisms associated with the roots, act as chemical signals that attract or repel specific microorganisms, the roots having a selective role in the structure of rhizospheric microbial communities under such situations (Bakker et al. 2012; Wu et al. 2012; Hortal et al. 2017; Christia and Bever 2018; Sasse et al. 2018). For the A. conizoydes monoculture and the competition between this species and B. pilosa, only 37 OTUs of bacteria and archaea and 13 of fungi were shared, while for the B. pilosa monoculture and the competition between A. conizoydes and B. pilosa, 161 OTUs of bacteria and archaea and 10 of fungi were shared (Fig. S2a and S2b). Especially for bacteria and archaea, the competition rhizobiome shares more OTUs with B. pilosa monoculture (161 OTUs) than with that of A. conizoydes, demonstrating the greater influence of B. pilosa in determining the structure of the bacterial and archaeal communities during competition (Fig. S2a). In fact, the invasion of B. pilosa has been shown

to be more influenced by the microbiota than by the nutritional characteristics of the soil, and the association of this species with soil mutualists is related to its greater competitive ability compared to the shrub Saussurea deltoidea (Cui and He 2009). Thus, the changes in the soil microbial communities reported in our work must have had a direct negative impact on the competitive ability and dry matter production of A. conizoydes when cultivated together with the other plants tested.

Our results for Shannon diversity show that the monoculture of A. conizoydes presents the lowest diversity of bacteria and archaeas when compared to the other treatments (Fig. 3e). The increase in OTU numbers for the bacterial and archaeal communities in the competition treatments between A. conyzoides and B. pilosa, may have negatively affected the accumulation of dry matter and the competitive ability of A. conyzoides. Under the same experimental conditions and using T-RFLP analyses to asses changes in soil microbial communities, Massenssini (2014) concluded that the microbial community structure of the competition between A. conizoydes and B. pilosa differs from the monocultures structure. The author considered A. conizoydes a weak competitor based on the decrease in microbial diversity revealed by T-RFLP richness, when he compared the competition treatments with A. conizoydes monoculture. Our results, based on the Illumina MiSeq sequencing, demonstrate a deeper view of this situation, proving the contrary, that there was an increase in the diversity by the Shannon index and the number of OTUs for bacteria and archaeas in competition (Fig. 3e and S2a), and a decrease in the number of fungal OTUs (Fig. S2b), demonstrating that changes in the microbial communities of competing plants are complex. In addition to diversity and richness, the composition of the root rhizobiome itself directly contribute to the outcome of such interactions. The new generation sequencing (NGS), including the Illumina MiSeq platform provides more detailed information about microbial communities from environmental samples than molecular fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) (Lee et al. 2011; Sun et al. 2014). In addition, studies to compare techniques such as T-RFLP and high throughput sequencing, such as pyrosequencing and Illumina MiSeq, have already shown opposite tendencies, as revealed in our study, being microbial communities much better characterized by the use of NGS (Lee et al. 2011; Wang et al. 2018).

In coexistence situations, the concentration of dissolved organic carbon in the roots may increase (Wu et al. 2012), and thus the recruitment of microorganisms with deleterious effects to one of the plants of the competition pair may be more evident. Plants have the

ability to recruit microorganisms with effects on plant growth and confer protection against pathogens, or also microorganisms with a pathogenic effect on animals and other plants (Bais et al. 2006; Zahar Haichar et al. 2014; Huang et al. 2014), which in a situation of stress by competition, can constitute a competitive advantage. In general, weeds are very dependent on the rhizospheric microbiota, requiring greater microbial diversity for optimal growth and development (Santos et al. 2012; Massenssini 2014; Melo et al. 2014). However, our results show that plant dependency on soil microorganimsms is more related to the specific composition of the rhizobiome and on the speficific functional roles that certain microbes play in the rhizosphere rather than on microbial diversity per si.

The monoculture of I. ramosissima and the competition between I. ramisissima and A. conizoydes presented the greatest Shannon diversity for bacteria and archaea, giving more weight to the richness component for the calculation of diversity indices. These treatments resulted in soil samples with the highest number of OTUs, 777 (177 exclusive) and 782 (184 exclusive), respectively (Fig. S2c and S2d). In the competition, I. ramosissima did not show changes in dry matter production when compared to the monoculture treatment (Fig. S1a), whereas A. conizoydes presented a significant decrease in dry matter ($p<0.01$) (Fig. S1b). Moreover, I. ramosissima monoculture also shares a greater number of OTUs of bacteria, archaeas, and fungi with the competition treatments than A. conizoydes, demonstrating the greater capacity of I. ramosissima in shaping the structure of the microbial community in competition in detriment of A. conizoydes (Fig. S2c and S2d).

Again, it is evident that higher number of bacterial and archaeal OTUs in competition situations are associated with a decreased competitive ability and dry matter production by A. conizoydes. Thus, this weed presented the lowest competitive ability and may be considered a weak competitor among the plant species analyzed in this study, being negatively influenced by bacterial and archaeal diversity with a greater weight for species richness.

4.2. Alteration of bacterial rhizosphere composition under competition

When analyzing the general profile of the distribution of taxa of bacteria and archaeae classified at the phylum, order, and genus level, very similar rhizobiomes were observed for all treatments. Among the most frequent taxa, no significant differences were observed in abundance for the different treatments (Fig. 4). An analysis of 118 weed species by the isolation of epiphytic bacteria from leaves, roots, and flowers of plants at different developmental stages showed that the effect of the plant species was not significant in

determining the weed bacterial community, since plants of different species showed very similar bacterial communities (Dobrovol'skaya et al. 2017). The most abundant phyla in all treatments were Proteobacteria and Actinobacteria. Studies with invasive plants comparing soils from invaded and non-invaded areas, demonstrated that invasions alter the soil microbial communities in the same direction, causing increases in the abundance of specific bacteria also belonging to the phyla Proteobacteria, Acidobacteria and Actinobacteria (Trognitiz et al. 2016).

Proteobacteria have been reported as one of the most abundant phyla found in the rhizosphere of several plants such as banana (Huang et al. 2015), maize (Bakker et al. 2015), rice (Edwards et al. 2015), wheat, oat, pea (Turner et al. 2013), and cotton (Qiao et al. 2017), among others. The phylum Proteobacteria is, in fact, the most abundant and most diverse bacterial phylum, widely distributed, composed of gram-negative bacteria, with ecological, phylogenetic, and pathogenic importance and with different morphologies and forms of obtaining energy (Mukhopadhya et al. 2012). Among the most abundant genera found in our samples, some belong to the phylum Proteobacteria, such as Sphingomonas, Bradyrhizobium, Acidibacter, Burkholderia-Caballeronia-Paraburkholderia and Phenylobacterium (Fig. 4c). These genera have enhanced growth-promoting functions that confer advantages during plant competition, such as Bradyrhizobium, which is a diazotrophic bacterium capable of supplying nitrogen to the associated plant (Vessey 2003; Shaharoona et al. 2006), Sphingomonas known as an indolacetic acid and siderophores producer, phosphate solubilizer, and protector against plant pathogens (Pereira and Castro 2014; Wemheuer et al. 2017), and Burkholderia, able to solubilize phosphate and produce secondary metabolites including antibiotics or antifungal compounds (Young et al. 2013; Wemheuer et al. 2017).

The second most abundant phylum in all the treatments was Actinobacteria, which are gram-positive, filamentous bacteria that play an important role in the decomposition of complex plant substrates, such as cellulose and chitin and are also capable of utilizing a range of simple carbon compounds. They are recognized for the production of secondary metabolites of industrial and commercial interest, and for the production of various types of antibiotics (Anandan et al. 2016). Among the most abundant genera found in our samples, some belong to the phylum Actinobacteria, such as Acidothermus, Conexibacter, Hamadaea, Jatrophihabitans, Gaiella and Streptomyces.

The genus Streptomyces is the most abundant phylum found in studies conducted with diverse soil samples (close to 70 %), and is recognized for the production of bioactive natural

products with potential applications. About 7,600 secondary metabolites with various functions have been described, including antibacterial, antifungal, antitumor, and antiparasitic compounds, enzymes and also bioherbicides (Anandan et al. 2016). The presence of this genus of weed-associated bacteria has already been described in a study that evaluated the taxonomic structure of epiphytic bacterial communities of the rhizosphere and phyllosphere of seven weeds (Dobrovol'skaya et al. 2017) and one of the herbicidal compounds produced by Streptomyces is Anisomycin that is marketed under the name Methozyphenone (Treichel and Forte 2016). The presence of a bacterium that is capable of producing so many metabolites, capable of affecting the adjacent microbial community and plants, undoubtedly has an important role in weed establishment, competition with other weeds and also with crops.

When analyzing all OTUs classified at the genus level, only three showed significant differences in their abundance between the treatments tested. Bdellovibrio showed greater abundance in the competition treatment with I. ramosissima and A. conyzoides and less abundance in that with A. conyzoides and B. pilosa. The genera Flexibacter and Domibacillus presented greater abundances in the competition between I. ramosissima and B. pilosa. Bdellovibrio had its abundance increased in the presence of an invasive exotic plant, Sorghum halepense that produces a phenolic allelochemical (Huang et al. 2017) and this genus is found within a very specific group of bacteria with a very interesting traits: its predates gram-negative bacteria and has the ability to influence the structure of the surrounding microbial community. Their presence, abundance, and richness are positively correlated with the diversity of microbiomes, and may act as motors for the microbial diversity of the hosts and their environments (Johnke et al. 2019). Here, Bdellovibrio was found in greater abundance in the competition treatment with I. ramosissima and A. conyzoides, the treatment with greatest microbial diversity.

4.3. Alteration of fungal rhizosphere composition under competition

The profile of taxa distribution for fungi classified at the phylum, order, and genus level, varied for all treatments. Among the most abundant taxa, only the phylum Ascomycota showed significant differences in abundance between the treatments, having less abundance in the I. ramosissima monoculture (Fig. 5 and S3). This is the largest fungal phylum known to exist and includes endophytic, saprophytic, mutalistic, and symbiotic fungi, such as mycorrhizal and lichen-forming species, animal and plant pathogens, and is present in various environments (Webster and Weber 2007).

Many weeds produce seeds in high abundance and with high dispersion, these seeds are readily available for association with soil microorganisms. In a study of the weeds Abutilon thesphrasti, Erichloa villosa, Polygonum pesnsylvanicum, and Ambrosia trifida, the authors proved that these seeds may serve as a carbon source for saprophytic fungi belonging to the Ascomycota phylum and further extrapolated the findings, stating that, in addition to providing nutrients for specific fungi, seeds can establish associations with them that does not involve its own biodeterioration (Chee-Sanford 2008). In addition, some Ascomycota species that are specifically associated with weeds can be used as bioherbicides, such as Lewia chlamidosporifoemans, which is a highly virulent pathogen of Euphorbia heterophylla, one of the worst and most aggressive weeds in soybean fields (Vieira and Barreto 2010).

5. Conclusion

The three weed species studied here when living in coexistence establish negative ecological interactions, competing with each other. Competion between weeds leads to changes in the rhizosphere microbial community composition and bacterial diversity. These changes are specific to certain taxa. Weeds have the ability to recruit specific taxa depending on each competion situation they are involved in. Such changes in the rhizobiome are directly related to a lower competitive ability and dry matter production by A. conizoydes during coexistence.

Acknowledgments

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Figures

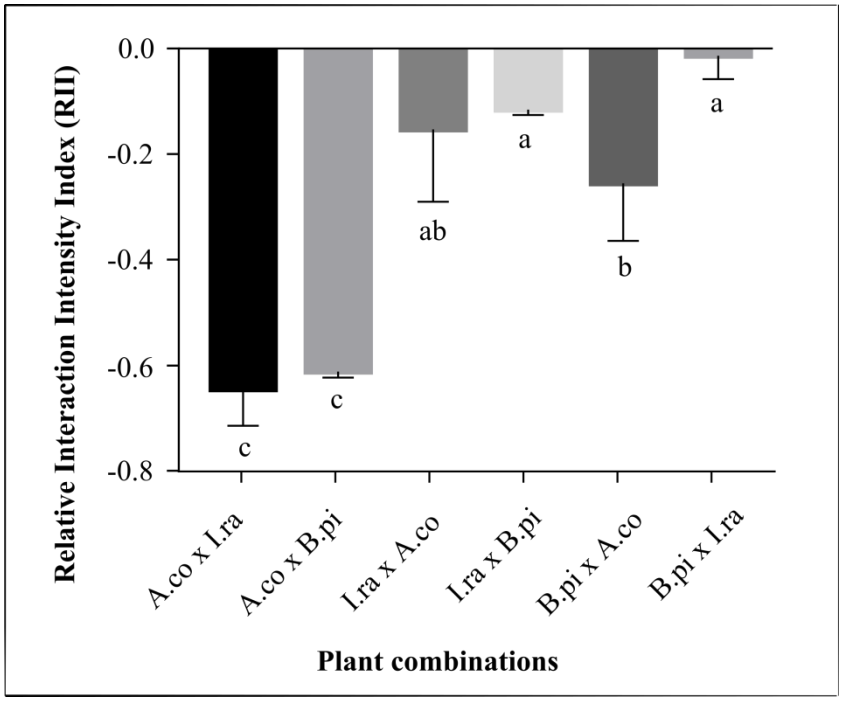

Fig. 1. Relative Interaction Index (RII) after 80 days of weed growth in a greenhouse in monoculture or in competion. The bars represent the standard deviation and different letters indicate significant differences between means based on the Tukey's test ($p < 0.05$). The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the names of two species.

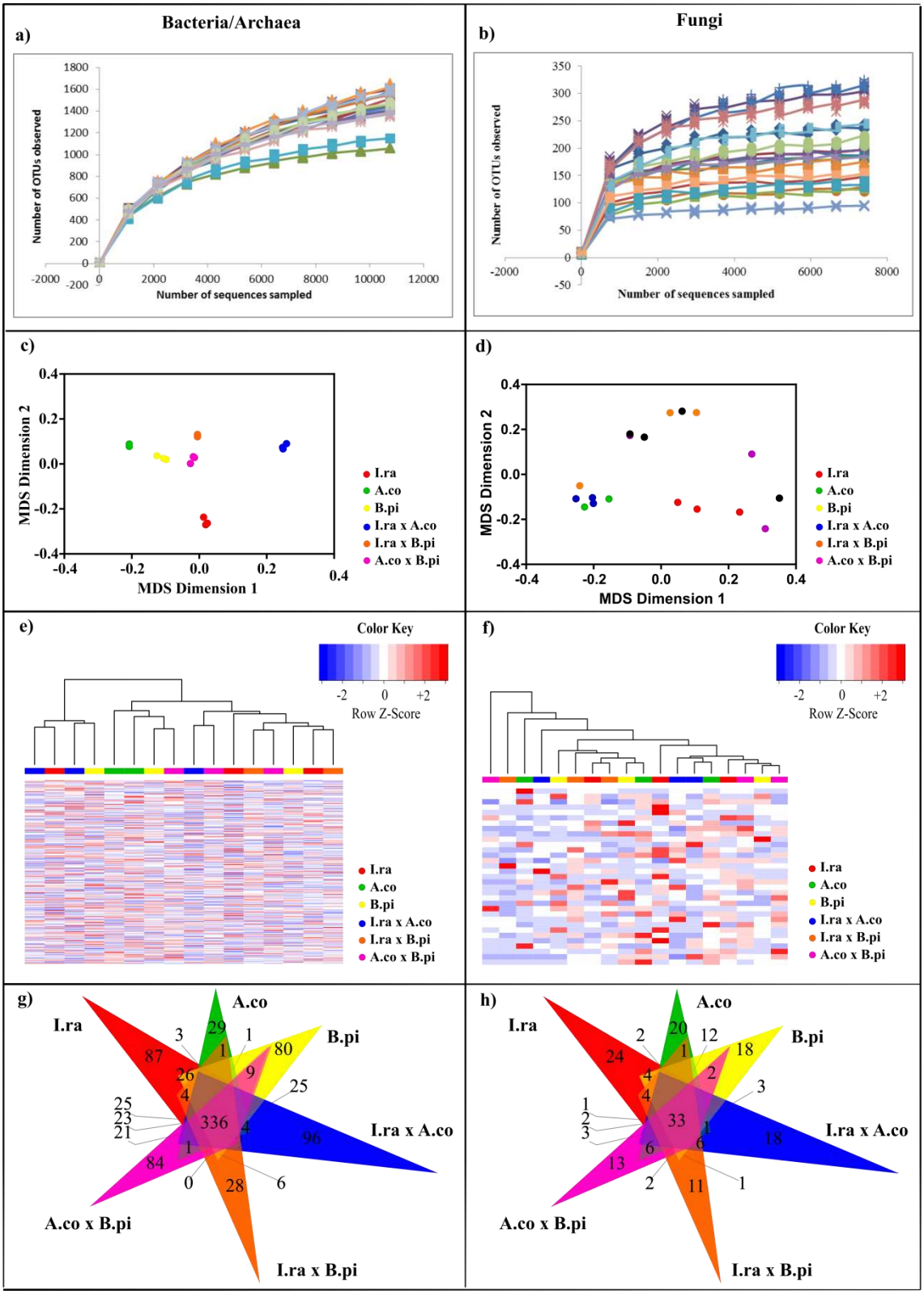

Fig. 2. Rarefaction curves for different treatments calculated based on OTUs defined as 97 % similarity level for **(a)** Bacteria and Archaea, and **(b)** Fungi. Multidimensional Scaling (MDS) for different soil treatments of weeds in monoculture and in competition. **(c)** MDS of Bacteria and Archaea, and **(d)** Fungi. The MDS was based in distance of Jaccard index. Distribuition of shared microbial OTUs among soil treatments **(e)** Bacterial and Archaeal, and **(f)** Fungi. The values of the relative abundances of each OTU were indicated by color intensity in heatmap. Venn diagram of OTUs from **(g)** Bacteria and Archaea, and **(h)** Fungi. Exclusive

and shared OTUs among treatments are based on 97 % similarity. The numbers inside the diagram indicate the numbers of OTUs. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species.

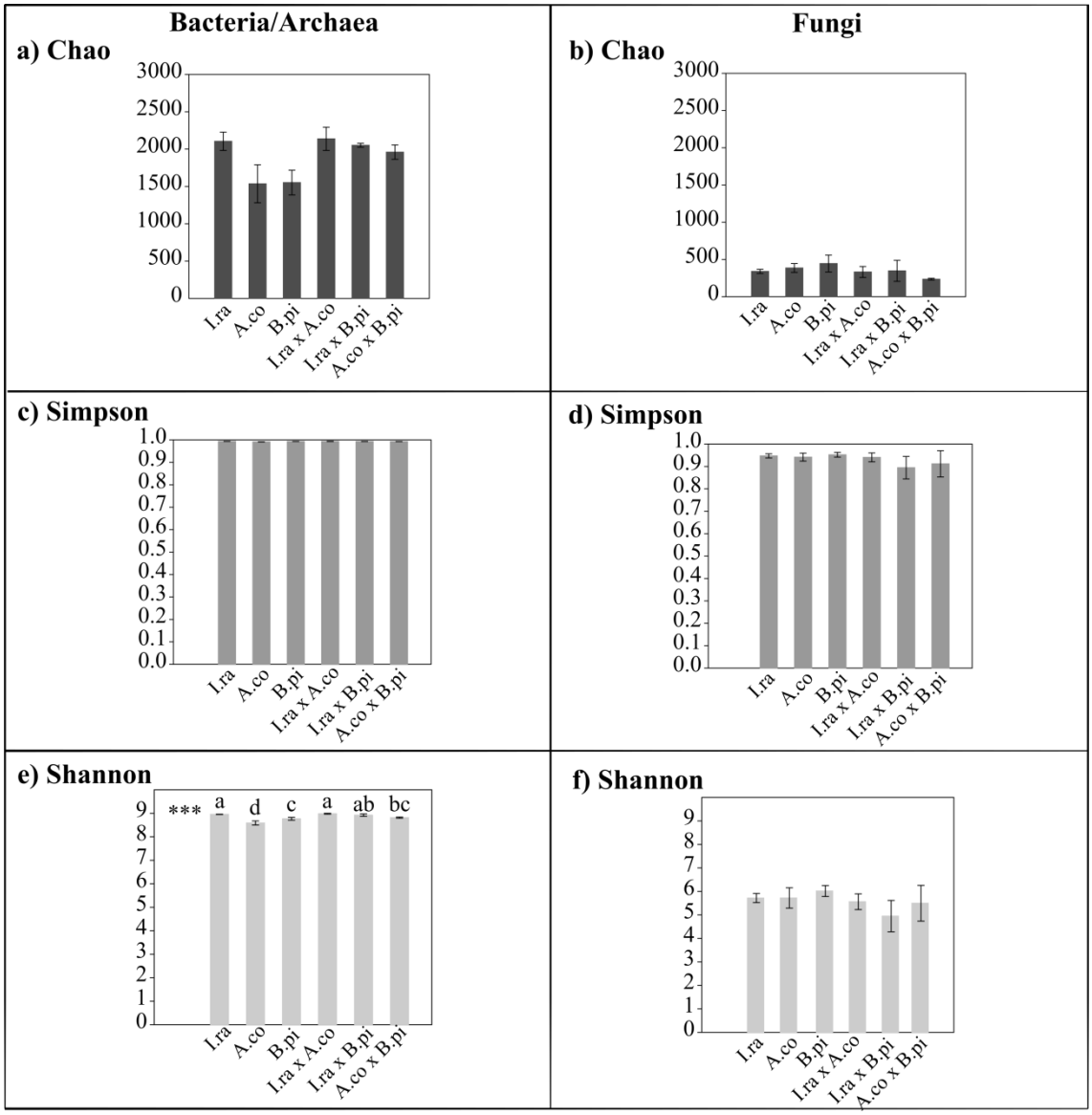

Fig. 3. Indices of diversity for each treatment of rhizosphere in soils cultivated with weeds in monoculture and in competition. **(a)** Chao index for Bacteria and Archaea, and **(b)** Fungi. **(c)** Simpson index for Bacteria and Archaea, and **(d)** Fungi. **(e)** Shannon index for Bacteria and Archaea, and **(f)** Fungi. The results for each treatment comprises the three replicates and the asterisks indicate that this index differs significantly between treatments by the Bonferroni´s test (*, ** and ***, significant to 5, 1 and 0,1 %, respectively by the ANOVA test). The bars represent the standard error. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species.

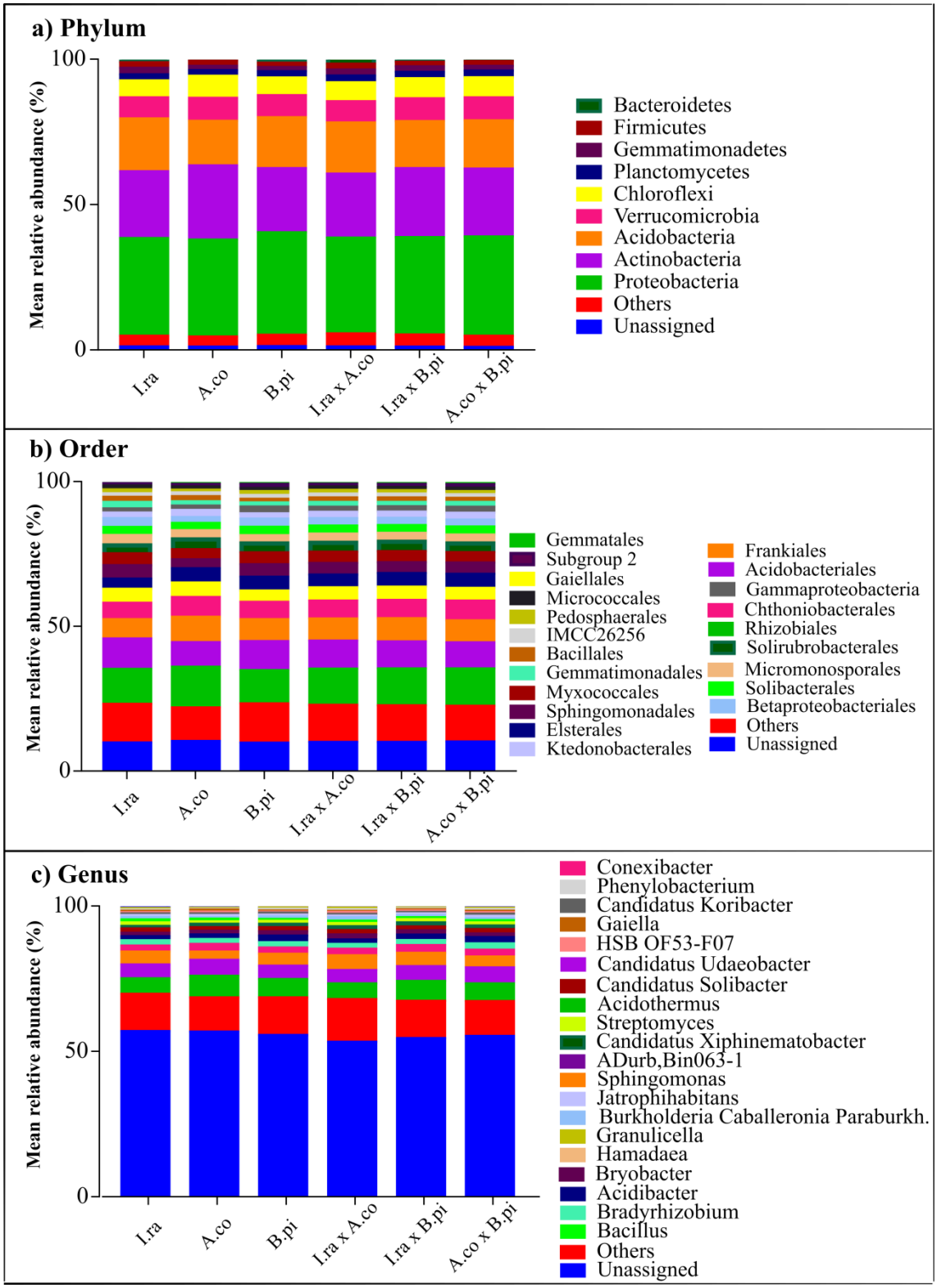

Fig. 4. Taxonomic distribution of OTUs of Bacteria and Archaea in soil treatments with weeds in monoculture and in competition. **(a)** At the phylum level, **(b)** at the order level and **(c)** at the genus level (95 % of similarity). The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species.

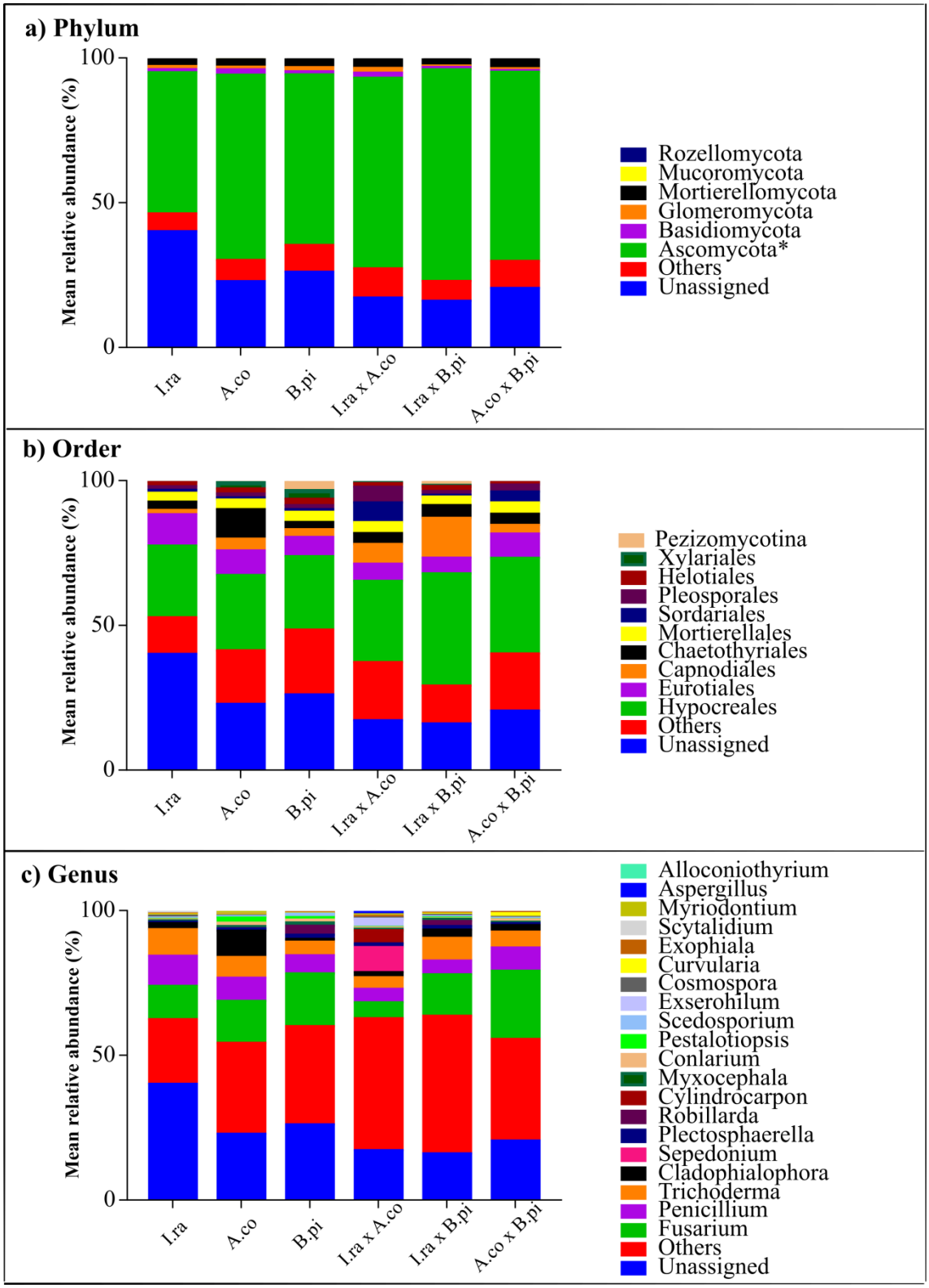

Fig. 5. Taxonomic distribution of OTUs of Fungi in soil treatments with weeds in monoculture and in competition. **(a)** At the phylum level, **(b)** at the order level and **(c)** at the genus level (95 % of similarity). The asterisks indicate that this OTUs differs significantly abundance between treatments by the Bonferroni´s test (*, ** and ***, significant to 5, 1 and 0,1 %, respectively by the ANOVA test). The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species.

Supplementary Data

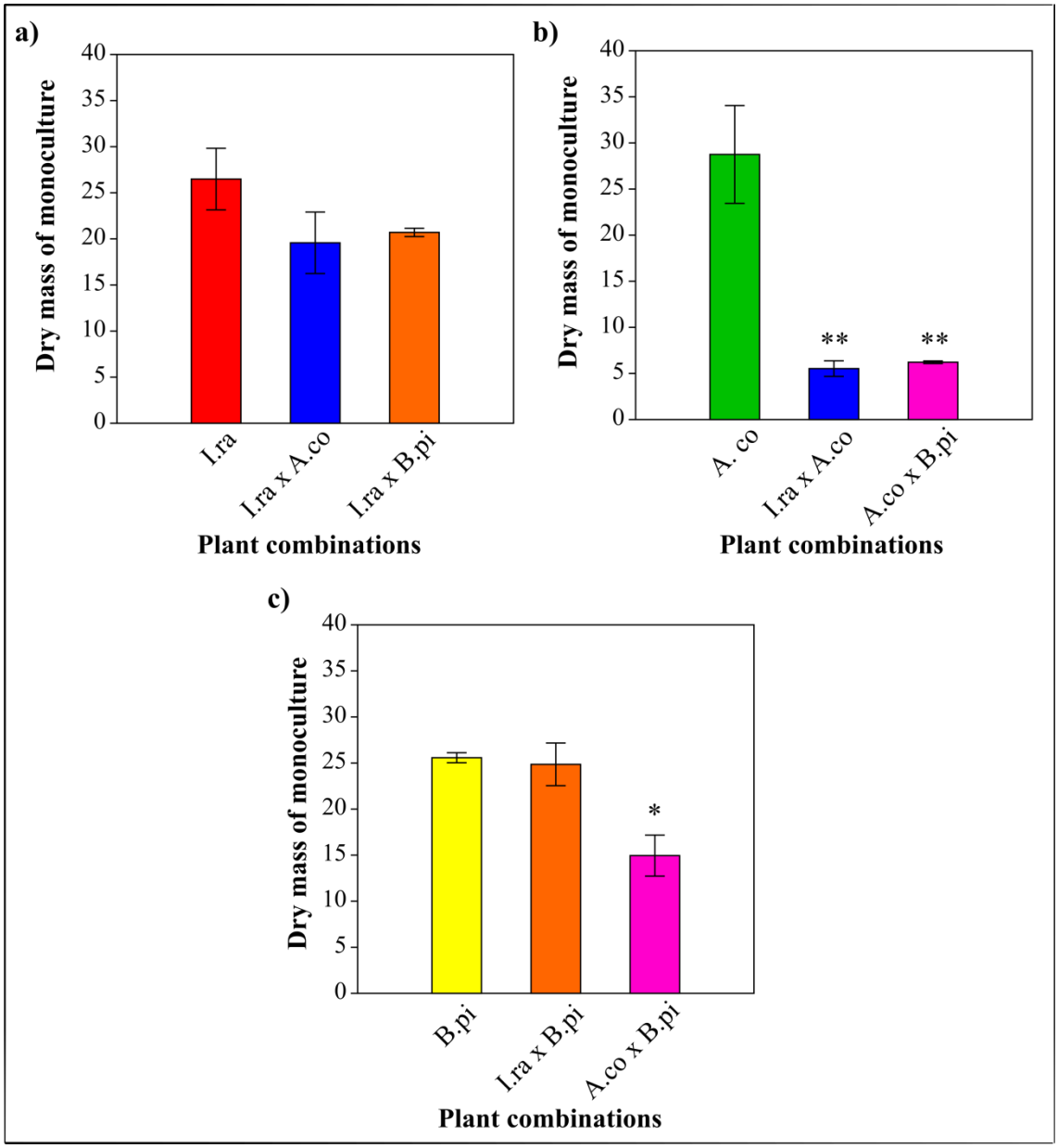

Fig. S1. Dry matter of weeds in monoculture and competition. **(a)** Comparison of dry matter of weed I. ramosissima in monoculture and when in competition with others weeds, **(b)** A. conizoydes and **(c)** B. pilosa. Asterisks indicate difference by the Dunnett´s test when compared to the first column of monocultures (*, ** and ***, significant to 5, 1 and 0,1 %, respectively by the ANOVA test). The bars represent the standard deviation. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species.

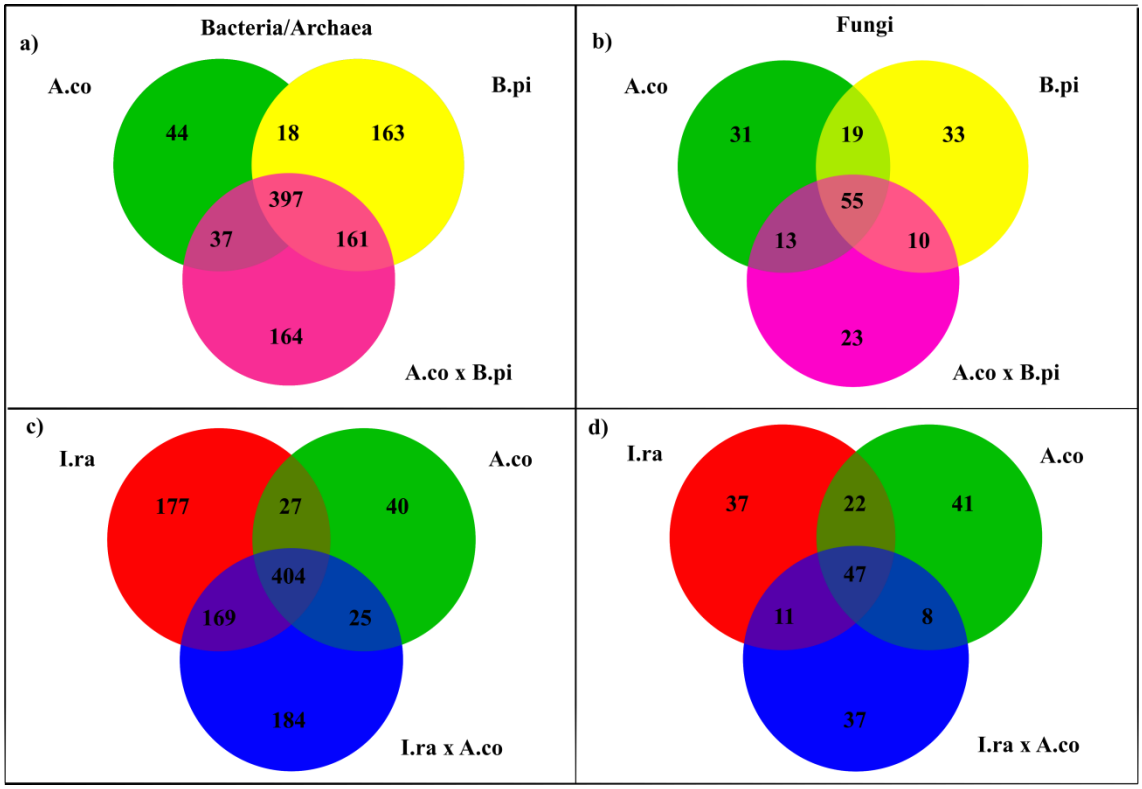

Fig. S2. Venn diagram of OTUs for Bacteria, Archaea, and Fungi in the soil treatments from weeds in monoculture and in competition. **a)** Bacteria and Archaea OTUs comparing the monocultures of A. conizoydes and B. pilosa, and the competition between these two weeds. **b)** Fungi OTUs comparing the monocultures of A. conizoydes and B. pilosa, and the competition between these two weeds. **(c)** Bacteria and Archaea OTUs comparing the monocultures of I. ramosissima and A. conizoydes, and the competition between these two weeds. **(d)** Fungi OTUs comparing the monocultures of I. ramosissima and A. conizoydes, and the competition between these two weeds. Exclusive and shared OTUs among treatments are based on 97 % similarity. The numbers inside the diagram indicate the numbers of OTUs. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species.

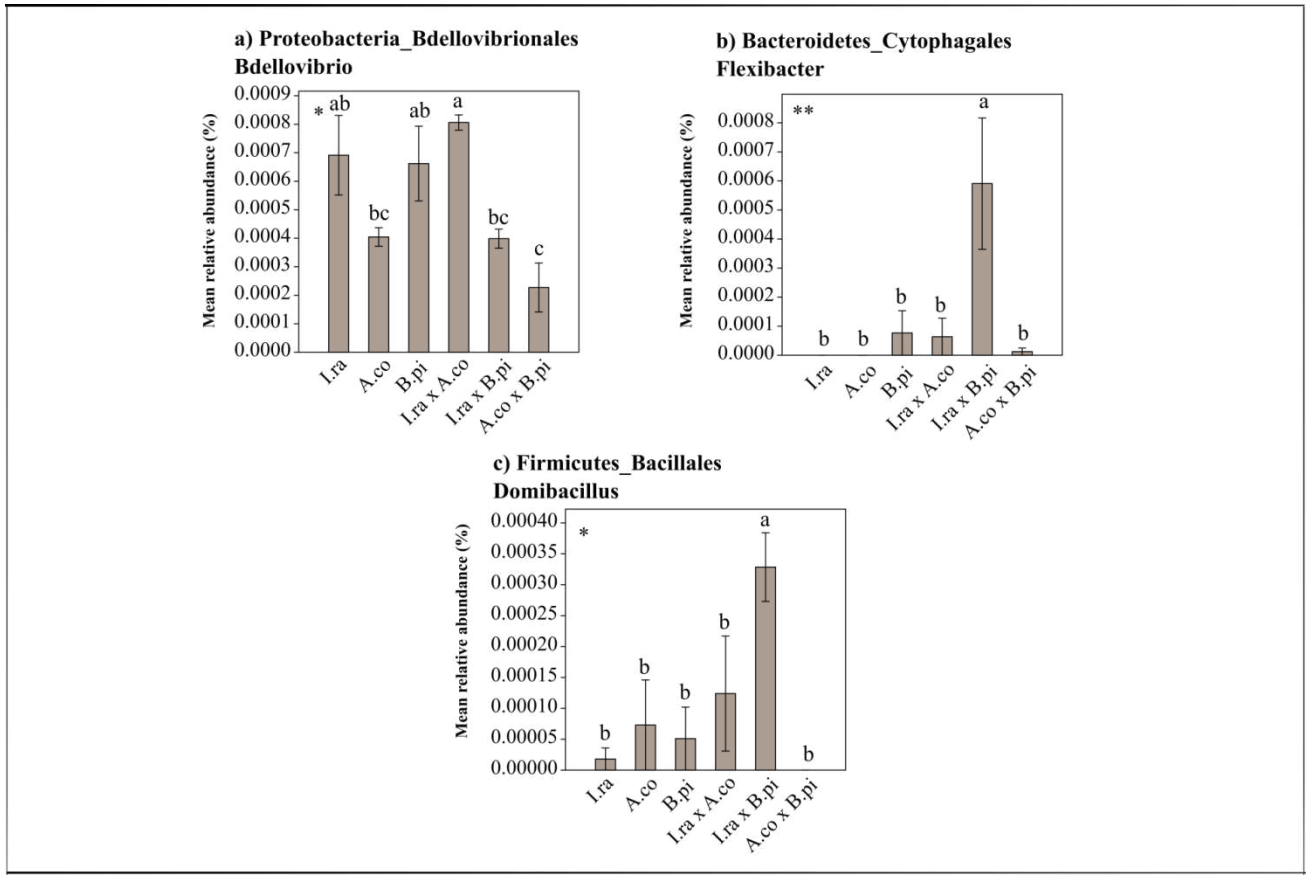

Fig. S3. Comparison of OTUs of Bacteria at genus level that significantly differed the abundance between soil treatments with weeds in monoculture and in competition. **(a)** genus Pajaroellobacter, belonging to order Myxococcales and to phyla Proteobacteria, **(b)** specie Bdellovibrio, belonging to order Bdellovibrionales and to phyla Proteobacteria, **(c)** specie Flexibacter, belonging to order Cytophagales and to phyla Bacteroidetes and **(d)** specie Domibacillus, belonging to order Bacillalesales and to phyla Firmicutes. Different letters indicate significant differences between means based on the Bonferroni´s test (*, ** and ***, significant to 5, 1 and 0,1 %, respectively by the ANOVA test). The bars represent the standard deviation. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by “x” between the name of two species.

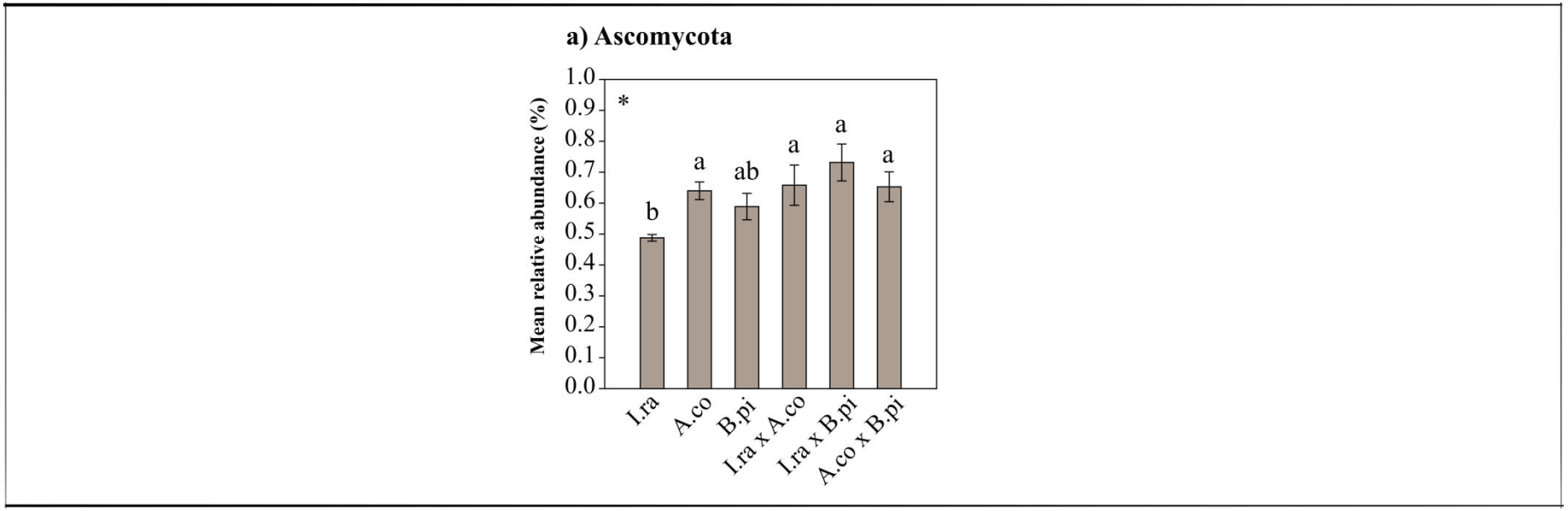

Fig. S4. Comparison of the most abundant OTU of Fungi at phylum level that significantly differed the abundance between soil treatments with weeds in monoculture and in competition. **(a)** Phylum Ascomycota. Different letters indicate significant differences between means based on the Bonferroni´s test (*, ** and ***, significant to 5, 1 and 0,1 %, respectively by the ANOVA test). The bars represent the standard deviation. The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species.

Table S1. Good's coverage index.

	I.ra	A.co	B.pi	I.ra x A.co	I.ra x B.pi	A.co x B.pi
Archaea [ns]	0.9844 ± 0.0237	0.9712 ± 0.0028	0.9353 ± 0.0193	0.9695 ± 0.0346	0.9760 ± 0.0001	0.9926 ± 0.0081
Bacteria [ns]	0.9858 ± 0.0006	0.9772 ± 0.0046	0.9792 ± 0.0082	0.9834 ± 0.0040	0. 9858 ±0.0006	0.9863 ± 0.0034
Fungi [ns]	0.9987 ± 0.0004	0.9989 ± 0.0003	0.9985 ± 0.0003	0.9990 ± 0.0002	0.9984 ± 0.0008	0.9982 ± 0.0018

[ns] The ns means that there was no significant difference between means (p>0.05).
The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species.

Table S2. Average of Chao, Simpson and Shannon index for each treatment of rhizosphere in soils cultivated with weeds in monoculture and in coexistence. The results for each treatment comprises the three replicates and the OTUs were define to 97 % of identity.

Bacteria/Archaea	**Chao** [a ns]	**Simpson** [b ns]	**Shannon** [c] ***
I.ra	2104.8320 ± 212.1032	0.9934 ± 0.0007	8.9562 ± 0.0124 a
A.co	1535.3590 ± 359.7179	0.9913 ± 0.0007	8.5897 ± 0.1228 d
B.pi	1551.7213 ± 286.6202	0.9931 ± 0.0006	8.7677 ± 0.1026 c
I.ra x A.co	2137.6823 ±268.9385	0.9933 ± 0.0013	8.9831 ± 0.0328 a
I.ra x B.pi	2051.2955 ± 36.7886	0.9930 ± 0.0007	8.9236 ± 0.0732 ab
A.co x B.pi	1959.5890 ± 166.4725	0.9925 ± 0.0004	8.8163 ± 0.0461 bc
Fungi	**Chao** [a ns]	**Simpson** [b ns]	**Shannon** [c ns]
I.ra	340.2547 ± 47.3311	0.9472 ± 0.0163	5.7177 ± 0.3369
A.co	386.0379 ± 102.1508	0.9416 ± 0.0309	5.7209 ± 0.7507
B.pi	445.5870 ± 196.7456	0.9522 ± 0.0186	6.0172 ± 0.4033
I.ra x A.co	332.3942 ± 127.3353	0.9406 ± 0.0343	5.5604 ± 0.5802
I.ra x B.pi	347.5278 ± 244.7515	0.8946 ± 0.0868	4.9483 ± 1.1566
A.co x B.pi	235.6111 ± 21.0252	0.9116 ± 0.1016	5.4958 ± 1.3186

[a] Non-parametric estimator used to predict species richness (total number of OTUs presents), based in singletons and doubletons (Chao1).

[b] Diversity index that considers the evenness of species more than richness, considering the common species. The 1-D index was calculated, the value ranges from 0 to 1 and increases as the diversity increases.
[c] Diversity index that indicates species richness, considering the abundance of individual taxa. A higher number indicate more diversity (H).
[ns] The ns means that there was no significant difference between means ($p>0.05$) of all indexes in all treatments.
[***] Means that there was significant difference between means ($p<0.001$) of all indexes in all treatments. Different letters indicate significant differences between means based on the Bonferroni´s test.
The plant species are: A.co – Ageratum conyzoides; B.pi – Bidens pilosa and I.ra – Ipomoea ramosissima. Coexistence treatments are indicated by "x" between the name of two species (Chiarucci et al. 2011; Kim et al. 2017; Lemos et al. 2011).

Table S3. Exclusive OTUs of Bacterial and Archaea present in each soil treatments[a].

Ipomoea ramosissima
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae; Polyangium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; 27F-1492R; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; Burkholderiaceae; Herbaspirillum
Bacteria; Actinobacteria; Acidimicrobiia; IMCC26256; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Solibacterales; Solibacteraceae (Subgroup 3); Bryobacter; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Oligoflexales; 0319-6G20
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae; Polyangium
Unassigned
Bacteria; Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; Burkholderiaceae; Ralstonia; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Sandaracinaceae; uncultured; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; Acidobacteriaceae (Subgroup 1); uncultured; uncultured bacterium
Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Paenibacillus; Paenibacillus validus
Bacteria; Chloroflexi; AD3; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; Ktedonobacteria; B12-WMSP1; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium
Bacteria; Chloroflexi; TK10; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Patescibacteria; Parcubacteria; Candidatus Jorgensenbacteria; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9
Bacteria; Chloroflexi; TK10; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Unassigned
Bacteria; Actinobacteria; MB-A2-108; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Solibacterales; Solibacteraceae (Subgroup 3); GOUTB8
Bacteria; Bacteroidetes; Bacteroidia; Sphingobacteriales; Sphingobacteriaceae; Mucilaginibacter
Bacteria; Chloroflexi; AD3; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Diplorickettsiales; Diplorickettsiaceae; Aquicella; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae
Bacteria; Proteobacteria; Gammaproteobacteria; Diplorickettsiales; Diplorickettsiaceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Micropepsales; Micropepsaceae; uncultured
Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; uncultured; uncultured Perlucidibaca sp,
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Thermomonosporaceae; Actinomadura
Bacteria; Proteobacteria; Gammaproteobacteria; Salinisphaerales; Solimonadaceae; Nevskia
Bacteria; Chloroflexi; JG30-KF-CM66; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Beijerinckiaceae; 1174-901-12; uncultured bacterium

Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Bacteroidetes; Bacteroidia; Sphingobacteriales; Sphingobacteriaceae; Mucilaginibacter
Bacteria; Actinobacteria; Acidimicrobiia; Acidimicrobiales; Acidimicrobiaceae; uncultured; uncultured bacterium
Unassigned
Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured
Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; metagenome; metagenome
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; HSB OF53-F07; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SM2D12; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Novosphingobium; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Myxococcaceae
Unassigned
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Bacteria; Planctomycetes; vadinHA49; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; uncultured; uncultured bacterium
Bacteria; Chloroflexi; P2-11E; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; uncultured; uncultured bacterium
Bacteria; Chloroflexi; Chloroflexia; Chloroflexales; Roseiflexaceae; uncultured; uncultured bacterium
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Beijerinckiaceae; Microvirga; uncultured bacterium
Bacteria; Acidobacteria; Subgroup 5; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Patescibacteria; Parcubacteria; Candidatus Jorgensenbacteria; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfarculales; Desulfarculaceae; uncultured
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; KD3-10; uncultured bacterium; uncultured bacterium
Bacteria; Acidobacteria; Thermoanaerobaculia; Thermoanaerobaculales; Thermoanaerobaculaceae; Subgroup 10; uncultured bacterium
Bacteria; Actinobacteria; Acidimicrobiia; IMCC26256; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Streptosporangiaceae; Microbispora
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Thermomonosporaceae; Actinoallomurus
Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Actinobacteria; Micromonosporales; Micromonosporaceae; Hamadaea
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae; Haliangium;

metagenome
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae; Haliangium; uncultured bacterium
Bacteria; Elusimicrobia; Lineage IIa; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Planctomycetes; BD7-11; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Elusimicrobia; Lineage IIa
Bacteria; Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; Nitrosomonadaceae; Ellin6067; uncultured beta proteobacterium
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Xiphinematobacteraceae; Candidatus Xiphinematobacter; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; RCP2-54; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Actinobacteria; Propionibacteriales; Nocardioidaceae; Actinopolymorpha
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae; Pajaroellobacter; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; uncultured
Unassigned
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; Acidobacteriaceae (Subgroup 1); Granulicella; uncultured bacterium
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; 1959-1; uncultured bacterium
Bacteria; Acidobacteria; Blastocatellia (Subgroup 4); Blastocatellales; Blastocatellaceae; JGI 0001001-H03; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Chlamydiae; Chlamydiae; Chlamydiales; Simkaniaceae; uncultured
Bacteria; Acidobacteria; Subgroup 17; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; uncultured
Bacteria; Actinobacteria; Acidimicrobiia; IMCC26256; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae; Haliangium; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; uncultured bacterium; uncultured bacterium
Ageratum conizoydes
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; Acidobacteriaceae (Subgroup 1); Acidipila
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; bacteriap25; uncultured bacterium; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; JG30-KF-AS9; uncultured bacterium; uncultured bacterium
Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Paenibacillus; Paenibacillus polymyxa
Bacteria; Proteobacteria; Alphaproteobacteria; Micropepsales; Micropepsaceae
Bacteria; Actinobacteria; Acidimicrobiia; Microtrichales; Ilumatobacteraceae; uncultured; uncultured bacterium

Bacteria; Planctomycetes; Planctomycetacia; Isosphaerales; Isosphaeraceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Reyranellales; Reyranellaceae; Reyranella; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; WPS-2; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; Candidatus Udaeobacter; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; bacteriap25; metagenome; metagenome
Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; 67-14; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Thermomonosporaceae; Actinoallomurus
Bacteria; Proteobacteria; Deltaproteobacteria
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; Candidatus Udaeobacter; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; mle1-27; uncultured delta proteobacterium; uncultured delta proteobacterium
Bacteria; Actinobacteria; Acidimicrobiia; IMCC26256; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; TK10; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; SC-I-84
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; uncultured
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Rubrobacteria; Rubrobacterales; Rubrobacteriaceae; Rubrobacter; uncultured bacterium
Bidens pilosa
Bacteria; Planctomycetes; Planctomycetacia; Isosphaerales; Isosphaeraceae; Aquisphaera; uncultured planctomycete
Bacteria; Chloroflexi; AD3; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; FCPU426; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Gammaproteobacteria Incertae Sedis; Unknown Family; Acidibacter; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; P3OB-42; uncultured bacterium; uncultured bacterium
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; Gemmatirosa
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium

Bacteria; Actinobacteria; Actinobacteria; Pseudonocardiales; Pseudonocardiaceae; Crossiella; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Archaea; Thaumarchaeota; Nitrososphaeria; Nitrososphaerales; Nitrososphaeraceae; Candidatus Nitrososphaera

Bacteria; Actinobacteria; Actinobacteria; Micromonosporales; Micromonosporaceae
Bacteria; Armatimonadetes; Fimbriimonadia; Fimbriimonadales; Fimbriimonadaceae; uncultured bacterium; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured planctomycete
Bacteria; Verrucomicrobia; Verrucomicrobiae; Pedosphaerales; Pedosphaeraceae; Pedosphaera; uncultured bacterium

Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Bradyrhizobium; uncultured bacterium

Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales
Bacteria; Bacteroidetes; Bacteroidia; Cytophagales; Microscillaceae; uncultured; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Bacteria; Actinobacteria; Acidimicrobiia; uncultured; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae; Haliangium; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Solibacterales; Solibacteraceae (Subgroup 3); Bryobacter; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfarculales; Desulfarculaceae; uncultured; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; uncultured; uncultured Acidobacteria bacterium; uncultured Acidobacteria bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae; Pajaroellobacter; uncultured bacterium
Archaea; Thaumarchaeota; Group 1,1c; uncultured archaeon; uncultured archaeon; uncultured archaeon; uncultured archaeon
Bacteria; Elusimicrobia; Lineage IIb; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium

Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae 1; Clostridium sensu stricto 12
Bacteria; Planctomycetes; Pla4 lineage; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium

Bacteria; Acidobacteria; Holophagae; Subgroup 7

Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Microbacteriaceae; Agrococcus
Bacteria; Acidobacteria; Acidobacteriia; Solibacterales; Solibacteraceae (Subgroup 3); Bryobacter; uncultured Acidobacteria bacterium
Bacteria; Chloroflexi; Ktedonobacteria; C0119; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium

Bacteria; Proteobacteria; Gammaproteobacteria; Diplorickettsiales; Diplorickettsiaceae; uncultured
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; uncultured; uncultured Ktedobacteria bacterium
Bacteria; Planctomycetes; Phycisphaerae; Tepidisphaerales; CPla-3 termite group; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Gammaproteobacteria Incertae Sedis; Unknown Family; Acidibacter; uncultured bacterium

Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; Roseomonas; uncultured Roseomonas sp,
Bacteria; Acidobacteria; Acidobacteriia; Solibacterales; Solibacteraceae (Subgroup 3); Candidatus Solibacter; uncultured Acidobacteria bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Lysobacter
Bacteria; Actinobacteria; Actinobacteria; Micromonosporales; Micromonosporaceae; uncultured; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Isosphaerales; Isosphaeraceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; Burkholderiaceae; Comamonas
Bacteria; Patescibacteria; Parcubacteria; Candidatus Jorgensenbacteria; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Thermomonosporaceae; Actinomadura
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Blfdi19; uncultured bacterium; uncultured bacterium
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; uncultured; uncultured bacterium
Bacteria; Bacteroidetes; Bacteroidia; Cytophagales; Microscillaceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae
Bacteria; Actinobacteria; Actinobacteria; Catenulisporales; Actinospicaceae; Actinospica
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; uncultured; metagenome; metagenome
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; uncultured; uncultured bacterium
Bacteria; Chloroflexi; AD3; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; Candidatus Udaeobacter; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Oligoflexales; 0319-6G20; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; uncultured
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae; Pajaroellobacter
Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; Koribacteraceae; Candidatus Koribacter; uncultured bacterium
Bacteria; Verrucomicrobia; Verrucomicrobiae; Pedosphaerales; Pedosphaeraceae; ADurb,Bin063-1
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; 67-14; uncultured bacterium; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Subgroup 2; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae; Haliangium; uncultured bacterium
Bacteria; Acidobacteria; Blastocatellia (Subgroup 4); 11-24; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; Candidatus Udaeobacter; uncultured bacterium

Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Xiphinematobacteraceae; Candidatus Xiphinematobacter
Unassigned
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; Gemmatimonas; uncultured bacterium
Bacteria; Acidobacteria; Blastocatellia (Subgroup 4); Blastocatellales; Blastocatellaceae; Blastocatella
Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; Acidiphilium; uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Subgroup 2; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; uncultured; uncultured bacterium
Bacteria; Patescibacteria; Parcubacteria; Candidatus Jorgensenbacteria; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9; Candidatus Adlerbacteria bacterium GW2011_GWC1_50_9
Bacteria; Proteobacteria; Alphaproteobacteria; Azospirillales; Inquilinaceae; Inquilinus; uncultured alpha proteobacterium
Bacteria; Actinobacteria; Actinobacteria; Micromonosporales; Micromonosporaceae
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured
Bacteria; Acidobacteria; Acidobacteriia; Subgroup 13
I. ramosissima x A. conizoydes
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; 1921-2; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; HSB OF53-F07; uncultured Ktedonobacter sp,
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; Gaiellaceae; Gaiella; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured planctomycete
Bacteria; Actinobacteria; Acidimicrobiia; IMCC26256; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Bdellovibrionales; Bdellovibrionaceae; Bdellovibrio; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; uncultured; uncultured bacterium
Unassigned
Bacteria; Chloroflexi; Ktedonobacteria; C0119; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Acidobacteria; Subgroup 6; uncultured Acidobacteria bacterium; uncultured Acidobacteria bacterium; uncultured Acidobacteria bacterium; uncultured Acidobacteria bacterium
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae; Conexibacter
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Sphingomonas
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; JG30-KF-AS9; uncultured bacterium; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Isosphaerales; Isosphaeraceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Phenylobacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Blastomonas; uncultured bacterium

Bacteria; Proteobacteria; Deltaproteobacteria; Oligoflexales; Oligoflexaceae; Oligoflexus; uncultured bacterium

Bacteria; Bacteroidetes; Bacteroidia; Cytophagales; Microscillaceae; uncultured

Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium

Bacteria; Actinobacteria; Actinobacteria

Bacteria; Actinobacteria; Actinobacteria; Frankiales; Sporichthyaceae; uncultured; uncultured bacterium

Bacteria; Bacteroidetes; Bacteroidia; Chitinophagales; Chitinophagaceae; uncultured; uncultured bacterium

Bacteria; Actinobacteria; Actinobacteria; Micromonosporales; Micromonosporaceae

Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; 1921-3; uncultured bacterium

Bacteria; Planctomycetes; Planctomycetacia; Isosphaerales; Isosphaeraceae; Singulisphaera

Bacteria; Acidobacteria; Acidobacteriia; Solibacterales; Solibacteraceae (Subgroup 3); Bryobacter; uncultured bacterium

Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; Acidicaldus

Bacteria; Actinobacteria; Actinobacteria; Catenulisporales; Actinospicaceae; Actinospica

Bacteria; Cyanobacteria; Oxyphotobacteria; Nostocales; Nostocaceae; Tolypothrix PCC-7601

Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Sphingomonas

Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; uncultured; uncultured bacterium

Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; uncultured; uncultured bacterium

Bacteria; Actinobacteria; Actinobacteria; Corynebacteriales; Mycobacteriaceae; Mycobacterium; uncultured bacterium

Bacteria; Chloroflexi; Ktedonobacteria; B12-WMSP1; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium

Bacteria; Acidobacteria; Acidobacteriia; Acidobacteriales; uncultured

Bacteria; Acidobacteria; Acidobacteriia; Solibacterales; Solibacteraceae (Subgroup 3); Bryobacter; uncultured Acidobacteria bacterium

Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; Chthoniobacter; uncultured bacterium

Bacteria; Cyanobacteria; Oxyphotobacteria; Chloroplast

Bacteria; Planctomycetes; Planctomycetacia; Isosphaerales; Isosphaeraceae; Singulisphaera

Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Beijerinckiaceae; Bosea; uncultured bacterium

Bacteria; Acidobacteria; Acidobacteriia; Solibacterales; Solibacteraceae (Subgroup 3); Paludibaculum; uncultured bacterium

Bacteria; Armatimonadetes; Armatimonadia; Armatimonadales; uncultured bacterium; uncultured bacterium; uncultured bacterium

Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; 1959-1; uncultured bacterium

Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured; uncultured bacterium; uncultured bacterium

Bacteria; Chlamydiae; Chlamydiae; Chlamydiales; Parachlamydiaceae; Candidatus Protochlamydia

Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Anaerocolumna

Bacteria; Acidobacteria; Acidobacteriia; Subgroup 2; uncultured bacterium; uncultured bacterium; uncultured bacterium

Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium

Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Ensifer

Bacteria; Actinobacteria; Acidimicrobiia; IMCC26256; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Gammaproteobacteria; Gammaproteobacteria Incertae Sedis; Unknown Family; Acidibacter
Bacteria; Bacteroidetes; Bacteroidia; Chitinophagales; Chitinophagaceae; uncultured
Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; uncultured; uncultured bacterium
Bacteria; Elusimicrobia; Lineage IIa
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Frankiaceae; Frankia
Bacteria; Bacteroidetes; Bacteroidia; Sphingobacteriales; env,OPS 17; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; uncultured; uncultured Ktedobacteria bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiales Incertae Sedis; uncultured; uncultured bacterium
Bacteria; Bacteroidetes; Bacteroidia; Sphingobacteriales; Sphingobacteriaceae; Mucilaginibacter
Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium
Archaea; Thaumarchaeota; Nitrososphaeria; Nitrososphaerales; Nitrososphaeraceae
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; Gemmatimonas; uncultured bacterium
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae; Solirubrobacter; uncultured bacterium
Bacteria; Chloroflexi; AD3; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; uncultured
Bacteria; Verrucomicrobia; Verrucomicrobiae; Pedosphaerales; Pedosphaeraceae; ADurb,Bin063-1; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; uncultured; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Novosphingobium
Bacteria; Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; Burkholderiaceae; Oxalobacter
Bacteria; Bacteroidetes; Bacteroidia; Chitinophagales; Chitinophagaceae; Flavisolibacter; uncultured bacterium
Bacteria; Bacteroidetes; Bacteroidia; Sphingobacteriales; Sphingobacteriaceae; Mucilaginibacter
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; Ktedonobacter; uncultured Ktedonobacter sp,
Bacteria; Planctomycetes; Phycisphaerae; Tepidisphaerales; WD2101 soil group; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; uncultured; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Rokubacteria; NC10; Rokubacteriales; uncultured bacterium; uncultured bacterium; uncultured bacterium
Unassigned
Bacteria; Bacteroidetes; Bacteroidia; Cytophagales; Spirosomaceae; Spirosoma
Bacteria; Armatimonadetes; uncultured; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; Gemmatirosa; uncultured bacterium
Bacteria; Chloroflexi; TK10; uncultured bacterium; uncultured bacterium; uncultured bacterium;

uncultured bacterium
Bacteria; Acidobacteria; Acidobacteriia; Subgroup 2; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; FBP; uncultured soil bacterium; uncultured soil bacterium; uncultured soil bacterium; uncultured soil bacterium; uncultured soil bacterium
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; Chthoniobacter; uncultured bacterium
Bacteria; Cyanobacteria; Oxyphotobacteria; Chloroplast; uncultured Dunaliella sp,; uncultured Dunaliella sp,; uncultured Dunaliella sp,
Bacteria; Proteobacteria; Gammaproteobacteria; Salinisphaerales; Solimonadaceae; Nevskia; uncultured bacterium
Bacteria; Firmicutes; Bacilli; Bacillales; Alicyclobacillaceae; Tumebacillus; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Micropepsales; Micropepsaceae; uncultured; metagenome
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; Candidatus Udaeobacter; uncultured bacterium
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; JG30-KF-AS9; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; 1959-1; uncultured Ktedobacteria bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
I. ramosissima x B. pilosa
Bacteria; Chloroflexi; TK10; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; KD4-96; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Bacteria; Firmicutes; Bacilli; Bacillales; Planococcaceae; Domibacillus
Bacteria; Bacteroidetes; Bacteroidia; Cytophagales; Spirosomaceae; Fibrella
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae; uncultured
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; G12-WMSP1; uncultured bacterium
Bacteria; WPS-2; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium
Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Paenibacillus
Bacteria; Acidobacteria; Acidobacteriia; Subgroup 13; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; TK10; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae; Haliangium; uncultured Nannocystineae bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae; Sorangium; Sorangium cellulosum
Bacteria; Acidobacteria; Blastocatellia (Subgroup 4); 11-24; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; AD3; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; WPS-2; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium

Bacteria; Chloroflexi; AD3; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; LD29; uncultured bacterium
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; uncultured; uncultured bacterium
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae; Conexibacter
Bacteria; Bacteroidetes; Bacteroidia; Cytophagales; Microscillaceae; Flexibacter
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Phenylobacterium
Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured
Bacteria; Planctomycetes; Planctomycetacia; Isosphaerales; Isosphaeraceae; Aquisphaera
Bacteria; Chloroflexi; KD4-96; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
A. conizoydes x B. pilosa
Bacteria; Chloroflexi; TK10; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; KD4-96; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Planctomycetes; Planctomycetacia; Gemmatales; Gemmataceae; uncultured; uncultured bacterium
Bacteria; Firmicutes; Bacilli; Bacillales; Planococcaceae; Domibacillus
Bacteria; Bacteroidetes; Bacteroidia; Cytophagales; Spirosomaceae; Fibrella
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae; uncultured
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae; G12-WMSP1; uncultured bacterium
Bacteria; WPS-2; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae; Acidothermus; uncultured bacterium
Bacteria; Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Paenibacillus
Bacteria; Acidobacteria; Acidobacteriia; Subgroup 13; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; TK10; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae; Haliangium; uncultured Nannocystineae bacterium
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae; Sorangium; Sorangium cellulosum
Bacteria; Acidobacteria; Blastocatellia (Subgroup 4); 11-24; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; AD3; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; WPS-2; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium
Bacteria; Chloroflexi; AD3; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium; uncultured Chloroflexi bacterium
Bacteria; Verrucomicrobia; Verrucomicrobiae; Chthoniobacterales; Chthoniobacteraceae; LD29; uncultured bacterium
Bacteria; Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; uncultured; uncultured bacterium

Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae; Conexibacter
Bacteria; Bacteroidetes; Bacteroidia; Cytophagales; Microscillaceae; Flexibacter
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Phenylobacterium
Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; uncultured; uncultured bacterium; uncultured bacterium
Bacteria; Proteobacteria; Alphaproteobacteria; Elsterales; uncultured
Bacteria; Planctomycetes; Planctomycetacia; Isosphaerales; Isosphaeraceae; Aquisphaera
Bacteria; Chloroflexi; KD4-96; uncultured bacterium; uncultured bacterium; uncultured bacterium; uncultured bacterium

[a] OTU identification was generated in the QIIME program by comparing OTUs sequences against the Silva 132 databases.

Table S4. Exclusive OTUs of Fungi present in each soil treatments[a].

Ipomoea ramosissima
Unassigned
Fungi; Glomeromycota; Glomeromycetes; Glomerales; Glomeraceae; unidentified; unidentified
Fungi; Ascomycota; Orbiliomycetes; Orbiliales; Orbiliaceae; Arthrobotrys
Unassigned
Unassigned
Unassigned
Unassigned
Unassigned
Unassigned
Unassigned
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae
Unassigned
Unassigned
Unassigned
Fungi; Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Penicillium
Unassigned
Unassigned
Unassigned
Fungi; Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Penicillium
Unassigned
Unassigned
Unassigned
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; unidentified; unidentified
Fungi; Ascomycota; Sordariomycetes; Microascales; Microascaceae
Ageratum conizoydes
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; Fusarium
Fungi; Ascomycota; Eurotiomycetes; Onygenales; Ajellomycetaceae
Fungi; Ascomycota; Sordariomycetes; Diaporthales; Diaporthaceae
Unassigned
Unassigned
Unassigned
Unassigned
Fungi; unidentified; unidentified; unidentified; unidentified; unidentified; unidentified
Unassigned

Unassigned
Fungi; Ascomycota; Sordariomycetes; Sordariomycetidae_ord_Incertae_sedis; Annulatascaceae; Conlarium; unidentified
Unassigned
Unassigned
Unassigned
Fungi; unidentified; unidentified; unidentified; unidentified; unidentified; unidentified
Fungi; unidentified; unidentified; unidentified; unidentified; unidentified; unidentified
Fungi; Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Penicillium; unidentified
Unassigned
Unassigned
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; Fusarium
Bidens pilosa
Unassigned
Unassigned
Unassigned
Unassigned
Unassigned
Fungi; Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Penicillium
Unassigned
Unassigned
Unassigned
Fungi; Ascomycota; Sordariomycetes; Hypocreales; unidentified; unidentified; unidentified
Unassigned
Fungi; Ascomycota; Sordariomycetes; Hypocreomycetidae_ord_Incertae_sedis; Plectosphaerellaceae; Plectosphaerella; Plectosphaerella_cucumerina
Fungi; Ascomycota; Sordariomycetes; Xylariales; Amphisphaeriaceae; Pestalotiopsis
Unassigned
Fungi; Ascomycota; Leotiomycetes; Helotiales; Helotiales_fam_Incertae_sedis; Scytalidium; Scytalidium_ganodermophthorum
Unassigned
Fungi; Ascomycota; Sordariomycetes; Xylariales; Amphisphaeriaceae; Pestalotiopsis
Fungi; Ascomycota
I. ramosissima x A. conizoydes
Unassigned
Fungi; Ascomycota; Sordariomycetes
Unassigned
Fungi; Glomeromycota; Glomeromycetes; Glomerales; Glomeraceae; unidentified; unidentified
Fungi; Ascomycota; Sordariomycetes; Sordariales; Sordariaceae
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; Fusarium; unidentified
Unassigned
Unassigned
Fungi; unidentified; unidentified; unidentified; unidentified; unidentified; unidentified
Unassigned
Fungi; unidentified; unidentified; unidentified; unidentified; unidentified; unidentified
Fungi; Ascomycota; Dothideomycetes; Pleosporales; Pleosporales_fam_Incertae_sedis; Berkleasmium; unidentified
Fungi; Ascomycota; Sordariomycetes

Fungi; unidentified; unidentified; unidentified; unidentified; unidentified; unidentified
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Hypocreales_fam_Incertae_sedis; Acremonium; Acremonium_persicinum
Fungi; Ascomycota
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Clavicipitaceae; Metarhizium; Metarhizium_carneum
Unassigned
I. ramosissima x B. pilosa
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Clavicipitaceae
Fungi; Ascomycota; Sordariomycetes; Sordariales; Lasiosphaeriaceae; Podospora; Podospora_pyriformis
Unassigned
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Bionectriaceae; unidentified; unidentified
Fungi; Ascomycota; Dothideomycetes; Capnodiales; Mycosphaerellaceae
Fungi; Ascomycota; Sordariomycetes
Unassigned
Fungi
Fungi
Fungi; unidentified; unidentified; unidentified; unidentified; unidentified; unidentified
Unassigned
A. conizoydes x B. pilosa
Fungi; Glomeromycota; Glomeromycetes; Archaeosporales; Ambisporaceae; Ambispora; Ambispora_appendicula
Fungi; Ascomycota; Sordariomycetes; Hypocreales; Hypocreaceae; Trichoderma; Trichoderma_harzianum
Fungi; Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae
Fungi; Ascomycota; Sordariomycetes; unidentified; unidentified; unidentified; unidentified
Fungi; Ascomycota; Sordariomycetes
Unassigned
Unassigned
Unassigned
Fungi; Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae; Aspergillus; Aspergillus_flavus
Fungi; Ascomycota; Eurotiomycetes; Chaetothyriales; Herpotrichiellaceae
Unassigned
Fungi; Ascomycota; Sordariomycetes; Sordariales; Chaetomiaceae; Chaetomium
Fungi; Glomeromycota; Glomeromycetes; Glomerales; Glomeraceae; unidentified; unidentified

[a] OTU identification was generated in the QIIME program by comparing OTUs sequences against UNITE databases.

CAPÍTULO 2

MICROBIAL DIVERSITY AND COMPOSITION IN *Zea mays* RHIZOSPHERE IN COEXISTENCE WITH WEEDS UNDER DIFFERENT FERTILITY LEVELS

MICROBIAL DIVERSITY IN Zea mays RHIZOSPHERE IN COEXISTENCE WITH WEEDS SUBJECTED TO DIFFERENT FERTILITY LEVELS

Abstract

Aims When two plants are under coexistence, the structure of the rhizospheric microbial community differs from that observed in monocultures. The possibility of manipulating microbial populations to favor crops in detriment of weeds may be an important strategy for weed control. Here, we have evaluated how soil fertilization affects the ecological interactions between Zea mays and weeds and their associations with rhizosphere microorganisms.

Methods The growth of two weed species, Amaranthus viridis and Bidens pilosa, and the crop Z. mays was evaluated in the greenhouse under two management conditions, natural soil fertility, with no fertilizer addition, and the correction of soil fertility, in monoculture and plant coexistence. Total DNA from microbial populations in the rhizosphere soil was extracted, followed by PCR amplification and sequencing using the Illumina MiSeq platform.

Results Maize showed greater competitive ability than weeds under natural soil fertility. After soil fertilization, no difference in competiveness was observed among the plants tested. Bacterial diversity was negatively affected by fertility and specific taxa were differently affected by the two factors evaluated, plant coexistence and soil fertilization. The fungal community did not change at α and β diversity in any of the samples analyzed.

Conclusions Significant changes in the rhizospheric microbial communities take place when maize coexists with weeds. Besides being affected by crop-weed interactions, the diversity of rhizosphere microrganisms is also influenced by fertilization practices. Changes in maize competiveness were associated with soil fertilization and changes in the structure of rhizosphere microbial communities.

Keywords: Zea mays, Amaranthus viridis, Bidens pilosa, Plant Coexistence, Fertility, Microbial Diversity, llumina MiSeq sequencing.

Abbreviations

AMF - Arbuscular Mycorrhizal Fungi

OTUs - Operational Taxonomic Units

RII - Relative Interaction Intensity Index (RII)

Z.ma or Z. mays - Zea mays hybrid BM 3061

A.vi or A. viridis - Amaranthus viridis

B.pi or B.pilosa - Bidens pilosa L.

x - Indicate coexistence treatments

1. Introduction

The physicochemical characteristics of the soil can interfere with the intensity and outcome of plant interactions. Sediment type, light intensity, water regime, salinity, and soil fertility levels can affect the development of a given plant species and, consequently, affect its competition interactions (Li et al. 2014; Melo et al. 2014; Ozaslan et al. 2016; Laurent et al. 2017; Florentine et al. 2018).

The main nutrients that affect crop growth are nitrogen, phosphorus, and potassium. They are important for the performance of various physiological functions of plants, directly affecting the yield of agricultural crops (Alley et al. 2009; Khan et al. 2013; Morais et al. 2018). Maize requires adequate amounts of nutrients for optimum growth and productivity during all growth stages. Nitrogen is responsible for the dark green coloration of healthy crops and nitrogen fertilization dose and timing directly affect maize grain growth and yield (Khan et al. 2013; Kumar et al. 2017; Morais et al. 2018). Phosphorus is the second essential nutrient for maize and its deficiency slows maize growth, reduces biomass production, and grain number (Khan et al. 2013; Kumar et al. 2017). Also, potassium is one of the most abundant cations in plants and when present at appropriate levels in the soil, promotes better maize harvests (Khan et al. 2013; Kumar et al. 2017).

Fertilizer application to soil, especially N and P, also directly affects weed growth. Under such situation, weeds compete better with maize for increased dry matter and soil cover. They absorb nutrients faster than crops and accumulate them at higher levels in their tissues (Yin et al. 2006; Zimdahl 2007; Pereira et al. 2012; Khan et al. 2013). Proper nutrient management plays a key role in reducing weeds in agricultural systems by altering competitiveness among plants. Fertilization control may lead to changes in the diversity and composition of the weed community that may favor crops (Everaarts 1992; Oliveira Procópio et al. 2005; Yin et al. 2006; Pereira et al. 2012; Khan et al. 2013; Kaur et al. 2018).

In a 14-year experiment with maize, it was concluded that changes in crop-associated weed community composition were primarily affected by P availability, followed by that of N. Under NPK fertilization, weed diversity was higher, but weed density was reduced, while decreased NPK applications decreased weed diversity (Yin et al. 2006). Weed control is generally more important than fertilization, as weed-infested areas already contain nutrients that were not used by the crop (Khan et al. 2013). The herbicide application in combination

with fertilizers showed promising results in weed control and maize grain yield (Ali et al. 2016). In addition, the effect of adding nitrogen fertilizer on weeds will depend on the fertilizer source, weed species, seed source and environmental conditions (Dyck et al 1995; Evans et al. 2003; Sweeney et al. 2008).

Plant species differ in their ability to absorb and accumulate nutrients in their tissues and the elemental relationships of plant tissues are associated with the adaptive and competitive success of a plant species in an ecosystem. In evaluating the elemental indices of maize in interspecific competition with Amaranthus viridis L, Bidens pilosa L. and Ipomoea grandifolia (Dammer) O'Donell, it was observed that competition had little influence on the nutrient content of maize, but reduced growth and the absorption of nutrients from weeds. Weeds have shown different strategies, B. pilosa and I. grandifolia are able to use nutrients more efficiently and A. viridis tends to accumulate nutrients in their tissues (Matos et al. 2019). All this information is important to better understand the dynamics of weed infestations and to improve agroecosystem management programs.

Indeed, soil fertility manipulation is a key tool for integrated weed management in crops, but so far it is not known how changing soil fertility influences and alters rhizospheric microbial communities in competitive situations, and how these changes may interfere with the results of competitive interactions between plants. Melo et al. (2014) evaluated microbial biomass, respiration rate and metabolic quotient of maize soil in competition with six weed species under different fertility levels. The authors concluded that biomass and microbial activity are altered by plant species, competition and soil fertility management and suggested that changes in microbial activity may be a strategy used by plant species to minimize the effects of competition.

Although there is evidence that the rhizosphere microbial community of competing plants is altered by different levels of soil fertilizer, little is known about which taxa are lost or recruited in these situations and how such changes can interfere with outcomes of coexistence between weeds and cultures. Evaluating the effect of fertility on crop-weed coexistence and on the diversity of rhizospheric microorganisms is critical to better understand such interactions and to plan management strategies. Thus, the aim of this work was to evaluate the changes in the rhizospheric microbial community of Zea mays in coexistence with weeds subjected to different fertility levels in a greenhouse experiment.

2. Materials and Methods

2.1. Assembly of the experiment

The experiments were conducted under greenhouse conditions at the Departamento de Microbiologia, Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil. Three plant species were used, one crop Zea mays hybrid BM 3061, and two weeds, Bidens pilosa and Amaranthus viridis. The plants were grown for 40 days in plastic pots containing 4.5 dm^{-3} of soil collected near an experimental area at UFV, with the following chemical and textural characteristics: pH: 5,5; P: 28,8 $mg.dm^{-3}$; K: 142 $mg.dm^{-3}$; Ca: 2,8 $cmol_c.dm^{-3}$; Mg: 0,9 $cmol_c.dm^{-3}$; Al: 0,1 $cmol_c.dm^{-3}$; H+Al: 4,29 $cmol_c.dm^{-3}$; SB: 4,1 $cmol_c.dm^{-3}$; t: 4,2 $cmol_c.dm^{-3}$; T: 8,4 $cmol_c.dm^{-3}$; V: 49 %; m: 2 %; MO: 2,52 $dag.Kg^{-1}$; P-rem: 35,1 $mg.L^{-1}$; Zn: 3,2 $mg.dm^{-3}$; Fe: 32 $mg.dm^{-3}$; Mn: 91 $mg.dm^{-3}$; Cu: 0,9 $mg.dm^{-3}$; B: 0,2 $mg.dm^{-3}$; Argila: 25%; Silte: 17%; Areia: 58%; Textura Argilo Arenosa (textura média).

The experimental design was completely randomized consisting of 10 treatments, three monocultures and two coexistence combinations at two nutritional levels each [natural soil fertility, with no fertilizer addition, and fertilized soil, according to Novais (1991)], with four replications. In the fertilized soil treatment, 40 $mg.dm^{-3}$ of $(NH_4)_2SO_4$ and 65 $mg.dm^{-3}$ of NH_4NO_3 were applied to the soil for the supply of N and S, and 291.71 $mg.dm^{-3}$ of $Ca(H_2PO_4)_2$ for the supply of P and Ca. There was no need for K application. Phosphate fertilization was performed before weed planting by incorporating the reagents above into the total soil volume. Nitrogen fertilization was divided equally into three applications (10, 20 and 30 days after germination of maize), via cover nutrient solution.

Seeding was done keeping a uniform distribution of plants on the soil surface in the pots according to each treatment. For the monocultures, fourteen plant seeds were sown, and after emergence, only 9 plants were left per pot. For the coexistence treatments, five maize seeds and ten weed seeds were sown and, after the emergence of seedlings, three maize and six weed plants were left per pot. Amaranthus viridis and B. pilosa seeds were sown seven days befores the maize seeds. This difference between sowing times was adopted so that weeds were not overtaken by maize early in the growing season.

The plants were irrigated once a day as necessary with distilled water. Once a week, the pots were weighed for moisture correction to 60 % of field capacity. After cultivation, roots and shoots were collected, dried at 60 ºC until constant weight to determine the total dry matter production. Rhizospheric soil, defined as soil that remains bound to roots after gentle agitation, was collected and stored at -4 ° C until analysis.

2.2. Plant interaction analysis

The Relative Interaction Intensity Index (RII) was used to determine the intensity of interactions between plants. This index was calculated as described by Armas et al. (2004): RII = (total dry matter of plant growing with competitor - total dry matter of plant growing in monoculture)/(total dry matter of plant growing with competitor + total dry matter of plant growing in monoculture). The RII is a measure of the strength of interaction between species and ranges from −1 to 1, is symmetrical around zero. Negative values indicate suppressive effects due to resource competition or other antagonistic effects; positive values indicate facilitation effects.

2.3. Extraction of total DNA from the soil and sequencing by Illumina MiSeq

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. The DNA samples were quantified using Qubit® 2.0 Fluorometer (Invitrogen by Life Technologies) and lyophilized.

The samples were sent to Argonne National Laboratory, USA; where the steps of amplification, library preparation, and sequencing were done. The V4 region of the bacterial and archaeal 16S rDNA gene was amplified using the 515f/806r primer set (Caporaso et al. 2012), and the fungal ITS region from rDNA was amplified with primers ITS2 and ITS1f (Smith and Peay 2014). The sequencing was done using the Illumina MiSeq, with reads paired-end 150 x 150 bp. The 16S and ITS amplicons were sequenced in separate runs.

2.4. Processing and analyses of sequencing data

The raw data were processed as described by the Brazilian Microbiome Project protocol (Pylro et al. 2014a, b) using the BMP Operating System (BMPOS) (Pylro et al. 2016) and the results were analyzed by the Quantitative Insights Into Microbial Ecology Software (Qiime v.1.9.1) (Caporaso et al. 2010). For the analyses of 16S rRNA gene sequencing data, briefly, the quality filtering of the sequences were performed using VSEARCH v.2.3.4 (Rognes et al. 2016). The truncated reads shorter than 240 bp and the reads with a number of expected errors higher than 1.0 were discarded. The filtered reads were dereplicated and unique sequences (singletons) were also removed using VSEARCH. Sequences with more than 97 % similarity were considered the same Operational Taxonomic Unit (OTU), and comparisons were made with the UPARSE v.7.1b (Edgar 2013). The chimeric sequences were identified and removed using UCHIME (Edgar et al. 2011). After

clustering, the sequences were aligned and the taxonomy of each OTU was assigned using the reference database SILVA release 132 by the uclust method.

For the analysis of fungal ITS region sequencing data, only the forward file (R1.fastq) was used. The quality filtering of the sequences were performed using USEARCH 8.1 (Edgar 2010) discarding reads with number of expected errors higher than 0.5. After the dereplication, the ITSx software was used to extract only the ITS1 region from fungi. The truncated reads shorter than 140 bp were removed and the chimeric sequences were removed using UCHIME (Edgard et al. 2011) and USEARCH. Sequences with similarity greater than 97 % were grouped in the same OTU and the taxonomy of each was assigned using UNITE (Kõljalg et al. 2013) as the reference database by the uclust method.

2.5. Diversity and statistical analysis

Alpha diversity was calculated using the Qiime software and three metrics were calculated: Chao, Simpson and Shannon indices. The rarefaction curves were generated based on the number of observed OTUs in each treatment. For beta diversity, Multidimensional Scaling (MDS) were carried out based on the presence and absence of OTU sampling using the Jaccard's distance. For the Venn diagram, OTUs present in at least three of the four replicates and with a read number greater than or equal to 2 were considered as present in a sample. For the construction of the graphs, multivariate analyses, and statistics the R software, package ggplot2 (Wickham and Chang 2009), GraphPad Prism 7, and PAST were used. To compare the total dry matter, root dry matter, shoot dry matter and number of leaves for Z. mays in the treatments with no fertilizer and with fertilizer the t test was used. To compare the diversity indexes and relative abundance of OTUs among the samples the t test was also adopted. A one-way ANOVAs followed by correction by Bonferroni´s was used to compare phyla and genera which differ in their abundances according to coexistence, fertility or both, using the abundance in monoculture as reference.

3. Results

3.1. Sequencing summary

The Illumina MiSeq sequencing for Z. mays rhizosphere soil in monoculture and in coexistence with weeds subjected to different levels of fertility generated 2,381,047 sequences for the 16S rRNA gene. After quality filtering, removal of singletons and chimera by UCHIME, 2,288,073 (96.1 %) reads remained, with an average of 58,668.538 reads for sample. Among these, 2,211,872 (96.67 %) were classified as OTUs belonging to bacterial

phyla, 45,022.75 (1.97 %) reads were classified as belonging to phylum Archaea, and 31,178.24 (1.36 %) reads were attributed as 'Unassigned' (data not shown).

In the ITS region sequencing, 2,963,561 sequences were generated. After quality filtering, singletonremoval, extraction by ITSx and chimera removal by UCHIME, 820,402 (27.68 %) reads remained, with an average of 20,510.05 reads per sample. Among these, 584,791.8 (71.28 %) were classified as OTUs belonging to fungal phyla by UNITE database, 94,827.97 (11.56 %) reads were classified as Unidentified Fungi, and 140,782.3 (17.16 %) were grouped as "Unassigned".

For Bacteria and Archaea, the rarefaction curve tended to reach a plateau (Fig. S1a). Interestingly, the Good's coverage index values was lower for Bacteria than for Archaea, indicating that more sequences would be necessary to retrieve all the sequence types present to better express diversity within the treatments for Bacteria (Tab. S1). For fungi, 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. S1b) and the Good's coverage index values were higher than 99 % (Tab. S1).

3.2. Interaction between Zea mays and weeds under different levels of fertility

The Relative Interaction Intensity Index (RII) varied according to the combination of plants in coexistence (Fig. 1). Negative values indicate competition interactions and positive values indicate facilitation between coexisting plants. Thus, our results demonstrated that in both treatments with weed coexistence, maize had its growth facilitated. Differently, in the interaction between maize and B. pilosa, increased soil fertility led to decreased facilitation. Weeds, on the other hand, were negatively affected by competition with maize (Fig. 1) in all treatments, except in that with B. pilosa in which the growth of this species was facilitated by maize in the treatments with soil fertilization (Fig. 1).

Four morphological characteristics of the studied plants were analyzed: total dry matter, root dry matter, shoot dry matter and number of leaves (Fig. S2). For monoculture plants, all parameters were positively affected by soil fertility, except B. pilosa root dry matter (Fig. S2b). For the interation between Z. mays and A. viridis, for both plants, root dry matter was not affected by fertilization (Fig. S2f). In the interaction between Z. mays and B. pilosa, total dry matter, root dry matter, and shoot dry matter of maize showed no significant differences for treatments with and without fertilization (Fig. S2i, S2j, and S2k). These variables, however, showed similar or greater values than those obtained for maize

monoculture. The weed B. pilosa did not show increases in root dry matter with soil fertilization (Fig. S2j).

3.3. Comparison of the rhizospheric microbial community structure, richness and diversity analyses of OTUs depending on fertility

The Multidimensional Scaling (MDS) was used to analyze the relationship among soil treatments using a presence and absence matrix of OTUs based in the Jaccard's distance (Fig. 2). For the Z.ma rhizospheric soil in monoculture (Fig. 2a) and in coexistence with A. viridis (Fig. 2c) or B. pilosa (Fig. 2e), and the B. pilosa rizospheric soil in monoculture (Fig. S3c), the bacterial and archaeal communities in the treatments with fertilization formed a more similar group than in the treatments without fertilization. For the archaeal and bacterial communities of A. viridis monoculture (Fig. S3a) and for the fungal communities of all treatments, it can be noted that the soil samples from the same treatment did not group together. This shows that beta diversity for these microbial communities does not explain differences between soil samples with or without fertilization.

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), showed that there was no significant difference by the t test ($p>0.05$) for all samples, when comparing those with and without fertilization (Table 1). The Shannon diversity index, based on species richness, considering the number of taxa and the abundance of individual taxa (Chiarucci et al. 2011) and the Simpson diversity index that considers the evenness of species more than richness, considering the common species (Lemos et al. 2011; Kim et al. 2017), demonstrated greater diversity of bacterial and archaeal community in the rhizospheric soil of Z. mays in monoculture without fertilization and in the coexistence between Z. mays and B. pilosa also without fertilization, when compared to the respective fertilization samples (Table 1). In addition, the Simpson index, by providing values closer to one, evidenced greater diversity for bacteria and archaea than for fungi in all the samples analyzed (Table 1).

3.4. Taxonomic composition of the microbial community depending on soil fertility

The OTUs of Bacteria found in the rhizosphere of Z. mays in monoculture with or without fertilization were classified in 33 different phyla, 179 orders, and 537 genera that received taxonomic attributions. The orders and genera that could not be assigned to any taxa were grouped as "uncultured bacterium", "metagenome", "unassigned" and "other". Archaeal OTUs were classified into 3 phyla, 3 orders and 2 genera. The dominant phyla of Bacteria

and Archaea were Proteobacteria (26.2 % of all reads), Actinobacteria (22.6 %), Acidobacteria (18.5 %), Verrucomicrobia (7.0 %), Chloroflexi (4.4 %), Planctomycetes (4.3 %), Firmicutes (4.0 %), Gemmatimonadetes (3.5 %), Bacteroidetes (2.8 %), Thaumarchaeota (Archaea, 2.1 %), Cyanobacteria (1.2 %), and other phyla with more than 1 % of relative abundance, making up 96.5 % of the total abundance of bacterial and archaeal OTUs (Fig. 2a). 1.3 % of the reads could not be taxonomically classified and were said to be "Unassigned". Other phyla were grouped into "Others", making up 2.2 % of the reads (Fig. 3a). The bacterial profile at the phylum level is very similar among the samples of rhizospheric soil with and without fertilization, and only one phyla showed significant differences in the relative abundances between treatments ($p<0.05$), Planctomycetes, which is more abundant in the soil samples from monocultures, without soil fertilization (Fig. S4a). At the genus level, the 20 most abundant were selected (Fig. 3b) and, among these, three showed significant differences in the relative abundances between treatments. Bryobacter (8.1 % of all reads, $p<0.01$) (Fig. S4c) and Sphingomonas (18.6 %, $p<0.05$) (Fig. S4d), both more abundant in the fertilization treatment samples, and Amycolatopsis (2.2 %, $p<0.05$) (Fig. S4b) more abundant in the soil samples without fertilization.

Similar to that observed for the maize monoculture and in coexistence treatment with Z. mays and A. viridis, the composition and relative abundance of bacterial OTUs were similar across all treatments. These OTUs were classified into 33 different phyla, 179 orders, and 537 genera, which received taxonomic attributions. Archaeal OTUs were classified into 3 phyla, 3 orders, and 2 genera that could be assigned to specifi taxa. The dominant phyla of Bacteria and Archaea were Proteobacteria (26.1 % of all reads), Actinobacteria (23.7 %), Acidobacteria (18.2 %), Verrucomicrobia (6.3 %), Chloroflexi (4.8 %), Planctomycetes (4.5 %), Gemmatimonadetes (3.5 %), Bacteroidetes (2.7 %), Firmicutes (2.6 %), Cyanobacteria (2.6 %), and Thaumarchaeota (Archaea, 1.8 %), and all phyla with more than 1 % of relative abundance, making up 96.7 % of the total abundance of bacterial OTUs (Fig. 3c). The 1.3 % of the reads that could not be taxonomically classified were said to be "Unassigned" and the others phyla were grouped into "Others", making up 2.1 % of the reads (Fig. 3c). No phylum showed significant differences in relative abundance compared to soil samples with and without fertilization. At the genus level, the 20 most abundant taxa were selected (Fig. 3d), among which, only one showed significant differences in the relative abundances between treatments ($p<0.05$), the genera Acidibacter (0.7 %) being more abundant in the soil samples without fertilization (Fig. S4e).

In the coexistence treatment with Z. mays and B. pilosa, the OTUs were classified into 33 different phyla, 179 orders, and 541 genera that received taxonomic attributions. Archaeal OTUs were classified into 3 phyla, 3 orders, and 2 genera. The dominant phyla of Bacteria and Archaea were Proteobacteria (27.1 % of all reads), Actinobacteria (20.1 %), Acidobacteria (18.5 %), Verrucomicrobia (7.0 %), Chloroflexi (4.6 %), Planctomycetes (4.4 %), Firmicutes (3.9 %), Gemmatimonadetes (3.6 %), Bacteroidetes (3.0 %), Thaumarchaeota (Archaea, 2.0 %) and Cyanobacteria (1.8 %), all phyla with more than 1 % of relative abundance, making up 96.0 % of the abundance of bacterial OTUs (Fig. 3e). The 1.3 % of the reads that could not be taxonomically classified were said to be "Unassigned" and the other phyla were grouped into "Others", making up 2.7 % of the reads (Fig. 3e). No phylum showed significant differences in relative abundance for soil samples with and without fertilization. At the genus level, the 20 most abundant were selected (Fig. 3f) and among these, three showed significant differences in the relative abundances between treatments, the genera Acidibacter (2.1 %, $p<0.01$) (Fig. S4f) and Haliangium (3.5 % all reads, $p<0.05$) (Fig. S4g) both were more abundant in the soil samples without fertilization, and Sphingomonas (20.7 %, $p<0.05$) (Fig. S4h) in soil samples with fertilization.

The taxonomic profile of fungal OTUs is visually more varied when comparing samples with and without fertilization, however, unlike that observed for bacteria and archaea, no significant differences were observed in the relative abundances of any OTU at the phylum and genera levels in any soil sample in monoculture or coexistence (Fig. 4), confirming the results shown by the MDS, that fertilization did not affect the fungal beta diversity between these samples.

3.5. Comparison of the rhizospheric microbial community structure and OTUs affected by coexistence and soil fertilization

When comparing samples of rhizospheric soil with and without fertilization in maize monoculture or in coexistence with A. viridis or B. pilosa, it was observed that most of the OTUs classified as archaea, bacteria, or fungi were shared between the treatments (Fig. 5). Interestingly, no exclusive OTUs were observed in any of the treatments analyzed. The same can be observed for A. viridis and B. pilosa rhizospheric soil samples in monoculture (Fig. S5). The rhizospheric soil of the coexistence treatment with Z. mays and B. pilosa, without fertilization, corresponded to that with the highest number of bacterial and archaeal OTUs, totalling 1,306 (Fig. 5c). Maize monoculture under fertilization corresponded to the treatment with the highest number of fungal OTUs, totalling 169 (Fig. 5b and 5d).

By comparing the two factors analyzed in this work, maize in coexistence with weeds and soil fertilization, it was possible to identify which OTUs are affected by one factor or the other. Comparing Z. mays monoculture and the coexistence of Z. mays and A. viridis, with or without fertilization and using the maize monoculture without fertilizer as reference, the phylum Firmicutes and the genus Bryobacter were affected only by plant coexistence. The phyla Armatimonadetes, Latescibacteria, and Rokubacteria were affected only by fertilization (the comparison between Z. mays and the coexistence between Z. mays and B. pilosa with fertilizer showed no significant differences ($p > 0.05$, data not shown), and the genus Candidatus Uedobacter and RB41 were affected by both plant coexistence and fertilization (Fig. 6).

Comparing maize monoculture and the coexistence between Z. mays and B. pilosa, with or without fertilization, the phylum Firmicutes and the genus Norcadioides were affected only by plant coexistence. The phyla Armatimonadetes, Latescibacteria, Rokubacteria, and the genus Bryobacter and Sphingomonas were affected only by soil fertilization (the comparison between Z. mays and the coexistence between Z. mays and B. pilosa with fertilizations showed no significant differences ($p > 0.05$), data not shown). The genus Acidibacter was affected by both plant coexistence and soil fertilizaton (Fig. 7).

4. Discussion

In this work, it was possible to characterize the diversity and composition of microbial communities present in maize rhizosphere in coexistence with weeds, submitted to different levels of fertility. The approach used, Illumina MiSeq sequencing, was efficient to demonstrate the changes in the rhizospheric microbiota due to the two factors analyzed, plant coexistence and soil fertilization. This study is the first report on the use of this approach to evaluate these interactions.

4.1. Ecological interaction between plants

To evaluate the interaction between the coexisting plants, the Relative Interaction Intensity Index (RII) was calculated. In the coexistence treatments with Z. mays and weeds, maize had its growth facilitated by the weeds ($RII > 0$) (Fig. 1). The change in soil fertility affected the interaction between the plants tested. In the coexistence between maize and B. pilosa, maize growth facilitation was decreased and B. Pilosa, which had been negetaivelly affected by competition with maize, had its growth facilitated by the latter species (Fig. 1).

Maize can be generally considered a strong competitor or with good competitive ability when coexisting with certain weed species, such as Commelina benghalensis, Brachiaria brizantha, Ipomoea ramosissima, Ageratum conyzoides, and Bidens pilosa (Faria et al. 2014; Massenssini 2014; Monteiro 2016). In some cases, facilitation of maize growth can occur and this is dependent on the soil microbial structure and competing weed species (Matos et al. 2019). Soil fertilization increases both crop and weed dry matter increment, generally leading to higher nutrient uptake and storage in the weed than in the crop tissues (Oliveira Procópio et al. 2005; Pereira et al. 2012; Khan et al. 2013). The two plant species tested in our experiment, A. viridis and B. pilosa, are characterized for being aggressive, with allelopathic properties, and especially efficient at capturing and using environmental resources, such as water and nutrients (Oliveira Procópio et al. 2005; Sanon et al. 2009; Santos and Cury 2011). These traits could explain the increased competitive ability of these weeds when grown together with maize under soil fertilization. With increasing doses of phosphorus in the soil, B. pilosa shows good responses with increasing dry matter accumulation (Oliveira Procópio et al. 2005). A. viridis not only has the ability to increase soil nutrient availability, but also to increase bacterial abundance and soil microbial activity (Sanon et al. 2009). Another important point is that interspecific competition changes the elemental ratios of the weed tissues, changing biomass quality as reported by Matos et al. (2019), but the magnitude of these changes is dependent on the plant species living in coexistence

Increased fertility by itself promotes increments in all the morphological variables analyzed in our work, i.e., total dry matter, shoot dry matter, root dry matter and number of leaves, as observed for the monocultures (Fig. S2a, S2b, S2c and S2d). In the treatments with coexistence of Z. mays with B. pilosa, dry matter accumulation in the soil without fertilization, was higher or similar to those measured for maize monoculture in the soil without fertilization (Fig. S2i, S2j, S2k and S2l). This indicates that B. pilosa is the species that least affected dry matter production by Z. mays and that fertilization, in the treatments of coexistence with this plant, little contributed to increases in total, root, and shoot dry matter of Z. mays. A previous study was conducted with Z. mays in interspecific coexistence with B. pilosa and A. viridis in the greenhouse (Matos et al. 2019). Competition did not influence shoot and root dry matter production of maize as observed in our experiment (Matos et al. 2019).

4.2. Alteration of microbial rhizosphere diversity depending on coexistence and fertility

Our results showed that maize monoculture showed greater bacterial and archaeal diversity by the Shannon and Simpson indices for the soil without fertilizer addition. The same was observed for the coexistence between maize and B. pilosa (Table 1). In an experiment with the aim of evaluating microbial taxa present in the rhizosphere of Zea mays in monoculture and in coexistence with Ageratum conyzoides, Ipomoea ramosissima and B. pilosa, bacterial and archeal communities in the rhizospheric soil of the monoculture of maize and of the coexistence between Z. mays and B. pilosa were the most similar among all treatments, sharing the largest number of OTUs (Monteiro 2016). This fact supports the changes in the diversity of bacteria and archaea as a function of fertility that were observed in our work. These changes occurred in the same direction as that observed by Monteiro (2016) and reflect the similarities of the microbial communities involved.

To evaluate the effects of organic (OM) and mineral (NPK) fertilization on microbial biomass, activity, and diversity, a study based on PLFA analyses in a long-term fertilization experiment with maize was conducted. The amounts of total, bacterial, gram-negative, and actinobacterial PLFAs were found to be highest in the OM + NPK treatment, the gram-positive and anaerobic PLFAs were highest in the OM treatment and aerobic and fungal PLFAs were highest in the NPK treatment. The authors further concluded that the Shannon's index was significantly affected by total N and the Simpson’s index was significantly correlated with available P, indicating that soil fertility positively affects microbial diversity in maize soils (Zhong et al. 2010). In a more recent long-term studies using Illumina MiSeq for sequencing 16S rDNA genes for bacteria and 18S rDNA gene for fungi, in soils subjected to fertilization with farmyard manure, crop residues, and chemical fertilizer (NPK), in winter wheat/summer maize rotation, concluded that the long-term application of organic and inorganic fertilizers could enrich the bacterial and fungal communities and promote its diversity and that maize yields are positively correlated with changes in the soil microbial community structure (Ma et al. 2017). Another important factor, already described, is that most microbial veriables were mainly correlated with soil organic carbon content rather than P and N, indicating that the application of P and N did not directly affect microbial veriables in the soil, but did so indirectly by increasing crop yields, thus promoting the accumulation of soil organic matter (Zhong and Cai 2007). Similarly, in our experiments, we have observed increases in the morphological variables evaluated both for maize monoculture and for the coexistence treatment with maize and B. pilosa, reflecting in the increase of bacterial and archaea diversity (Fig. S2).

The fungal community was not significantly affected both at the level of α- (Chao, Shannon, and Simpson indexes) (Table 1) and β-diversity (Fig. 2 and S3). Similar results were observed in a study on maize rhizobiome in monoculture or in coexistence with A. conizoydes, I. ramosissima and B. pilosa (Monteiro 2016), demonstrating that coexistence did not significantly alter the rhizospheric soil fungal community for these plant combinations. N fertilization in the long-term had a negative effect on the biomass of arbuscular mycorrhizal fungal (AMF) in the soil associated with maize (Jeske et al. 2018). Decreases in the AMF biomass were higher with increased N fertilization rates. Conversely, nutrient deficiency in soil increases associations with AMF (Wu et al. 2005; Sarabia et al. 2017) and the use of different fertilization treatments can shape the microbial community in the same soil (Ma et al. 2017), being soil bacteria more sensitive than fungi to the fertilization practices (Ai et al. 2018). Here, we did not observe significant differences in soil fungal communities as a function of fertility, and this is probably due to the amount of NPK fertilizer used, which was only sufficient to correct the natural soil fertility in our short-term experiment the greenhouse.

4.3. Bacterial OTUs affected by coexistence and soil fertility

The phyla Armatimonadetes, Latescibacteria, Rokubacteria were affected only by fertilization in both coexistences situations evaluated, when maize monoculture without fertilization was used as reference (Fig. 6 and 7). In all cases, there was a significant reduction in the abundance of these taxa due to the factors evaluated. The phylum Armatimonadetes, formerly known as phylum candidate OP10, comprises, within known isolates, of gram-negative, pigment-producing bacteria with oligotrophic characteristics (Tamaki et al. 2011; Lee et al. 2014; Araujo et al. 2019). Their oligotrophic character may problably explain the significant decrease in the abundance of this group in the rhizospheric soil of Z. mays competing with A. viridis and B. pilosa in the treatments with fertilization

The phylum Rokubacteria, formerly known as candidate phylum SPAM, comprises bacteria with large genomes, high GC content, globally distributed, and with a versatile myxotrophic metabolism, which constitutes a generalistic metabolic strategy in oligotrophic environments, where it was first found (Becraft et al. 2017). Its metabolic adaptation to environments with low nutrient availability may also explain the decreased abundance in the fertilization treatments evaluated in this study. The scarce information on genomes, metabolic characteristics, and the absence of important metabolic pathways in the most recently discovered bacterial phyla, named as candidate phyla (Becraft et al. 2017), make up a challenge to better understand the change in abundance as fertility increases of the soil.

The phylum Fimicutes had its abundance negatively affected only as a function of coexistence in the two coexistence treatments evaluated, Z. mays with A. viridis and Z. mays with B. pilosa (Fig. 6 and 7). This phylum plays a fundamental role in initiating the degradation of complex substrates, such as plant cell walls, mucin, and starch particles, i.e., characteristics of r strategists (Flint et al. 2012; Chávez-Romero et al. 2016; Araujo et al. 2019). The increase in the abundance of this phylum has already been reported by comparing bulk soil with rhizospheric soil of maize, due to increases in nutrient availability around the roots (Araujo et al. 2019). However, when two plants are in coexistence, exudate composition will be different from that observed for plants in monoculture, and this supply of different organic compounds in the exudates released by plants may promote the recruitment of specific microbiota in the rhizosphere of plants in competition (Marschner et al. 2004; Bais et al. 2006; Zahar Haichar et al. 2014; Monteiro 2016; Christian and Bever 2018; Sasse et al. 2018). Due to their characteristics as r strategist, this phylum, which is favored in high nutrient but low competition environments, would be expected to increase its abundance due to the increased nutrient availability. This was not the case in our experiments since increased nutrient availability may also increase microbial competition for energy and carbon sources, which may have been detrimental to the Firmicutes phylum.

Comparing the rhizospheric soil samples of the maize monuculture with the coexistence between Z. mays and A. viridis as a function of fertility, we found that the abundance of genera that showed significant differences between the treatments varied as follows: Bryobacter and Candidatus Udaeobacter had their abundance increased as a function of coexistence and fertilization. The reverse was found for the genus RB41 which showed reduced abundance as a function of both factors. The Bryobacter genus is composed of gram-negative, strict aerobic, chemoorganotrophic bacteria capable of hydrolyzing various heteropolysaccharides and having various sugars as preferred growth substrates (Kulichevskaya et al. 2010). Contrary to the pattern previously observed for the phylum Firmicutes, increased release of common and rarer carbon sources in the soil due to the combination of competing plants, may have favored the increased abundance of this genus. The same hypothesis can be used to explain the increased abundance of Candidatus Udaeobacter. Because of its aerobic heterotrophy, relatively small genome, and multiple putative auxotrophies (Brewer et al. 2017), the abundance of this genus was positively affected by increased soil fertility and increased availability of new organic carbon sources in the rhizosphere promoted by plant coexistence. The genus RB41 has shown to be the most sensitive biomarker responding negatively to fertilization (Ai et al. 2018). In our work, RB41

abundance was negatively affected by fertilization and also by increased sources of nutrients available in the rhizosphere in plant coexistence situations.

Comparing the rhizospheric soil samples of maize monuculture with the coexistence treatment with Z. mays and B. pilosa as a function of fertility, we have observed that the genus Nocardiodes had its abundance decreased as a function of competition; the genera Bryobacter and Sphingomonas had higher abundance in soil samples with fertilization, and Acidibacter genus had its abundance increased as a function of plant coexistence and decreased as a function of soil fertility (Fig. 7). As previously described, the Bryobacter genus increased its abundance due to fertilization, possibly due to increases in the availability of nutrient sources, since it is a bacterium that has a high metabolic plasticity for carbon sources. The genera Nocardioides and Sphingomonas have been previously described as present only in the maize rhizosphere in coexistence with A. conyzoides, when compared with the rhizospheric soils of maize monoculture, with coexistence between maize and I. ramosissima, and maize in coexistence with B. pilosa (Monteiro 2016). These genera were described as plant growth promoters (Sheng et al. 2012; Pereira and Castro 2014) and are present here in all samples analyzed. Further information on their metabolic characteristics are necessary to infer the changes in abundances found here. Manure application, soil organic matter and Zn, Pb, and as quantities are positively correlated with Acidibacter (Ai et al. 2018). In our work, in fact, fertilization has positively affected the abundance of this genus. This genus has also been described as exclusive to the Z. mays rhizosphere in coexistence between B. pilosa (Monteiro 2016), and here we observed that coexistence with the same weed species had a negative effect on its abundance. More information on the functionality of Acidibacter is needed to understand such changes.

5. Conclusion

The coexistence between the maize and the weeds evaluated demonstrated that the crop has its growth facilitated in both levels of soil fertilization. The addition of fertilizer in the soil promoted the change of ecological relationships from negative to positive, when the weed B. pilosa was in coexistence with maize. The Illumina MiSeq sequencing revealed the taxonomic composition of microbial communities present in the rhizosphere of Z. mays in coexistence with A. viridis and B. pilosa, subjected the different fertility levels. Bacterial diversity was negatively affected by fertility and specific taxa were differently affected by the two factors evaluated, coexistence and fertility. The fungal community did not change at α and β diversity in any of the samples analyzed.

Acknowledgments

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Figures

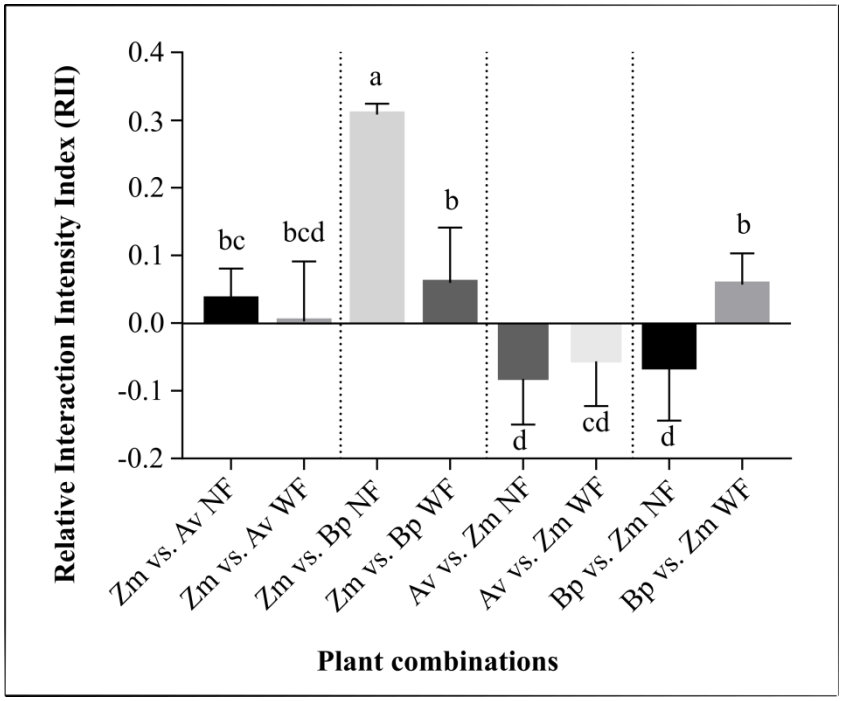


Fig. 1. Relative Interaction Index (RII) after 40 days of weed growth in a greenhouse in monoculture or in competion. The bars represent the standard deviation and different letters indicate significant differences between means based on the Tukey's test ($p<0.05$). The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

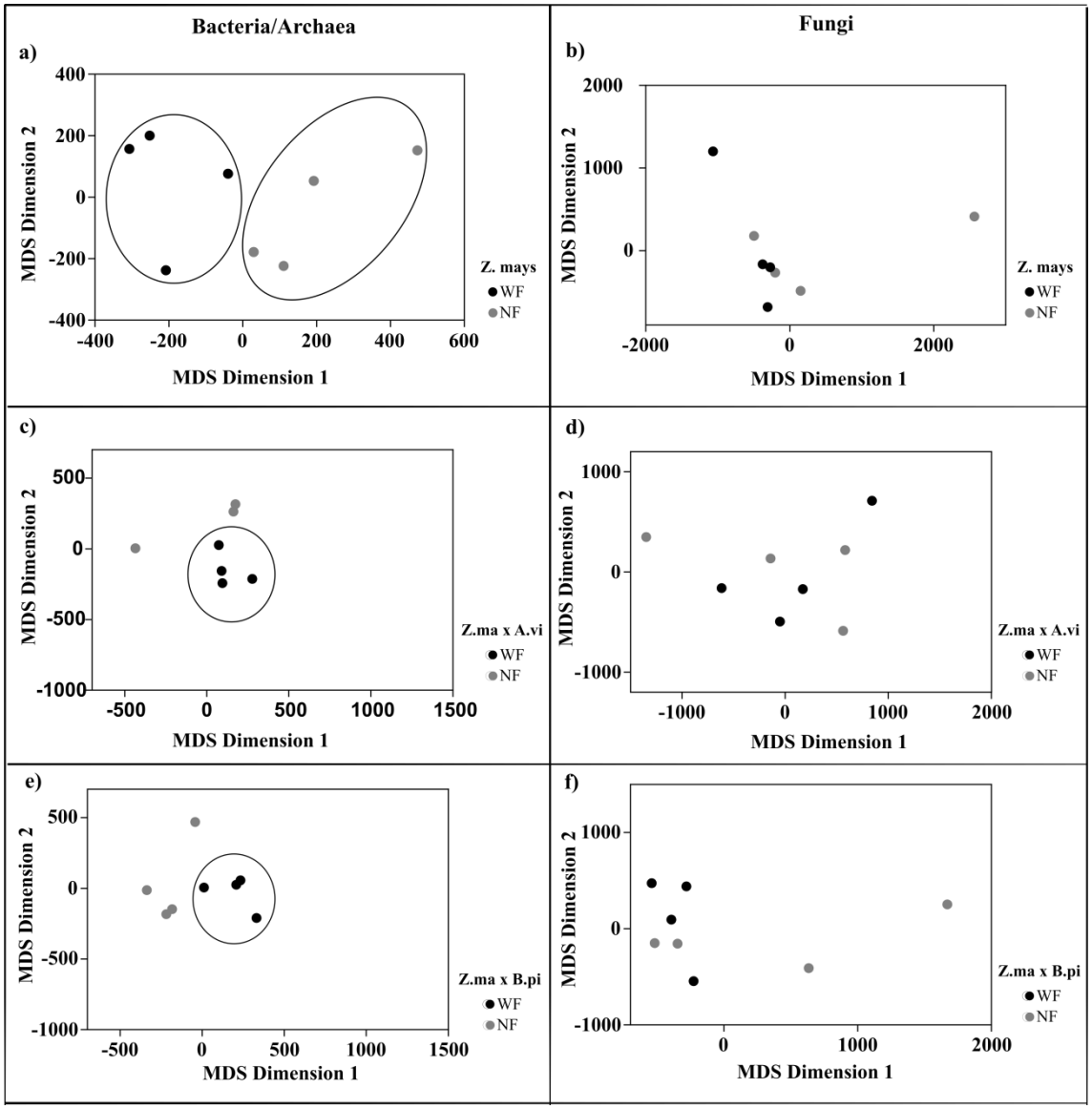
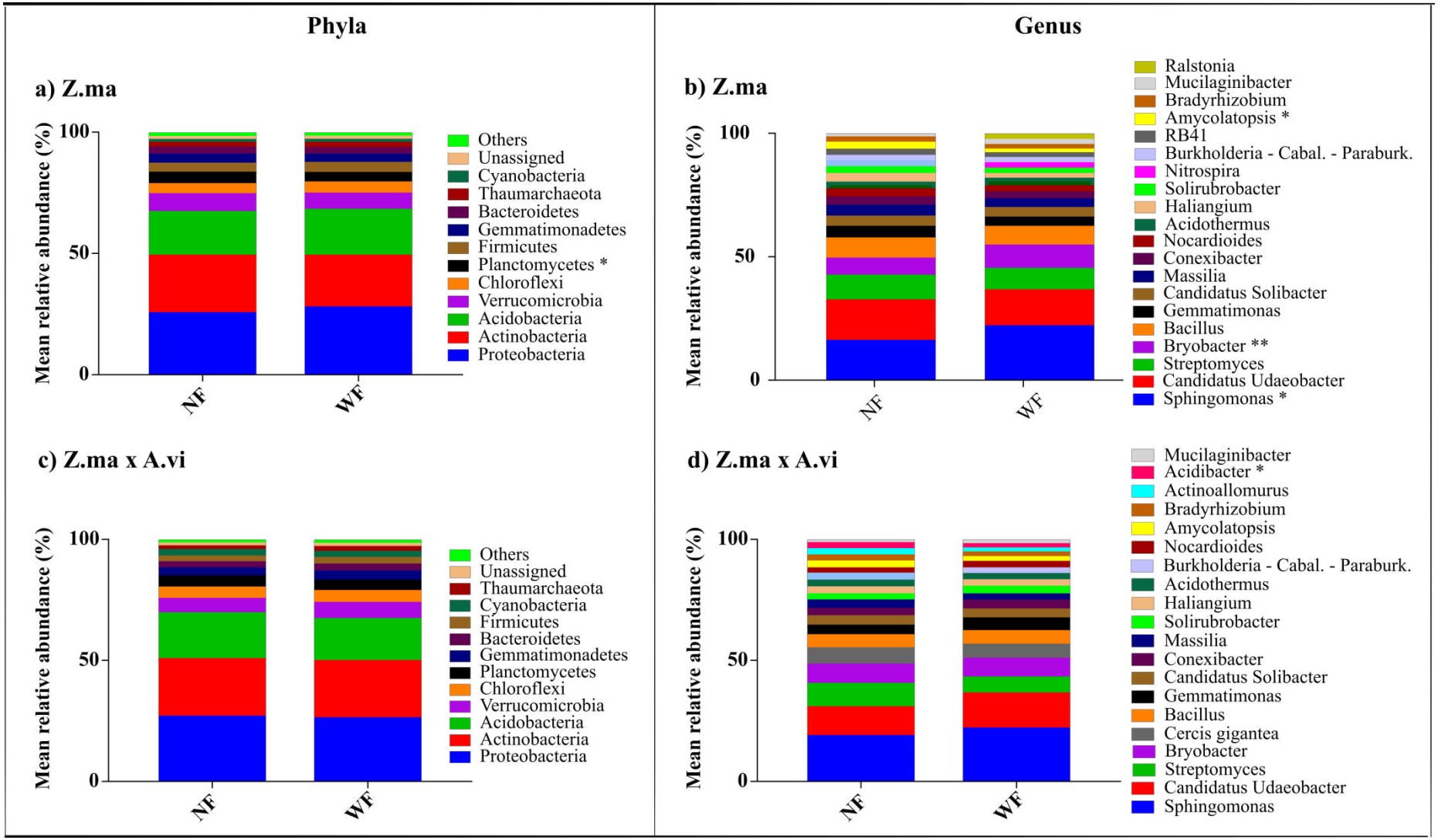


Fig. 2. Multidimensional Scaling (MDS) comparing soil microbial community as a function of fertility. **(a)** Bacteria and Archaea of Zea mays in monoculture and **(b)** Fungi, **(c)** Bacteria and Archaea of Z. mays in coexistence with Amaranthus viridis and **(d)** Fungi, and **(e)** Bacteria and Archaea of Z. mays in coexistence with Bidens pilosa and **(f)** Fungi. The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa.
Coexistence treatments are indicated by "x" between the name of two species.


Phyla
a) Z.ma
Mean relative abundance (%)
100
50
0
NF
WF
Others
Unassigned
Cyanobacteria
Thaumarchaeota
Bacteroidetes
Gemmatimonadetes
Firmicutes
Planctomycetes *
Chloroflexi
Verrucomicrobia
Acidobacteria
Actinobacteria
Proteobacteria
c) Z.ma x A.vi
Mean relative abundance (%)
100
50
0
NF
WF
Others
Unassigned
Thaumarchaeota
Cyanobacteria
Firmicutes
Bacteroidetes
Gemmatimonadetes
Planctomycetes
Chloroflexi
Verrucomicrobia
Acidobacteria
Actinobacteria
Proteobacteria
Genus
b) Z.ma
Mean relative abundance (%)
100
50
0
NF
WF
Ralstonia
Mucilaginibacter
Bradyrhizobium
Amycolatopsis *
RB41
Burkholderia - Cabal. - Paraburk.
Nitrospira
Solirubrobacter
Haliangium
Acidothermus
Nocardioides
Conexibacter
Massilia
Candidatus Solibacter
Gemmatimonas
Bacillus
Bryobacter **
Streptomyces
Candidatus Udaeobacter
Sphingomonas *
d) Z.ma x A.vi
Mean relative abundance (%)
100
50
0
NF
WF
Mucilaginibacter
Acidibacter *
Actinoallomurus
Bradyrhizobium
Amycolatopsis
Nocardioides
Burkholderia - Cabal. - Paraburk.
Acidothermus
Haliangium
Solirubrobacter
Massilia
Conexibacter
Candidatus Solibacter
Gemmatimonas
Bacillus
Cercis gigantea
Bryobacter
Streptomyces
Candidatus Udaeobacter
Sphingomonas

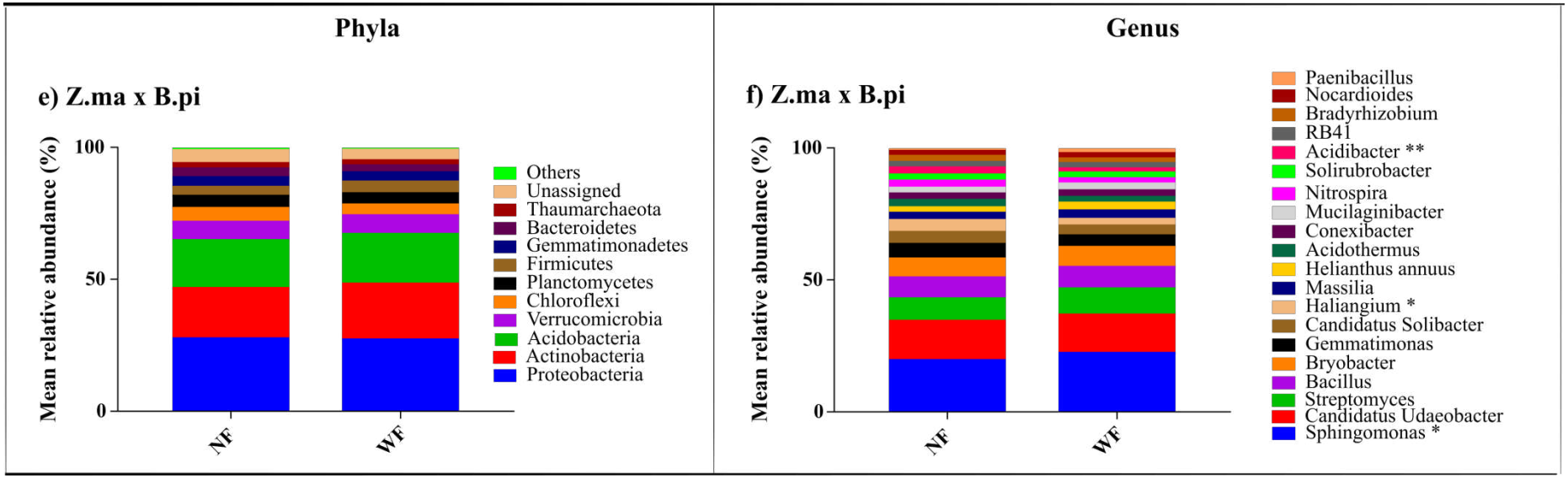
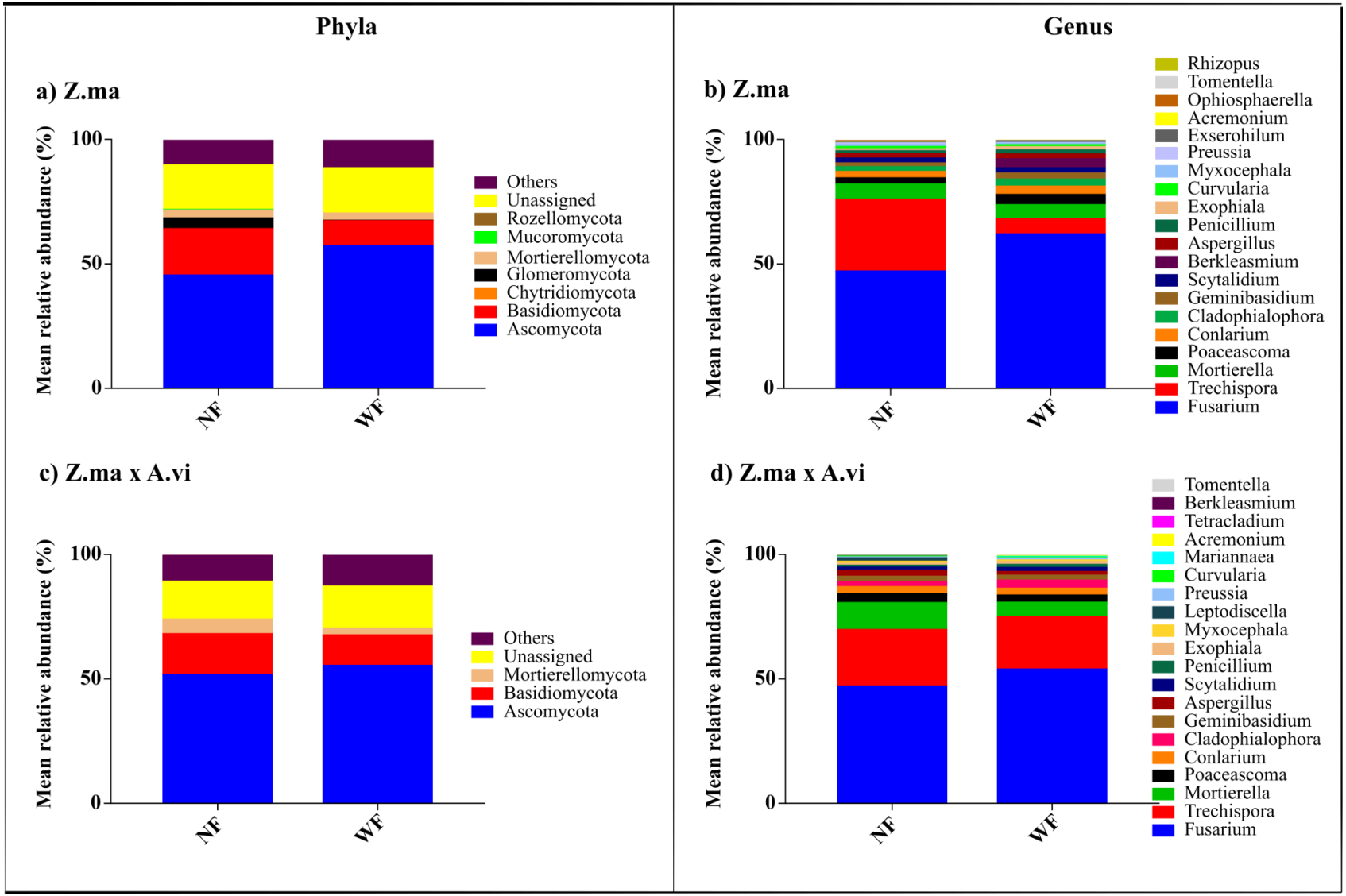


Fig. 3. Taxonomic distribution of OTUs of Bacteria and Archaea in rhizospheric soil treatments. **(a)** At the phylum level of monoculture of Zea mays, **(b)** at the genus level (95 % of similarity) of monoculture of Z. mays, **(c)** at the phylum level of coexistence between Z. mays and Amaranthus viriris, **(d)** at the genus level (95 % of similarity) of coexistence between Z. mays and A. viriris, **(e)** at the phylum level of coexistence between Z. mays and Bidens pilosa, and **(d)** at the genus level (95 % of similarity) of coexistence between Z. mays and B. pilosa. The asterisks indicate that this OTUs differs significantly relative abundance between treatments by the t test (*, ** and ***, significant to 5, 1 and 0,1 %, respectively). The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by “x” between the name of two species.


Phyla
a) Z.ma
Mean relative abundance (%)
100
50
0
NF
WF
Others
Unassigned
Rozellomycota
Mucoromycota
Mortierellomycota
Glomeromycota
Chytridiomycota
Basidiomycota
Ascomycota
c) Z.ma x A.vi
Mean relative abundance (%)
100
50
0
NF
WF
Others
Unassigned
Mortierellomycota
Basidiomycota
Ascomycota
Genus
b) Z.ma
Mean relative abundance (%)
100
50
0
NF
WF
Rhizopus
Tomentella
Ophiosphaerella
Acremonium
Exserohilum
Preussia
Myxocephala
Curvularia
Exophiala
Penicillium
Aspergillus
Berkleasmium
Scytalidium
Geminibasidium
Cladophialophora
Conlarium
Poaceascoma
Mortierella
Trechispora
Fusarium
d) Z.ma x A.vi
Mean relative abundance (%)
100
50
0
NF
WF
Tomentella
Berkleasmium
Tetracladium
Acremonium
Mariannaea
Curvularia
Preussia
Leptodiscella
Myxocephala
Exophiala
Penicillium
Scytalidium
Aspergillus
Geminibasidium
Cladophialophora
Conlarium
Poaceascoma
Mortierella
Trechispora
Fusarium

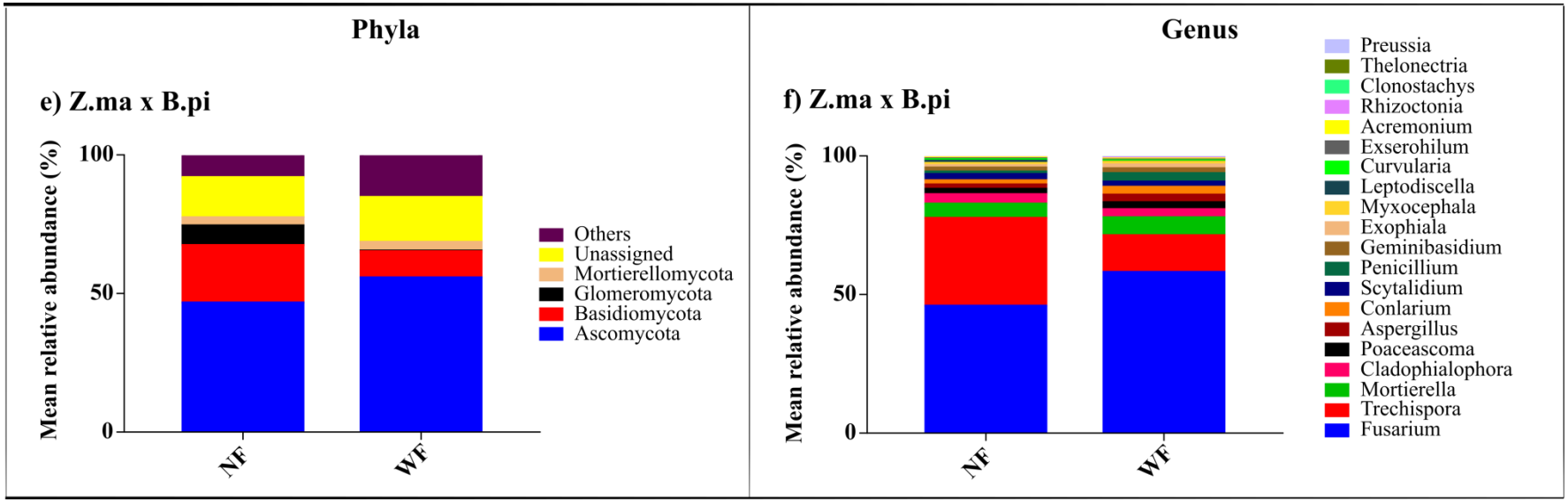


Fig. 4. Taxonomic distribution of OTUs of Fungi in rhizospheric soil treatments. **(a)** At the phylum level of monoculture of Zea mays, **(b)** at the genus level (95 % of similarity) of monoculture of Z. mays, **(c)** at the phylum level of coexistence between Z. mays and Amaranthus viriris, **(d)** at the genus level (95 % of similarity) of coexistence between Z. mays and A. viriris, **(e)** at the phylum level of competition between Z. mays and Bidens pilosa, and **(d)** at the genus level (95 % of similarity) of coexistence between Z. mays and B. pilosa. The asterisks indicate that this OTUs differs significantly relative abundance between treatments by the t test (*, ** and ***, significant to 5, 1 and 0,1 %, respectively). The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by “x” between the name of two species.

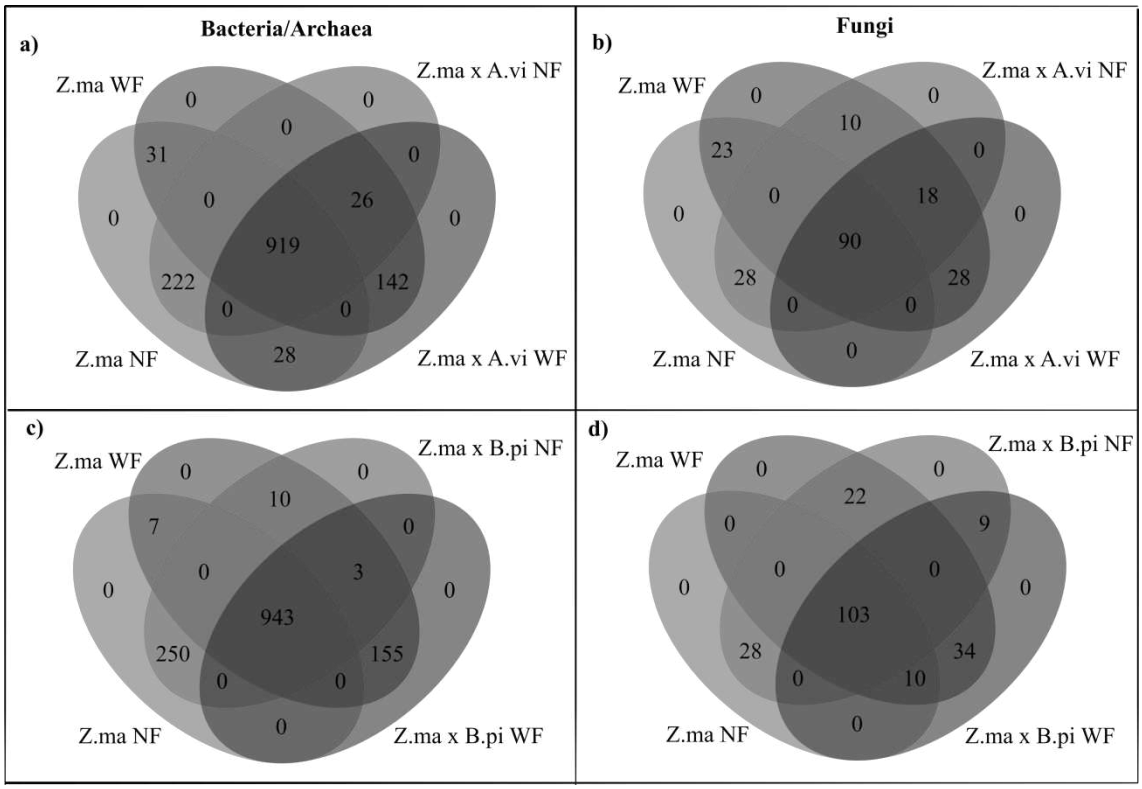


Fig. 5. Venn diagram of OTUs from **(a)** Bacteria and Archaea of Zea mays in monoculture and the coexistence between Z. mays and Amaranthus viridis, with and without fertilizer, and **(b)** Fungi in the same conditions. **(c)** Bacteria and Archaea of Zea mays in monoculture and the coexistence between Z. mays and Bidens pilosa, with and without fertilizer, and **(d)** Fungi in the same conditions. Exclusive and shared OTUs among treatments are based on 97 % similarity. The numbers inside the diagram indicate the numbers of OTUs. The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

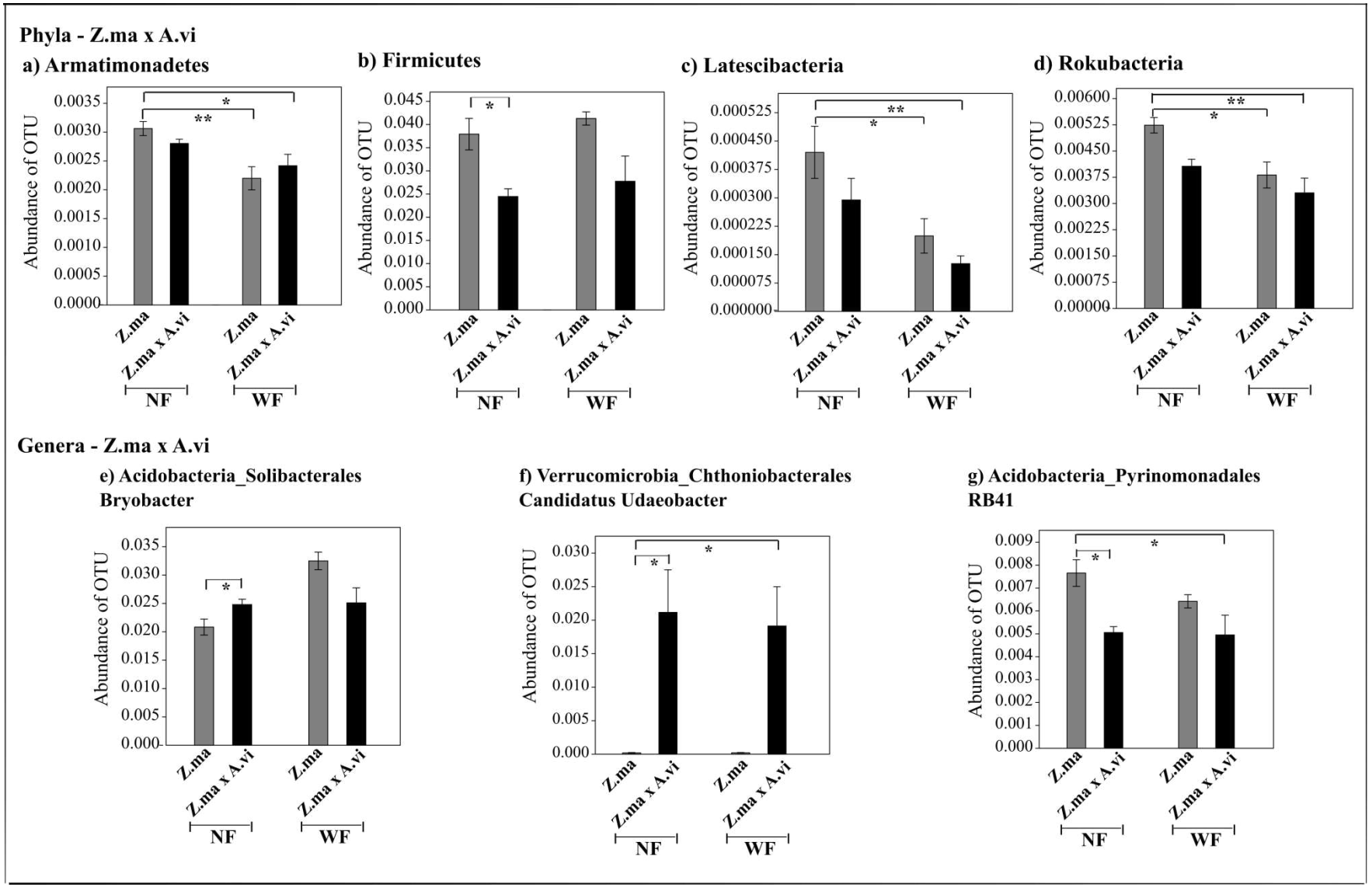


Fig. 6. Important phyla and genera of Bacteria present in the rizhospheric soil that showed significant differences in their relative abundances when comparing monoculture of Zea mays and the coexistence between Z. mays and Amaranthus viridis, subjected to different levels of fertility.

Phyla: **(a)** Phylum Armatimonadetes, **(b)** Phylum Firmicutes, **(c)** Phylum Latescibacteria and **(d)** Phylum Rokubacteria. Genera: **(e)** genus Bryobacter, belonging to order Solibacterales and to phyla Acidobacteria, **(f)** genus Candidatus Udaeobacter, belonging to order Chthoniobacterales and to phyla Verrucomicrobia and **(g)** genus RB41, belonging to order Pyrinomonadales and to phyla Acidobacteria. The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays and A.vi – Amaranthus viridis. Coexistence treatments are indicated by "x" between the name of two species.

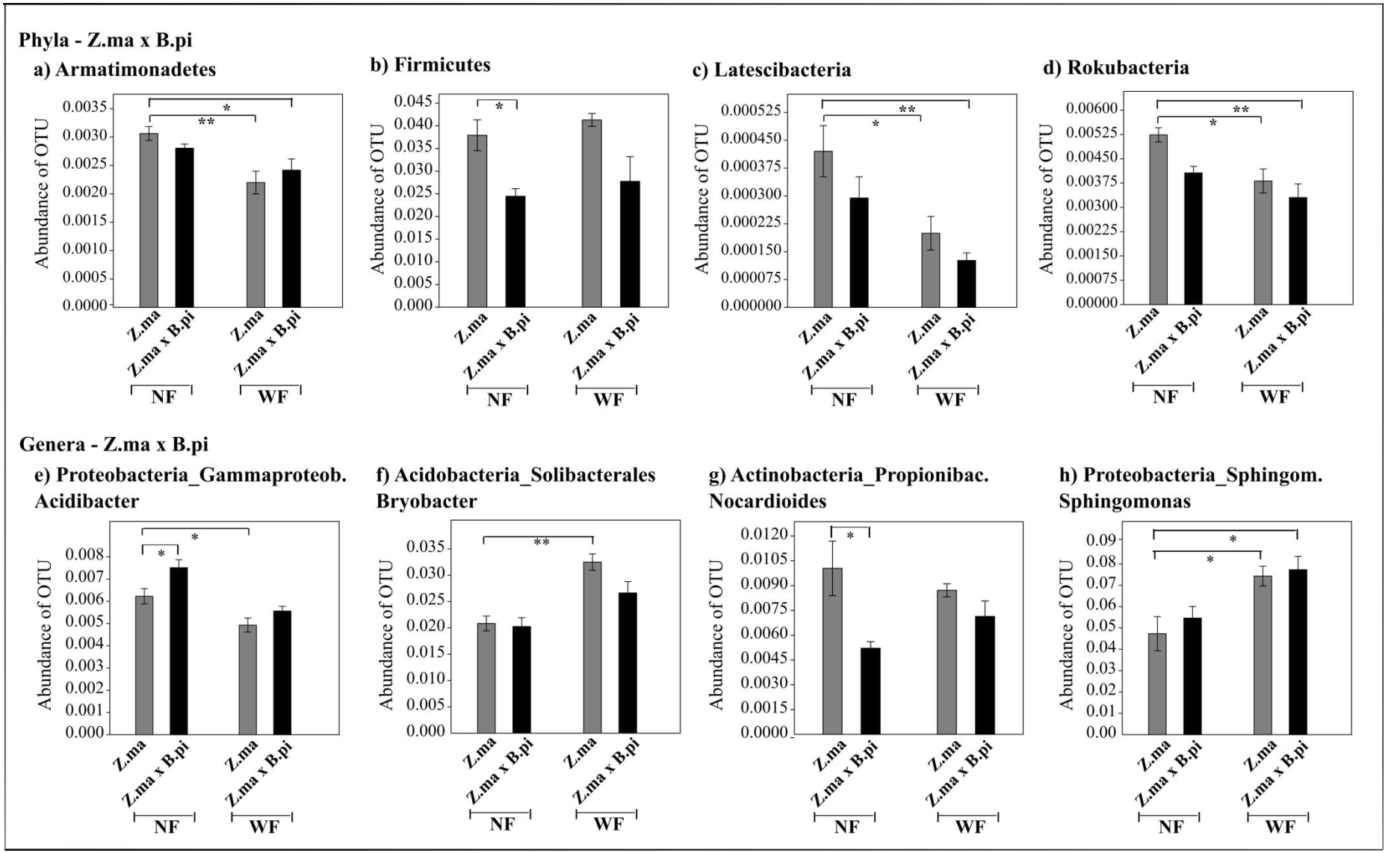


Fig. 7. Important phyla and genera of Bacteria present in the rizhospheric soil that showed significant differences in their relative abundances when comparing monoculture of Zea mays and the coexistence between Z. mays and Bidens pilosa, subjected to different levels of fertility.

Phyla: **(a)** Phylum Armatimonadetes, **(b)** Phylum Firmicutes, **(c)** Phylum Latescibacteria and **(d)** Phylum Rokubacteria. Genera: **(e)** genus Acidbacter, belonging to order Gammaproteobacterales and to phyla Proteobacteria, **(f)** genus Bryobacter, belonging to order Solibacterales and to phyla Acidobacteria, **(g)** genus Norcadioides, belonging to order Propionibacterales and to phyla Actinobacteria and **(h)** genus Sphingomonas, belonging to order Sphingomonadales and to phyla Proteobacteria. The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

Tables

Table 1. Indexes of diversity for each treatment of rhizosphere in soils cultivated with and without fertilizers. Chao, Shannon and Simpson indexes for Bacteria, Archaea, and Fungi. The results for each treatment comprises the four replicates and the asterisks indicate that this index differs significantly between treatments by the t test (*, ** and ***, significant to 5, 1 and 0,1 %) and the ns means that there was no significant difference between means (p>0.05) of all indexes in all fertilizers treatments. The plant species are: Z.ma – Zea mays; A.vi – Amaranthus viridis and B. pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

Bacteria/Archaea	**Fertilization condition**	**Chao** [a ns]	**Shannon** [b]	**Simpson** [c]
Z.ma	NF	5365.2570 ± 165.4674	9.7638 ± 0.0676 **	0.9961 ± 0.0005 *
	WF	5255.0167 ± 147.4785	9.5083 ± 0.0751	0.9950 ± 0.0006
A.vi	NF	4846.9591 ± 713.5306	9.5615 ± 0.1883	0.9953 ± 0.0009
	WF	4997.9957 ± 368.8088	9.4383 ± 0.0656	0.9948 ± 0.0004
B.pi	NF	5134.1542 ± 275.2989	9.6898 ± 0.2498	0.9959 ± 0.0011
	WF	4946.5388 ± 405.6275	9.5588 ± 0.0293	0.9954 ± 0.0004
Z.ma x A.vi	NF	5216.2582 ± 387.9768	9.6097 ± 0.0924	0.9956 ± 0.0002
	WF	47990978 ± 174.7345	9.4924 ± 0.1241	0.9951 ± 0.0008
Z.ma x B.pi	NF	5256.2371 ± 397.0918	9.7970 ± 0.184 ***	0.9963 ± 0.0001 **
	WF	5088.0557 ± 140.6493	9.4300 ± 0.1049	0.9948 ± 0.0006
Fungi	**Fertilization condition**	**Chao** [a ns]	**Shannon** [b ns]	**Simpson** [c ns]
Z.ma	NF	527.7897 ± 46.4121	5.4219 ± 0.5237	0.9041 ± 0.0370
	WF	548.6063 ± 35.0497	5.3614 ± 0.6495	0.8893 ± 0.0508
A.vi	NF	549.5860 ± 97.1510	5.1698 ± 0.2314	0.8824 ± 0.0220
	WF	516.1618 ± 97.0871	5.2344 ± 0.3079	0.9949 ± 0.0019
B.pi	NF	482.2995 ± 140.4336	5.4586 ± 0.3779	0.9146 ± 0.0216
	WF	578.1674 ± 35.2132	5.4344 ± 0.1035	0.8782 ± 0.0217
Z.ma x A.vi	NF	521.7979 ± 76.7872	5.4347 ± 0.4715	0.9169 ± 0.0099
	WF	548.6075 ± 81.8910	5.2288 ± 0.8021	0.8832 ± 0.0803

Z.ma x B.pi	NF	544.7265 ± 59.1869	5.4754 ± 0.4645	0.9111 ± 0.0100
	WF	516.5540 ± 95.2210	5.6157 ± 0.2025	0.9186 ± 0.0195

[a] Non-parametric estimator used to predict species richness (total number of OTUs presents), based in singletons and doubletons (Chao1).
[b] Diversity index that indicates species richness, considering the abundance of individual taxa. A higher number indicate more diversity (H).
[c] Diversity index that considers the evenness of species more than richness, considering the common species. The 1-D index was calculated, the value ranges from 0 to 1 and increases as the diversity increases.
(Chiarucci et al. 2011; Kim et al. 2017; Lemos et al. 2011).
* The asterisks means that there was significant difference between means with and without fertility ($p<0.05$).
** The asterisks means that there was significant difference between means with and without fertility ($p<0.01$).
*** The asterisks means that there was significant difference between means with and without fertility ($p<0.001$).

Supplementary Data

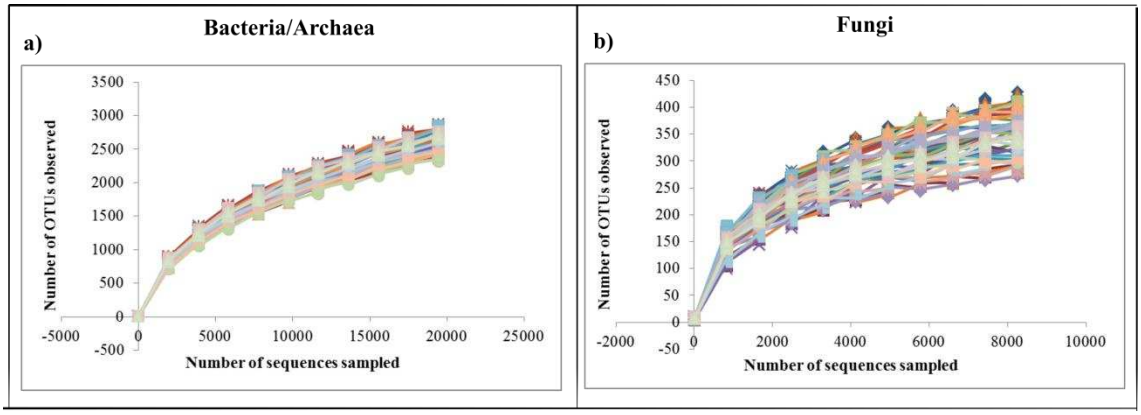


Fig. S1. Rarefaction curves for different treatments calculated based on OTUs defined as 97 % similarity level for **(a)** Bacteria and Archaea, and **(b)** Fungi.

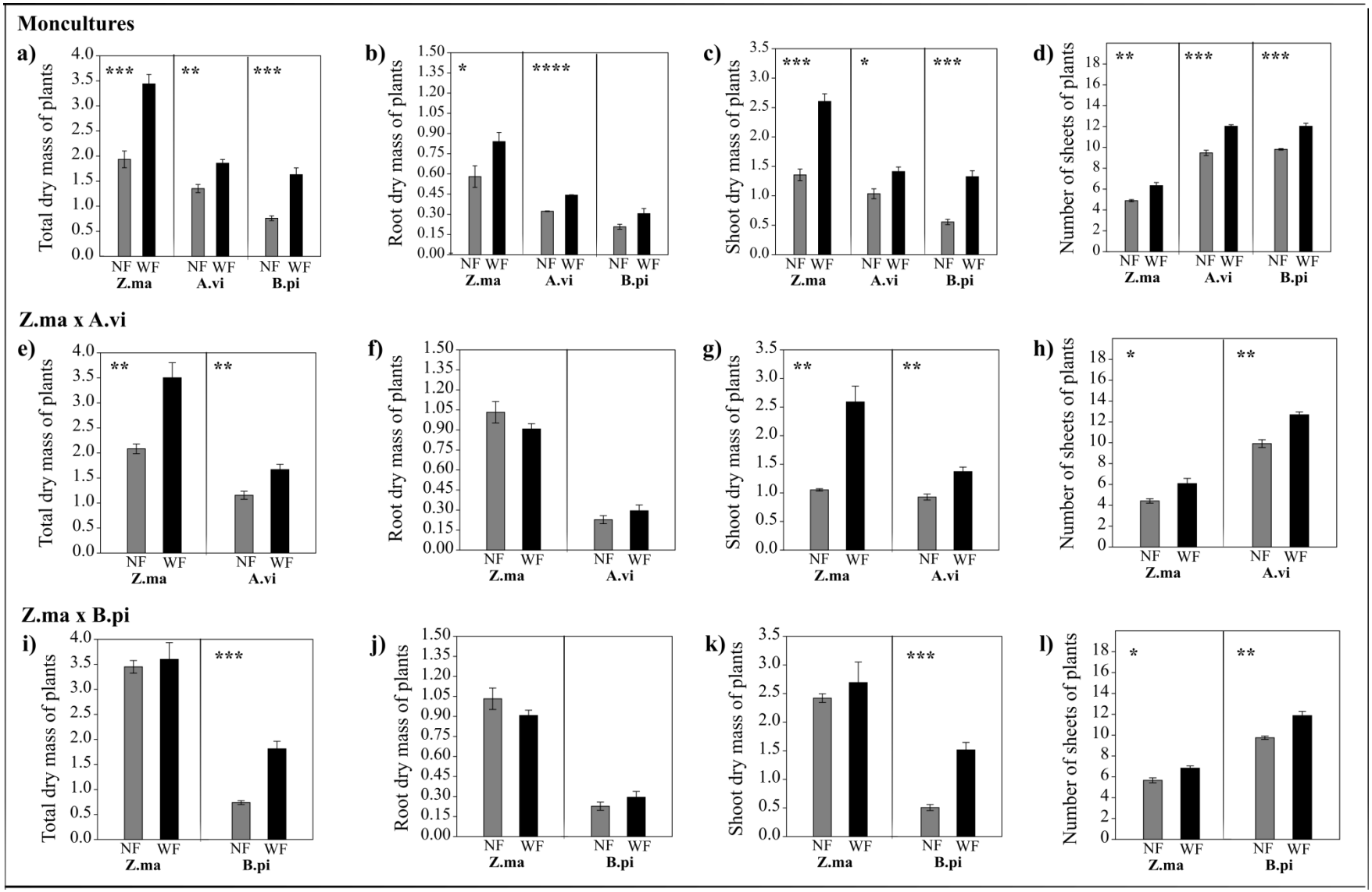


Fig. S2. Morphological characteristics of Zea mays and weeds in monoculture and in coexistence under to different fertility levels. The fist, analysis of monocultures of Zea mays, Amaranthus viridis and Bidens pilosa for **(a)** total dry matter, **(b)** root dry matter, **(c)** shoot dry matter and

(d) number of sheets. Analyzes of each plant in coexistence between Z. mays and A. viridis **(e)** total dry matter, **(f)** root dry matter, **(g)** shoot dry matter and **(h)** number of sheets, and analyzes of each plant in coexistence between Z. mays and B. pilosa **(i)** total dry matter, **(j)** root dry matter, **(k)** shoot dry matter and **(l)** number of sheets. Asterisks indicate different between paired bars by the t test (*,**, *** and **** significant to 5, 1, 0.1 % and 0.01 % respectively). The bars represent the standard deviation. The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

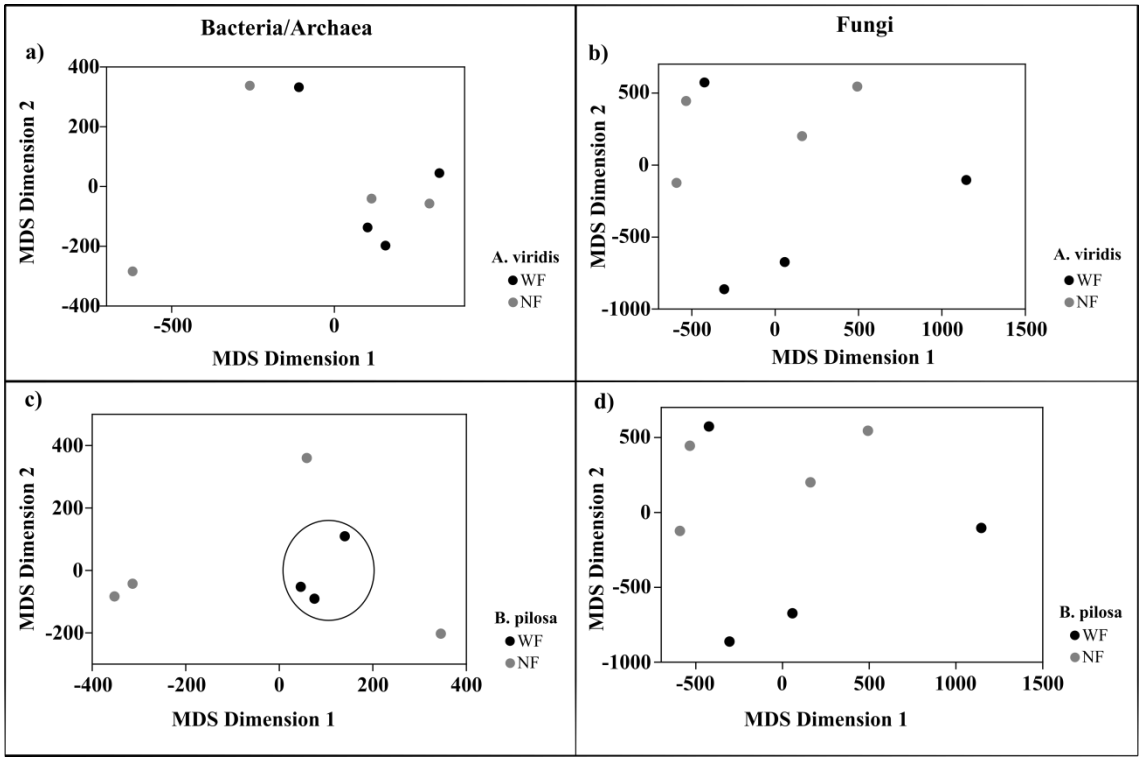


Fig. S3. Multidimensional Scaling (MDS) comparing soil microbial community as a function of fertility. **(a)** Bacteria and Archaea of Amaranthus viridis in monoculture and **(b)** Fungi, **(c)** Bacteria and Archaea of Bidens pilosa in monoculture and **(d)** Fungi. The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are:
A.vi – Amaranthus viridis and B.pi – Bidens pilosa.

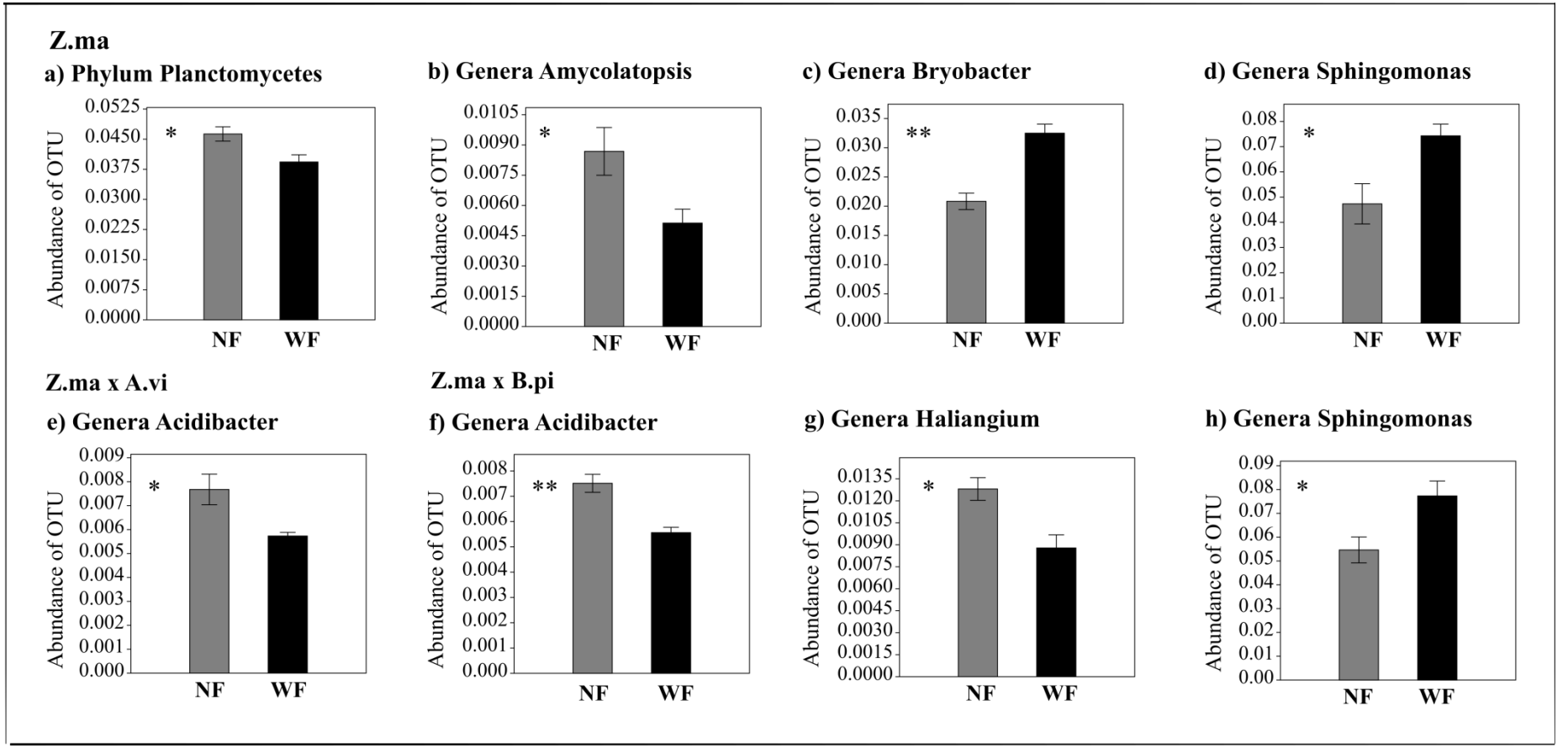


Fig. S4. Comparison of OTUs of Bacteria that significantly differed the relative abundance in rhizospheric soil with or without fertilizer in Zea mays in monoculture **(a)** Phylum Planctomycetes and Genus **(b)** Amycolatopsis, **(c)** Bryobacter and **(d)** Sphingomonas; in coexistence between Z. mays and Amaranthus viridis **(e)** Genera Acidibacter, and in coexistence between Z. mays and Biden pilosa, genus **(f)** Acidibacter, **(g)** Haliangium and **(h)** Sphingomonas. Asterisks indicate different between paired bars by the t test (*,**, *** and **** significant to 5, 1, 0.1 % and 0.01 % respectively). The bars represent the standard deviation. The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

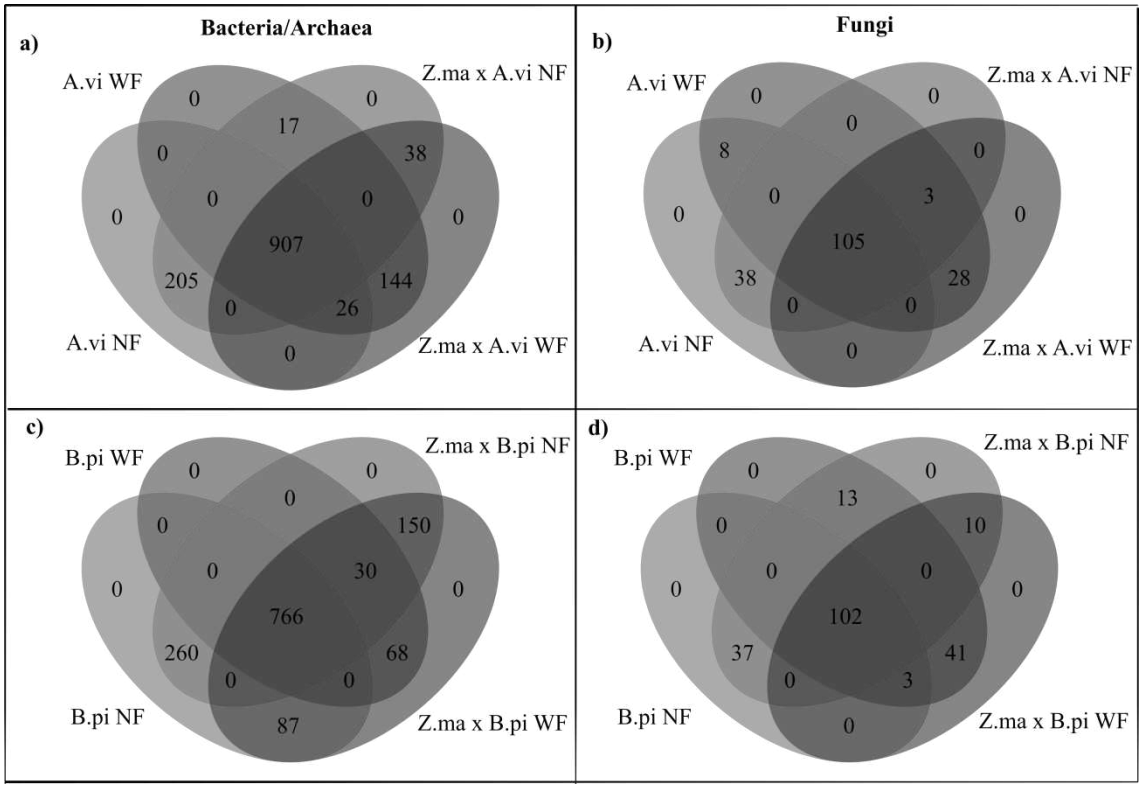


Fig. S5. Venn diagram of OTUs from **(a)** Bacteria and Archaea of Amaranthus viridis in monoculture and the coexistence between Zea mays and A. viridis, with and without fertilizer, and **(b)** Fungi in the same conditions. **(c)** Bacteria and Archaea of Bidens pilosa in monoculture and the coexistence between Z. mays and B. pilosa, with and without fertilizer, and **(d)** Fungi in the same conditions. Exclusive and shared OTUs among treatments are based on 97 % similarity. The numbers inside the diagram indicate the numbers of OTUs. The subtitle means: WF – with fertilizer and NF – no fertilizer and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

Table S1. Good's coverage index

Tratament	Fertilization condition	Archaea	Bacteria	Fungi
Z.ma	NF	0.9945 ± 0.0015	0.9788 ± 0.0012 **	0.9965 ± 0.0008
	WF	0.9958 ± 0.0020	0.9832 ± 0.0017	0.9948 ± 0.0018
A.vi	NF	0.9940 ± 0.0035	0.9708 ± 0.0147	0.9941 ± 0.0020
	WF	0.9934 ± 0,0023	0.9809 ± 0.0031	0.9949 ± 0.0019
B.pi	NF	0.9958 ± 0.0038	0.9813 ± 0.0017	0.9950 ± 0.0021 *
	WF	0.9956 ± 0.0035	0.9788 ± 0.0037	0.9919 ± 0.0006
Z.ma x A.vi	NF	0.9923 ± 0.0037	0.9788 ± 0.0016	0.9967 ± 0.0010 *
	WF	0.9945 ± 0.0030	0.9776 ± 0.0024	0.9940 ± 0.0018
Z.ma x B.pi	NF	0.9942 ± 0.0020	0.9766 ± 0.0075	0.9948 ± 0.0023
	WF	0.9934 ± 0.0023	0.9823 ± 0.0005	0.9945 ± 0.0014

* The asterisks means that there was significant difference between means with and without fertility ($p<0.05$).
** The asterisks means that there was significant difference between means with and without fertility ($p<0.01$).
The plant species are: A.vi – Amaranthus viridis; B.pi – Bidens pilosa and Z. – Zea mays. Coexistence treatments are indicated by "x" between the name of two species.

CAPÍTULO 3

MICROBIAL DIVERSITY AND COMPOSITION IN *Zea mays* RHIZOSPHERE IN COMPETITION WITH WEEDS UNDER DISTINCT SOIL ELECTRIC CONDUCTIVITIES

MICROBIAL DIVERSITY AND COMPOSITION IN Zea mays RHIZOSPHERE IN COMPETITION WITH WEEDS UNDER DISTINCT SOIL ELECTRIC CONDUCTIVITIES

Abstract

Aims Changes in soil electrical conductivity (EC) may occur as a consequence of agricultural practices, such as manure and fertilizer application. We have hypothesized that rhizosphere microorganims are sensitive to EC changes in the soil leading to distinct outcomes of the ecological interactions between plants. Here, we have evaluated the outcomes of Zea mays-weed interactions as a function of soil EC associated with changes in the structure of the rhizosphere microbial communities.

Methods The growth of two weed species, Amaranthus viridis and Bidens pilosa, and the crop Z. mays, was evaluated in the greenhouse under two management conditions, monoculture and plant coexistence, and distinct level of soil EC, natural soil electrical conductivity and adjusted electrical conductivity by adding NaCl salt. Total DNA from microbial populations in the rhizosphere soil was extracted, followed by PCR amplification and sequencing using the Illumina MiSeq platform.

Results Increases in soil EC caused significant changes in bacterial diversity and in the structure and composition of bacterial and fungal taxa. These were associated to changes in the outcome of mayze-weed interactions. For the interaction between maize and B. pilosa, the ecological relations between these plants changed from competition to facilitation as EC was increased. This could be attributed to the changes in soil microbial structure rather than to soil EC itself since changes in this chemical variable did affect plant growth.

Conclusions Changes in soil EC caused changes in the maize rhizobiome in monoculture and in coexistence with weeds. The soil microbiota was more sensitive to EC change than the evaluated plants. Therefore, a shift from competition to facilitation could be attributed to the existence of disctint microbial communities under natural and increased EC.

Keywords: Amaranthus viridis, Bidens pilosa, Electrical Conductivity, Plant Coexsitence, Microbial Diversity, llumina MiSeq sequencing, 16S rRNA and ITS.

Abbreviations

OTUs - Operational Taxonomic Units

RII - Relative Interaction Intensity Index (RII)

Z.ma or Z. mays - Zea mays hybrid BM 3061

A.vi or A. viridis - Amaranthus viridis

B.pi or B.pilosa - Bidens pilosa L.

x - Indicate coexistence treatments

1. Introduction

Changes in soil electrical conductivity may occur during soil management and some of the main drawbacks that arise from irrigation water quality or inadequate crop management is the gradual increase of EC or soil salinization (Ribeiro et al. 2005). The application of manure and commercial fertilizers can influence soil EC, later leading to soil salinization (Johnson et al. 2003; Gomes et al. 2008; Carmo and Silva 2016). The greater the amount of fertilizer applied to the soil, the greater the value of EC (Mota et al. 2006). Irrigation water salinity will affect plants depending on species and even genotypes, maize being considered a moderately salinity-sensitive species, with a threshold of water salinity of 1.1 dS m^{-1} to 1.7 dS m^{-1} (Ayres and Westcot 1991). The use of saline water in maize irrigation can cause nutritional imbalance and competitive inhibition in nutrient absorption (Oliveira et al. 2007; Sousa et al. 2010). When using irrigation water with EC ranging from 0.8 to 3.6 dS m^{-1}, an increase of soil salinity was observed after ninety days of plant cultivation, inhibiting K accumulation in the leaves and Mg and P in the grains (Sousa et al. 2010). Early development of maize plants is also affected by the salinity level of irrigation water, inhibiting stem diameter and number of leaves emitted by the plants (Oliveira et al. 2009). The dry matter content of shoots and roots is also negatively affected (Garcia et al. 2007; Blanco et al. 2008; Sousa et al. 2010).

In addition to changing soil physical and chemical properties and decrease crop productivity, changes in soil EC directly affect soil microorganisms, influencing the soil microbial community structure, decreased soil basal respiration, biomass carbon, and microbial metabolic coefficient, as well as increased arbuscular mycorrhizal fungal spore production (Pereira et al. 2004; Jesus Bezerra et al. 2010; Andronov et al. 2012; Yan et al. 2015; Kim et al. 2016). In a study evaluating changes in soil microbial community structure as a function of different EC levels using T-RFLP, the authors concluded that some bacterial and archaeal taxa are shared by all samples and the fungal community profile is more variable (Andronov et al. 2012). In another recent study carried out with soil samples from greenhouses, pyrosequencing analyses revealed that, despite the heterogeneity of environmental variables, the bacterial communities of these soils were particularly influenced

by EC and that taxa classified at the phylum level responded to EC in different ways (Kim et al. 2016).

Soil physicochemical characteristics and rhizospheric microorganisms may interfere with the intensity and outcome of plant interactions (Massenssini 2014; Massenssini et al. 2014, 2015; Monteiro 2016; Matos et al. 2019). Abiotic factors, besides playing a decisive role in the composition of plant communities, have an indirect role in modulating plant interactions (Massenssino et al. 2015). In addition, changes in plant-associated microbial community structure is determinant for the outcome of ecological interactions established by coexisting plants, as recently described by Matos et al. (2019). Thus, we have hypothesized that i) changes in soil EC lead to changes in the rhizobiome and, therefore, in the outcome of maize-weed interactions, and ii) that the soil microbial community is more sensitive to slight but significant changes in soil electrical conductivity than plants. Therefore, this study aimed at evaluating the outcomes of Zea mays-weed interactions as a function of soil EC associated with changes in the structure of the rhizosphere microbial communities.

2. Materials and Methods

2.1. Assembly of the experiment

The experiments were conducted under greenhouse conditions at the Departamento de Microbiologia, Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil. Three plant species were used: one crop Zea mays hybrid BM 3061, and two weeds, Bidens pilosa and Amaranthus viridis. The plants were grown for 40 days in plastic pots containing 4.5 dm^{-3} of soil collected in a UFV experimental area, having the following chemical and textural characteristics: pH: 5.5; P: 28.8 $mg.dm^{-3}$; K: 142 $mg.dm^{-3}$; Ca: 2.8 $cmolc.dm^{-3}$; Mg: 0.9 $cmolc.dm^{-3}$; Al: 0.1 $cmolc.dm^{-3}$; H + Al: 4.29 $cmolc.dm^{-3}$; SB: 4.1 $cmolc.dm^{-3}$; t: 4.2 $cmolc.dm^{3}$; T: 8.4 $cmolc.dm^{-3}$; V: 49 %; m: 2 %; MO: 2.52 $dag.kg^{-1}$; P-rem: 35.1 $mg.L^{-1}$; Zn: 3.2 $mg.dm^{-3}$; Fe: 32 $mg.dm^{-3}$; Mn: 91 $mg.dm^{-3}$; Cu: 0.9 $mg.dm^{-3}$; B: 0.2 $mg.dm^{-3}$; Clay: 25 %; Silt: 17 %; Sand: 58 %; Sandy Clay Texture (medium texture).

The experimental design was completely randomized and consisted of 10 treatments, three monocultures and two competition combinations, under two conditions of soil EC (natural soil electrical conductivity - no salt, and adjusted electrical conductivity - with salt), with four replications. The NaCl P.A. was used to change the electrical conductivity of the soil. For this, a test was previously performed to verify the amount of NaCl to be added to the soil to achieve the expected conductivity, following Ayers and Westcot (1976) and 2.43 grams of NaCl per kg of soil was used to rise to reach the expected conductivity. The NaCl

application was performed 12 days after weed planting. The saline solution was applied on the surface of the soil without allowing contact of the solution with the weed seedlings.

The fertilization was, then, performed according to the recommendations of Novais (1991). We applied 40 $mg.dm^{-3}$ of $(NH_4)_2SO_4$ and 65 $mg.dm^{-3}$ of NH_4NO_3 were as N and S sources, and 291.71 $mg.dm^{-3}$ of $Ca(H_2PO_4)_2$ for the supply of P and Ca. Considering the natural K content of the soil, no application of this nutrient was done. Phosphate fertilization was performed before weed planting by incorporating the fertilizer into the total soil volume. Nitrogen fertilization was divided into three equivalent applications (10, 20, and 30 days after germination of maize plants), via cover nutrient solution.

Seeding was done so as to keep a uniform distribution of plants on the soil surface according to each treatment. For monocultures, 12 seeds were sown for maize and 20 for the weed species and, after seedling emergence, nine plants were left per pot. For the coexistence treatments, six maize seeds and ten weed seeds were sown. After emergence, three maize and six weed seedlings were left per pot. Weed seeds were sown seven days before those of maize. This difference in sowing times was adopted so that the weed seedlings would not overtake maize early during the conduction of the experiment.

The plants were irrigated with distilled water once a day as needed. Once a week, the pots were weighed for moisture calibration and field capacity correction to 60 %. After cultivation, the plants were cut close to the soil. Rhizospheric soil, defined as the soil that remains bound to roots after gentle agitation, was collected and stored at -4 ° C until analysis. The roots were then, thoroughly washed with tap water. Roots and shoots were dried at 60 ºC until constant weight to determine the dry matter.

The EC measurements of the soil samples were made following the recommendations of Claessen (1997) using a conductivity meter. A portion of 50 g of soil was weighed and mixed with 100 ml of deionized water and allowed to stand overnight. The following day, the mixture was filtered on filter paper and the solution was used to determine EC in siemens per meter (S/m).

2.2. Plant interaction analysis

The Relative Interaction Intensity Index (RII) was used to determine the intensity of interactions between plants. This index was calculated as described by Armas et al. (2004): RII = (total dry matter of plant growing with competitor - total dry matter of plant growing in monoculture)/(total dry matter of plant growing with competitor + total dry matter of plant growing in monoculture). The RII is a measure of the strength of interaction between species,

ranges from −1 to 1, and is symmetrical around zero. Negative values indicate suppressive effects due to resource competition or other antagonistic effects; positive values indicate facilitation effects.

2.3. Extraction of total DNA from soil and sequencing by Illumina MiSeq

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. The DNA samples were quantified using Qubit® 2.0 Fluorometer (Invitrogen by Life Technologies) and lyophilized.

The samples were sent to Argonne National Laboratory, USA, where the steps of amplification, library preparation, and sequencing were done. The V4 region of the bacterial and archaeal 16S rDNA gene was amplified using the 515f/806r primer set (Caporaso et al. 2012), and the fungal ITS region from rDNA was amplified with primers ITS2 and ITS1f (Smith and Peay 2014). The sequencing was done using Illumina MiSeq, with reads paired-end 150 x 150 bp. The 16S and ITS amplicons were sequencing in separate runs.

2.4. Processing and analyses of sequencing data

The raw data were processed as described by the Brazilian Microbiome Project protocol (Pylro et al. 2014a, b) using the BMP Operating System (BMPOS) (Pylro et al. 2016) and the results were analyzed by the Quantitative Insights Into Microbial Ecology Software (Qiime v.1.9.1) (Caporaso et al. 2010). For the analyses of 16S rRNA gene sequencing data, briefly, the quality filtering of the sequences were performed using VSEARCH v.2.3.4 (Rognes et al. 2016). The truncated reads shorter than 240 bp and the reads with number of expected errors higher than 1.0 were discarded. The filtered reads were dereplicated and unique sequences (singletons) were also removed using VSEARCH. Sequences with more than 97 % similarity were considered the same Operational Taxonomic Unit (OTU), and comparisons were made with the UPARSE v.7.1b (Edgar 2013). The chimeric sequences were identified and removed using UCHIME (Edgar et al. 2011). After clustering, the sequences were aligned and the taxonomy of each OTU was assigned using the reference database SILVA release 132 by the uclust method.

For the analysis of fungal ITS region sequencing data, only the forward file (R1.fastq) was used. The quality filtering of the sequences were performed using USEARCH 8.1 (Edgar 2010) discarding reads with a number of expected errors higher than 0.5. After the dereplication, the ITSx software was used to extract only ITS1 region from fungi. The

truncated reads shorter than 140 bp was removed and the chimeric sequences were removed using UCHIME (Edgar et al. 2011) and USEARCH. Sequences with similarity greater than 97 % were grouped in the same OTU and the taxonomy of each was assigned using UNITE (Kõljajg et al. 2013) as the reference database by the uclust method.

2.5. Diversity and statistical analysis

Alpha diversity was calculated using the Qiime software and three metrics were calculated: Chao, Simpson, and Shannon indices at genera level. The rarefaction curves were generated based on the number of observed OTUs in each treatment. For beta diversity, Multidimensional Scaling (MDS) were carried out based on the presence and absence of OTU sampling using Jaccard's distance. For the Venn diagram, the OTU present in at least two of the three replicates and with reads number greater than or equal to 2 were considered as present in a sample, and the diagrams were created using the online tool jvenn (Bardou et al. 2014). The clustered hierarchical heatmaps were made only with OTUs shared among all samples. The construction of the graphs, multivariate analyzes, and statistics were done using the R software, package ggplot2 (Wickham and Chang 2009), GraphPad Prism 7, and PAST.

To compare the RII index between all the samples one-way ANOVAs were used followed by the Tukey's test. To compare the RII index for each competition situation with and without salt addition, the t test was used. To compare EC between soils with and without salt addition a one-way ANOVA was used followed by the Tukey's test. To compare the total dry matter, root dry matter, shoot dry matter, and number of leaves of Z. mays at different EC levels the t test was used. To compare the diversity indexes and relative abundance of OTUs among the samples the t test was adopeted. To compare phyla and genera which differ in their abundances according to competition, EC levels or both using the abundance in monocultures as reference, a one-way ANOVA followed by correction by Bonferroni´s test was adopted.

3. Results

3.1. Ecological interaction between Zea mays and weeds under distinct EC levels

EC values, in siemens per meter (S/m) for each treatment of plant coexistence were determined and shown to be significantly higher after sat addition (Fig. S1). The EC values for all situations of coexistence between the plants that remained with the basal EC of the soil, were statistically equal, with an average of 0.0077 S/m. The mean values for the coexistence between maize and B. pilosa in the treatment that the EC of soil was changed by adding NaCl, was 0.0262 S/m. For all other situations of coexistence in which the EC of soil was changed,

the average statistically equal for all the treatments was 0.0187 S/m. However, this significant increase in EC was not sufficient to provoke changes in total, root, and shoot dry matter and number of leaves of the plants (Fig. S2).

The RII varied according to the combinations of plants in coexistence (Fig. 1). Negative values indicate competition interactions between plants. Our results revealed that competition between Z. mays and A. viridis was the most intense (Fig. 1). In the other coexistence situations, competition between plants could be observed, except when maize was grown with B. pilosa (Fig. 1). In this situation, maize was negatively affected by competition with the weed in both levels of soil EC. The weed B. pilosa, at the natural level of soil EC, was also under competition. However, as EC was increased, B. piolsa growth was facilitated by coexistence with maize (Fig. 1).

3.2. Sequencing summary

The Illumina MiSeq sequencing for Z. mays rhizosphere soil in monoculture and in competition with weeds subjected to two different levels of EC generated 2,563,402 sequences for the 16S rRNA gene. After quality filtering, removal of singletons, and removal of chimeras by UCHIME, 2,451,616 (95.6 %) reads remained, with an average of 6,2085.50 reads per sample. Among these, 2,401,171 (97.94 %) reads were classified as OTUs belonging to Bacteria, 19,020.2 (0.78 %) reads were classified as Archaea and 31,424.4 (1.28 %) reads were attributed as Unassigned (data not shown).

For the sequencing of ITS region 2,254,171 sequences were generated. After quality filtering, removal of singletons, extraction by ITSx, and removal of chimeras by UCHIME, 490,723 (21.77 %) reads remained, with an average of 11,916 reads per sample. Among these, 369,864.3 (75.37 %) reads were classified as OTUs belonging to fungal phyla by the UNITE database, 20,908.05 (4.26 %) reads were classified as Unidentified Fungi, and 99,950.67 (20.37 %) were attributed as Unassigned.

For Bacteria and Archaea, the rarefaction curve tended to reach a plateau (Fig. S3a). Interestingly, the Good's coverage index values were lower for Bacteria than for Archaea, showing that more sequences would be necessary to retrieve all the sequence types of Bacteria present to better express diversity within the treatments (Tab. S1). For Fungi, 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. S3b) and the Good's coverage index values were higher than 99 % (Tab. S1).

3.3. Comparison of the rhizospheric microbial community structure, richness and diversity analyses of OTUs depending on EC

The Multidimensional Scaling (MDS) was used to analyze the relationship among soil treatments using a presence and absence matrix of OTUs based in the Jaccard's distance (Fig. 2). For the rhizospheric community of bacteria, archaea, and fungi of Z. mays monoculture (Fig. 2a and 2b), A. viridis monoculture (Fig. S4a and S4b), and the competition treatment with Z. mays and A. viridis (Fig. 2c and 2d), from the soils with both leves of EC formed distinct and well defined groups. The same was observed for the bacterial and archaeal communities in the competition treatment with maize and B. pilosa (Fig. 2e) and in that corresponding to the B. pilosa monoculture (Fig. S4c). For the fungal community under the same treatments, only samples with lower EC formed a defined group (Fig. 2f and Fig. S4d). Salt-added samples were dispersed in MDS.

The Chao richness index showed no significant difference by the t test ($p>0.05$) for the all samples analyzed, comparing both levels of EC (Table 1). The Shannon diversity index demonstrated greater diversity of the bacterial and archaeal communities in samples without salt addition in the rhizospheric soil of Z. mays monoculture ($p<0.05$) and the fungal community in the A. viridis one ($p<0.05$), when compared to salt-added samples (Table 1). The Simpson diversity index also showed no significant differences between samples with and without alteration of EC ($p>0.05$), in all treatments (Table 1), but the Simpson index, generally, showed greater diversity of bacteria and archaea than fungi in all analyzed samples, with values closer to one (Table 1).

3.4. Taxonomic composition of the microbial community depending as a function of soil EC

The OTUs of Bacteria found in the rhizosphere of Z. mays in monoculture with or without added salt were classified in 29 different phyla, 179 orders, and 427 genera that received taxonomic attribution. The orders and genera that did not receive taxonomic attribution are those taxa classified as "uncultured bacterium", "metagenome", "unassigned", and "other". The OTUs of Archaea were classified into 3 phyla, 4 orders, and 1 genus, Candidatus Nitrososphaera that received taxonomic attribution (data not show). The dominant phyla of Bacteria were Proteobacteria (31.1 % of all reads), Acidobacteria (18.1 %), Actinobacteria (17.5 %), Verrucomicrobia (8.2 %), Bacteroidetes (4.6 %), Planctomycetes (4.3 %), Gemmatimonadetes (4.2 %), Chloroflexi (4.0 %), Firmicutes (2.1 %), and all phyla with more than 1 % relative abundance, making up 93.9 % of the abundance of bacterial

OTUs (Fig. 3a). 1.5 % of the reads was not taxonomically classified and were said to be "Unassigned" and the others phyla were grouped into "Others", making up 4.6 % of the reads (Fig. 3a). Five phyla of bacteria were significantly affected by changes in soil EC. Actinobacteria ($p<0.001$) and Chloroflexi ($p<0.01$) were more abundant in the samples with natural soil EC and the phyla Bacteriodetes ($p<0.01$), Verrucomicrobia ($p<0.01$), and Acidobacteria ($p<0.05$) were more abundant in samples with salt addition, with higher EC (Fig. S5). At the genus level, the 20 most abundant were selected (Fig. 3b) and of these, five showed significant differences in the relative abundances between treatments. Haliangium (1.5 % of all reads, $p<0.05$), Conexibacter (0.7 %, $p<0.001$) and Acidothermus (0.5 %, $p<0.01$) (Fig. S5), both most abundant in samples with basal EC, and Candidatus Udaebacter (4.8 %, $p<0.05$) and Nitrospira (0.8 %, $p<0.01$) (Fig. S5), in samples with higher EC.

The Fungal OTUs were classified into 6 different phyla, 39 orders, and 87 genera. The orders and genera that did not receive taxonomic attribution were classified as "Others". The dominant phyla of Fungi were Ascomycota (46.3 % of all reads), Basidiomycota (14.1 %), Glomeromycota (11.5 %), Zygomycota (6.7 %), and all phyla with more than 1 % relative abundance, making up 78.6 % of the abundance of fungal OTUs (Fig. 4a). The 18.4 % of the reads were not taxonomically classified and were said to be "Unassigned". The other phyla were grouped into "Others", making up 3.0 % of the reads (Fig. 4a). Only one phylum had its abundance significantly altered by the change EC [Zygomycota ($p<0.05$) (Fig S5)], being negatively affected by increased EC. At the genus level, the 20 most abundant ones were selected (Fig. 4b) and among these, two showed significant differences in the relative abundances between treatments. The abundances of the genera Mortierella (6.6 %, $p<0.05$) and Exophiala (0.2 %, $p<0.05$) (Fig. S5) were significantly reduced with increased soil EC.

In the competition between Z. mays and A. viridis, the composition and relative abundance of Bacterial OTUs was more similar among treatments with few changes due to soil EC levels. These OTUs were classified into 39 different phyla, 166 orders and 411 genera, which received taxonomic attributions. The OTUs of Archaea were classified into 3 phyla, 3 orders, and 2 genera, Candidatus Nitrososphaera and Methanobrevibacter that received taxonomic attributions (data not show). The dominant phyla of Bacteria were Proteobacteria (30.5 % of all reads), Acidobacteria (17.0 %), Actinobacteria (16.3 %), Verrucomicrobia (9.1 %), Bacteroidetes (5.4 %), Planctomycetes (4.5 %), Chloroflexi (4.3 %), Gemmatimonadetes (4.1 %), Cyanobacteria (1.7 %) Firmicutes (1.3 %), and all phyla with more than 1 % relative abundance, making up 94.3 % of the abundance of bacterial OTUs (Fig. 3c). 1.6 % of the reads was not taxonomically classified and were said to be

“Unassigned” and the others phyla were grouped into “Others”, making up 4.1 % of the reads (Fig. 3c). Only two phyla showed significant differences in relative abundance at the two EC values, Acidobacteria ($p<0.01$) (Fig S6) was more abundant in the samples with basal soil EC and Proteobacteria ($p<0.05$) (Fig S6) was more abundant in the samples with higher EC. At the genus level, the 20 most abundant ones were selected (Fig. 3d) and among these, three showed significant differences in the relative abundances between treatments, the genera Candidatus Solibacter (4.5 %, $p<0.01$), Bryobacter (2.3 %, $p<0.05$), and Bradyrhizobium (0.6 %, $p<0.05$) (Fig. S6) were more abundant in the samples with basal soil EC.

The Fungal OTUs were classified into 6 different phyla, 42 orders, and 85 genera. The orders and genera that did not receive taxonomic attribution were classified as “Others”. The dominant phyla of Fungi were Ascomycota (46.68% of all reads), Basidiomycota (21.9 %), Zygomycota (5.8 %), and all phyla with more than 1 % relative abundance, making up 74.5 % of the abundance of fungal OTUs (Fig. 4c). 17.7 % of the reads were not taxonomically classified and were said to be “Unassigned” and the others phyla were grouped into “Others”, making up 7.7 % of the reads (Fig. 4c). At the genus level, the 20 most abundant were selected (Fig. 4d). In this competition, no fungal phylum or genus were significantly changed by increased soil EC.

In the competition treatment with Z. mays and B. pilosa, the OTUs were classified into 28 different phyla, 165, and 421 genera that received taxonomic attributions. The OTUs of Archaea were classified into 3 phyla, 2 orders, and 1 genera which received taxonomic attribution, Candidatus Nitrososphaera (data no show). The dominant phyla of Bacteria were Proteobacteria (28.0 % of all reads), Actinobacteria (19.3 %), Acidobacteria (17.8 %), Verrucomicrobia (9.2 %), Gemmatimonadetes (4.6 %), Chloroflexi (4.4 %), Planctomycetes (4.4 %), Bacteroidetes (4.3 %), Firmicutes (1.6 %) Patescibacteria (1.0 %), and all phyla with more than 1 % relative abundance, making up 94.7 % of the abundance of bacterial OTUs (Fig. 2e). 1.3 % of the reads was not taxonomically classified and were said to be “Unassigned” and the other phyla were grouped into “Others”, making up 2.7 % of the reads (Fig. 3e). Four phyla showed significant differences in relative abundance compared to soil samples with and without added salt, Verrucomicrobia ($p<0.01$) and Gemmatimonadetes ($p<0.05$) (Fig S7) were more abundant in the samples with basal soil EC, and Actinobacteria ($p<0.001$) and Chloroflexi ($p<0.05$) (Fig S7) were more abundant in the samples with higher EC. At the genus level, the 20 most abundant were selected (Fig. 3f) and among these, five showed significant differences in the relative abundances between treatments, the genus Candidatus Solibacter (1.4 %, $p<0.05$) and Nitrospira (0.8 %, $p<0.01$) (Fig S7) were more

abundant in the samples with basal soil conductivity and Conexibacter (0.8 % all reads, $p<0.05$), Acidothermus (0.6 %, $p<0.05$), and Nocardioides (0.5 %, $p<0.01$) (Fig S7) both were more abundant in the soil samples with added salt. The Fungal OTUs were classified into 6 different phyla, 43 orders, and 101 genera. The orders and genera that did not receive taxonomic attributions were classified as "Others". The dominant phyla of Fungi were Ascomycota (52.3 % of all reads), Basidiomycota (20.1 %), Zygomycota (2.1 %), Glomeromycota (1.3 %) and all phyla with more than 1 % relative abundance, making up 75.8 % of the abundance of bacterial OTUs (Fig. 4e). The 21.5 % of the reads was not taxonomically classified and were said to be "Unassigned" and the other phyla were grouped into "Others", making up 2.7 % of the reads (Fig. 4e). Only one phylum was had its abundance significantly altered by the change in the electrical conductivity of the soil, Ascomycota ($p<0.01$) (Fig S7) which presented greater abundance in samples with higher EC. At the genus level, the also 20 most abundant are shown (Fig. 4f) and of these, two showed significant differences in the relative abundances between treatments, the genera Penicillium (1.7 %, $p<0.05$) and Xilaria (0.2 %, $p<0.01$) (Fig S7) had their relative abundances negatively affected by increased EC.

3.5. Comparison of the rhizospheric microbial community structure and OTUs affected by competition and soil electric conductivity

When comparing samples of rhizospheric soil with and without added salt in maize monoculture or in competition with A. viridis or B. pilosa, it was observed that most of the OTUs classified as Archaea, Bacteria, or Fungi are shared between treatments (Fig. 5). When comparing samples from maize monoculture and from competition between maize and A. viridis, with and without alteration of soil EC, maize monoculture at the lower EC presented the highest number of bacterial and archaeal OTUs, totaling 1,520. Of which 146 were exclusive to this treatment (Fig 5a). For the rhizospheric community of fungi, the monoculture of maize at higher EC showed the highest number of OTUs, 70, with 15 being exclusive to this treatment (Fig. 5b). The comparison of maize monoculture and competition between maize and B. pilosa, with and without alteration of soil EC, showed that maize monoculture at basal soil EC showed the highest number of OTUs of bacteria and archaea, 1519, with 131 exclusive (Fig. 5c). The competition between maize and B. pilosa at higher EC values showed the the highest number of fungal OTUs, 81, 12 exclusive OTUs (Fig. 5d). The figure S8 compares OTUs from competition treatments between maize and weeds, with

and without added salt, with A. viridis (Fig. S8a and S8b) and B. pilosa monocultures (Fig. S8c and S8d).

By comparing the two factors analyzed in this work, maize competition with weeds and soil EC, it was possible to identify which OTUs were affected by each one of the factors analyzed. Comparing Z. mays monoculture and the competition of Z. mays and A. viridis, with or without added salt and using the maize monoculture with soil basal EC as reference (Fig. S9), the phylum Bacteriodetes and Firmicutes had their abundances changed by the two factors evaluated. The phylum Cyanobacteria and Verrucomicrobia were positively affected only by coexistence between plants. The phylum Acidobacteria was positively affected and Actinobacteria and Chloroflexi were negatively affected by the change in soil EC. Some genera also had their abundance affected by the factors analyzed here (Fig. 6). The genera Conexibacter (Fig. 6b) and Solirubrobacter (Fig. 6c) were affected by the two factors, coexistence and change in soil EC, and the genera Bacillus (Fig. 6a) and Exophila (Fig. 6d) were negatively affected only by coexistence between plants, being the genus Exophila the only significantly altered Fungi taxa.

Comparing maize monoculture and the competition between Z. mays and B. pilosa, with or without added salt and using the maize monoculture with soil basal EC as reference (Fig. S10), the phylum Actinobacteria, Ascomycota, and Zygomycota had their abundances altered by the two factors evaluated. The phylum Firmicutes was negatively affected and Gemmatimonadetes and Verrucomicrobia were positively affected only by coexistence between plants. The phylum Bacteriodetes was positively and Chloroflexi was negatively affected by the change in soil EC. The genera that had their abundances affected by some factor analyzed in this study were Haliangium (Fig. 6e), Conexibacter (Fig. 6f), MND1 (Fig. 6g), Nitrospira (Fig. 6h), Solirubrobacter (Fig. 6j) and the genus of Fungi Mortierella (Fig. 6k) that had their abundances altered by the both factors evaluated. The genera Pseudolabrys (Fig. 6i) and the genus Penicillium of Fungi (Fig. 6l) were positively affected only by coexistence between plants.

4. Discussion

4.1. Ecological interaction between plants in coexistence under distinct levels of electric conductivity

In this study, we have added NaCl to the soil to simulate a subtle but significant change in soil EC, reproducing the initial stages of soil salinization by the use of saline water, fertirigation, and application of organic and inorganic fertilizers during crop management.

Here, changes in EC did not affect the morphological variables of the analyzed plants (Fig. S2), since the final EC values ranged from twenty-seven to six times less than would be necessary to cause damage to maize (Ayres and Westcot 1991). Despite this, the increased EC was significant (Fig. S1) and sufficient to promote changes in the structure of the rhizosphere microbial community (Fig. 3 and 4) and in the ecological interactions between the plants studied in our work (Fig. 1). Thus, soil microbiota was shown to be more responsive to changes in soil EC than plants, and can be used as a soil quality indicator to verify the progression of salinization in soils (Andronov et al. 2012).

To evaluate the interaction between coexisting plants, the RII was calculated. The coexistence between Z. mays and A. viridis the led to competition between the two species (RII<0) (Fig. 1). Maize was more affected by this interaction than the weed, considering the more negative values of RII recorded for the crop. Among all plant combinations evaluated in this work, the Z. mays and A. viridis interaction was the one that showed the highest suppressive influence between the species tested. In another work, the intentional soil sterilization followed by microbiota reconstitution with a non-sterile soil suspension evidenced that A. viridis facilitated maize growth, especially in the with soil microbiota reconstitution (RII>0) (Matos et al. 2019). However, it was shown that soil microbiota reconstitution was effective in restructuring the soil bacterial community, but not that of fungi, and that the extent of facilitation depended on the structure of the microbial community (Matos et al. 2019). Here, unlike previous studies, the interaction between Z. mays and A. viridis was one of competition (Fig. 1) and the fungal rhizobiome was not altered due to distinct soil EC (Fig. 4c and 4d). Reconstitution of soil microbiota was more effective for restablishing the bacterial communities, raising the level of facilitation (RII>0) (Matos et al. 2019), whereas the presence of an unchanged fungal community induced competition (RII<0). Thus, in the interaction between maize and A. viridis, it is likely that bacteria were more related to facilitation, and fungi, to competition. These inferences are possible, because here in our work, no significant differences in RII were observed between the soils with lower and higher EC (Fig. 1), and this was accompanied by no significant changes in the fungal rhizobiome (Fig. 4c and 4d).

The coexistence treatment with maize and B. pilosa showed very interesting results. Maize, regardless of changes in the soil EC, was affected by competition with weeds (RII<0) (Fig. 1). B. pilosa was also affected by competition with maize when soil EC was not changed (RII<0). However, the addition of salt with the resulting higher soil EC led weed growth facilitation by maize (RII>0). In fact, Z. mays competed with B. pilosa (RII<0) when grown in

coexistence and the weed affected the increase of the dry matter of the maize, when compared to maize monoculture (Monteiro 2016). As already shown, facilitation depended on the structure of the soil microbial community (Matos et al. 2019) and here, the change in soil EC, which was greater in the coexistence between maize and B. pilosa, generated significant changes in the bacterial (Fig. 3e and 3f) and fungal (Fig. 4e and 4f) rhizobiomes, whereas specific taxa had their relative abundances affected as a function of soil EC alteration (Fig. 6 and S10). Thus, it is interesting to note that the management that causes changes in soil EC, such as the use of manure, commercial fertilizers, or saline irrigation water (Johnson et al. 2003; Ribeiro et al. 2005; Mota et al. 2006; Gomes et al. 2008; Carmo and Silva 2016) alter rhizospheric microorganisms and consequently ecological interactions between plants. The use of a commercial organic sulfur, potassium, and magnesium fertilizers leads to linear increases in soil EC with increasing fertilizer doses (Gomes et al. 2008). It has also been shown that even the application distance of phosphorus and potassium fertilizers in relation to maize seeds significantly affect soil EC (Bevilaqua et al. 1996). Liming has also been shown to increase soil EC (Carmo and Silva 2016). There is not yet a universal equation that relates electrical conductivity data to the total dry matter of maize, which is specific for each soil (Carmo and Silva 2016), however, here, in our work, it is clear that changes in soil EC cause changes in the plant rhizobiome in such way as to modify the outcome of crop-weed interaction. At higher EC values, shifts in plant-pant interaction leads to facilitation of B. pilosa growth when in coexistence with maize.

Although the same NaCl dose was used to alter the electrical conductivity of the soil in all treatments of coexistence between plants, the coexistence between Z. mays and B. pilosa showed a higher final soil EC value than the other treatments (Fig. S1). In fact, the coexistence between maize and B. pilosa causes an increase in the priming effect of the soil, which is the decomposition of soil organic matter due to the addition of substrates in the soil (Matos et al. 2019a, b). Such decomposition increases the amount of ions in solution, which would justify the higher EC value in this combination of plant.

4.2. Alteration of microbial rhizosphere diversity as a function of soil EC

Our results showed that maize and A. viridis monoculture showed greater bacterial and archaeal diversity by the Shannon index for the soils without salt addition (Table 1). In these cases, the increase of soil EC negatively affected bacterial diversity. The analysis of microbial diversity by T-RFLP in saline soils with different EC values demonstrated that there was no significant change in the general diversity of microbial populations, although structural

changes in the soil microbial community were observed (Andronov et al. 2012). In pasture soils, no changes in the Shanon index were detected for the soil bacterial community by DGGE profiling as a consequence of soil salinization (Zhang et al. 2015). These findings demonstrate that the use of the high throughput analyses by Illumina MiSeq sequencing was efficient to detect differences in the diversity of Bacteria, Archaea, and Fungi associated with maize and A. viridis rhizosphere grown in monoculture and submitted to two different levels of soil EC. In fact, the approach used here provided more detailed information on the microbial communities of environmental sample than approaches such as DGGE and T-RFLP (Lee et al. 2011; Wang et al. 2018), and confirmed that even slight but significant changes in soil EC were capable of provoking changes in soil microbial diversity.

The variations in microbial diversity observed in our work cannot be inferred to be propotional to changes in EC, i.e., it was not possible to infer that increasing EC causes a decrease in microbial diversity or vice versa (Table 1 and Fig. S1). Two other studies using pyrossequencing has also demonstrated that salinity affects soil microbial diversity, but as in our work, no linear correlation could be established (Hollister et al. 2010; Canfora et al. 2014). This suggests that other factors, such as the plant species tested, must also play a role in changing the rhizosphere microbial communities. In our work, we have observed that changes in the composition and structure of the rhizosphere microbial communities as a function of soil EC were more important than changes in microbial diversity, as demonstrated in other studies (Hollister et al. 2010; Canfora et al. 2014; Zhang et al. 2015; Kim et al. 2016; Wang et al. 2019).

4.3. Microbial OTUs affected by soil electrical conductivity

The abundance of specific taxa of Bacteria associated with the plants tested in our experiment was changed as a function of soil EC and coexistence with another species (Fig. 3, 4, S5, S6 and S7). For maize monoculture, taxa, such as the phyla Chloroflexi, Acidobacteria, and Verrucomicrobia, and the genera Acidothermus, Conexibacter, and Nitrospira (Fig. 3a and 3b) had their abundances changed when comparing the samples from soil at the two EC levels. Interestingly, when in coexistence with weeds, these same taxa had their abundance altered, but in opposite directions, as a function of soil EC. For example, the phylum Acidobacteria presented greater abundance in the samples with higher EC for the monocultures, while for the treatment with coexistence of maize with A. viridis, the highest abundances were recorded for the tretaments with basal soil EC (Fig 3, S5 and S6). A similar pattern was also observed for the taxa Chloroflexi, Acidothermus, and Conexibacter, for

which greater abundance was recorded for the monoculture treatments with basal soil EC, while for the competition between maize and B. pilosa, the greater abundance values were recorded for samples with increased soil EC (Fig. 3, S5 and S7). For the taxa Verrucomicrobia and Nitrospira, which were more abundant in the monoculture soil samples with higher EC, showed greater abundance in the soils from coexistence treatments and with basal soil EC (Fig. 3, S5 and S7).

These results demonstrate that it is not possible to establish a general pattern for changes in microbial abundances of microorganisms as a function of EC. It is true that changes in the structure of the microbial community do occur, but, here, they were determined on a case by case basis by the soil EC and plant combination. The heat map of the figures S11 and S12, shows the changes in the abundances of the main phyla and genera of Bacteria and Fungi as a function of the factors analyzed.

In this work, the two factor analyzed were important and determinant for the rhizobiome structure, acting together on many taxa of bacteria and fungi to regulate their abundances (Fig. 6, S9 and S10). Coexisting plants possess distinct microbial community structures compared to their own monocultures (Massenssini 2014; Massenssini et al. 2014; Monteiro 2016; Matos et al. 2019). Depending on the plant species that are coexisting, specific microorganisms may be recruited that can benefit a one or both members of the interaction, improving or hindering their competitive ability (Massenssini 2014; Monteiro 2016). Microbial taxa may be exclusive to a particular plant combination, and even the most common ones, which are present in the soil in various plant coexistence situations, may have their abundance affected by the plant species involved (Monteiro 2016).

Therefore, to better understand the effects of EC on the structure of the soil microbial community, we verified how each factor analyzed here, coexistence or soil EC, was affecting the abundance of specific taxa that showed significant differences in abundance between treatments (Fig. 6, S9 and S10). Comparing the competition between Z. mays and A. viridis and taking the maize monoculture at lower soil EC as a reference, we have found that the phyla Acidobacteria, Actinobacteria, and Chloroflexi had their abundances altered exclusively as a function of soil EC (Fig. S9). In the competition between maize and B. pilosa, compared maize monoculture at lower soil EC, the phyla Bacteriodetes and Chloroflexi were also affected exclusively by soil EC changes (Fig. S10).

These phyla have been reported to be the most abundant in saline soils (Hollister et al. 2010; Canfora et al. 2014; Kim et al. 2016) and were also among the most abundant in the rhizosphere of maize monoculture and weed-maize competition (Monteiro 2016). In

experiments evaluating changes in soil microbial communities as a function of a salinity gradient, the abundance of Acidobacteria phylum was negatively correlated with increased salinity (Hollister et al. 2010; Canfora et al. 2014; Kim et al. 2016). The opposite was observed in our experiment. Here, this phylum showed increased abundance at the higher soil EC level tested. It should be emphasized that the previous experiments reported here were made with soil samples taken from saline environments and that other variables, besides the salinity gradient, were taken into account for establishing the determinant factors for shaping the structure of soil microbial communities, such as water content, organic carbon, exchangeable cations, and pH.

The phylum Actinobacteria is a group widely distributed among all soil types reported and has been reported to be tolerant to high salinity levels in the soil. Actinobacteria have been reported to grow even in soils with more than 5 % NaCl (Andronov et al. 2012). Here, we have observed that the abundance of this group was negatively affected by soil EC. Similar results have been reported in the literature for a hypersaline soil (Hollister et al. 2010). The phylum Bacteriodetes has been shown to have a positive correlation with increasing salinity gradients in the soil (Canfora et al. 2014; Kim et al. 2016). In our work, this phylum increased in abundance with increasing soil EC. The phylum Chloroflexi, known for its phototrophic potential (Canfora et al. 2014) had its abundance decreased at higher EC. For this taxon, negative correlations between abundance and soil salinity has been reported (Kim et al. 2016).

No Archaea taxa were affected exclusively by changes in soil EC (Fig. 6, S9 and S10). Historically, Archaeae are related to extreme environments, being more dominant than bacteria in the occupation of ecological niches in saline environments (Andronov et al. 2012). This taxon contains more tolerant species that undergo changes in the expression of specific genes to deal with osmotic stress (Canfora et al. 2014). In our work, changes in soil EC were subtle and, probably, not sufficient to cause significant changes in the archaeal rhizospheric community.

Fungi showed significant alterations in specific taxa, such as that observed for Ascomycota abundance with increased soil EC (Fig. S7). In general, fungi are more osmotically tolerant than bacteria at higher salt concentrations (Rath et al. 2016), and similar to what was observed for Archaea, subtle changes in EC may not have been sufficient to cause profound changes in the abundance of specific fungal taxa.

Our results have shown that a number of microbial taxa were affected by soil EC, even at low salinity levels. These may represent microbial groups that are more sensitive to

changes in soil EC and plant combinations. The taxa that were not affected by the imposed EC conditions may constitute microbial groups with higher plasticity and more adaptable to changes in soil physico-chemical properties.

5. Conclusion

Subtle changes in the electrical conductivity of the soil are capable of promoting significant changes in the diversity of bacteria and in the composition of the rhizobioma even at levels of EC in which the plants are not affected. It was not possible to establish a clear relationship between the increase in soil EC and the increase or decrease in the abundance of specific microbial taxa, since such variations in abundance will depend on the factors involved, variation in soil EC and plant species in coexistence. However, such variations in the rhizobioma directly promotes changes in ecological interactions between plants, as observed for the coexistence between maize and B. pilosa, which varied from competition to facilitation with the increase in soil EC. The shif from competition to facilitations is determined by changes in the rhizosphere microbial communities brought about by increased soil EC.

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Figures

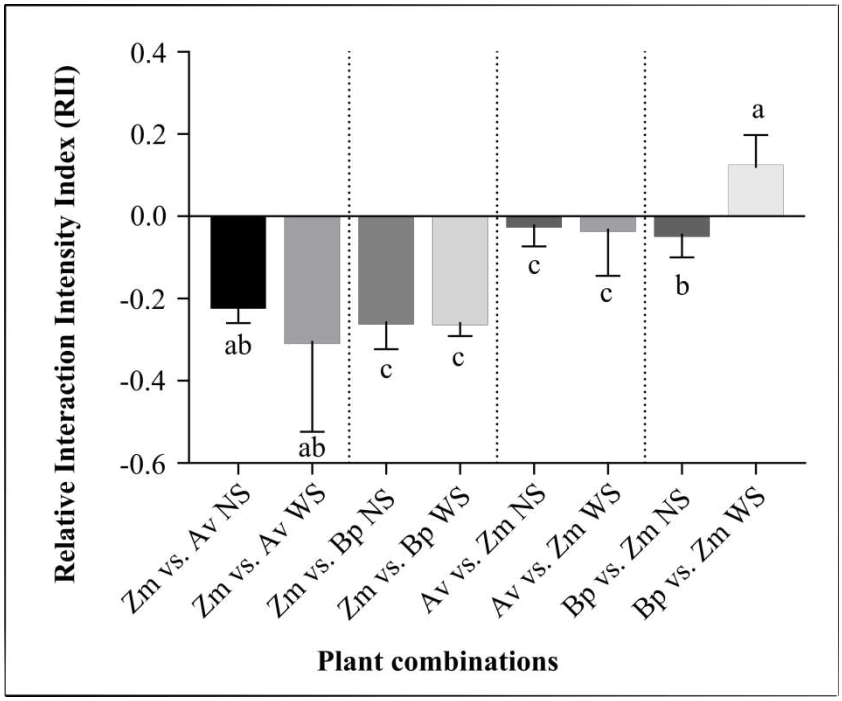


Fig. 1. Relative Interaction Index (RII) after 40 days of weed growth in a greenhouse in monoculture or in competion. The bars represent the standard deviation and different letters indicate significant differences between means based on the Tukey's test ($p<0.05$). The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

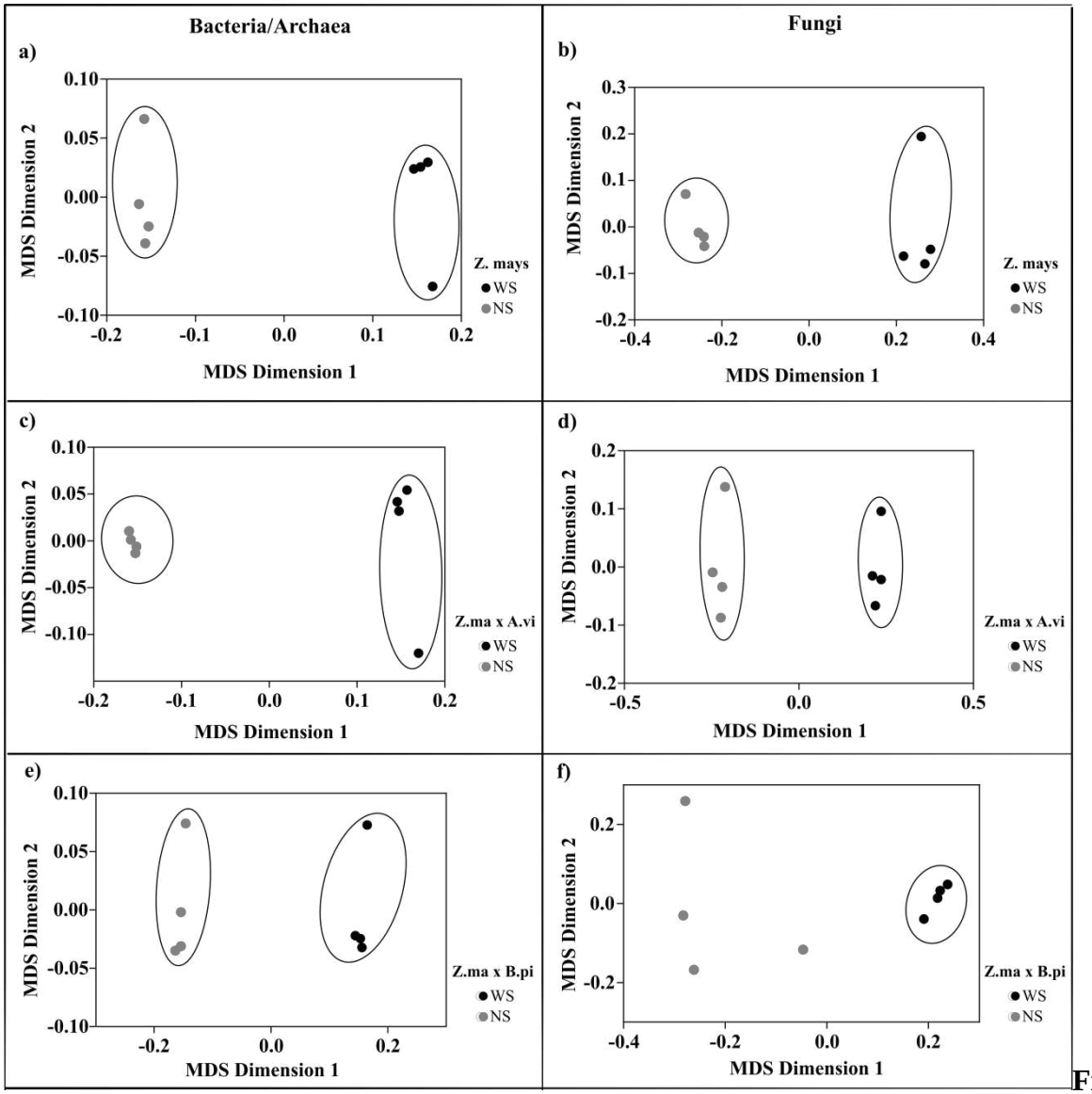
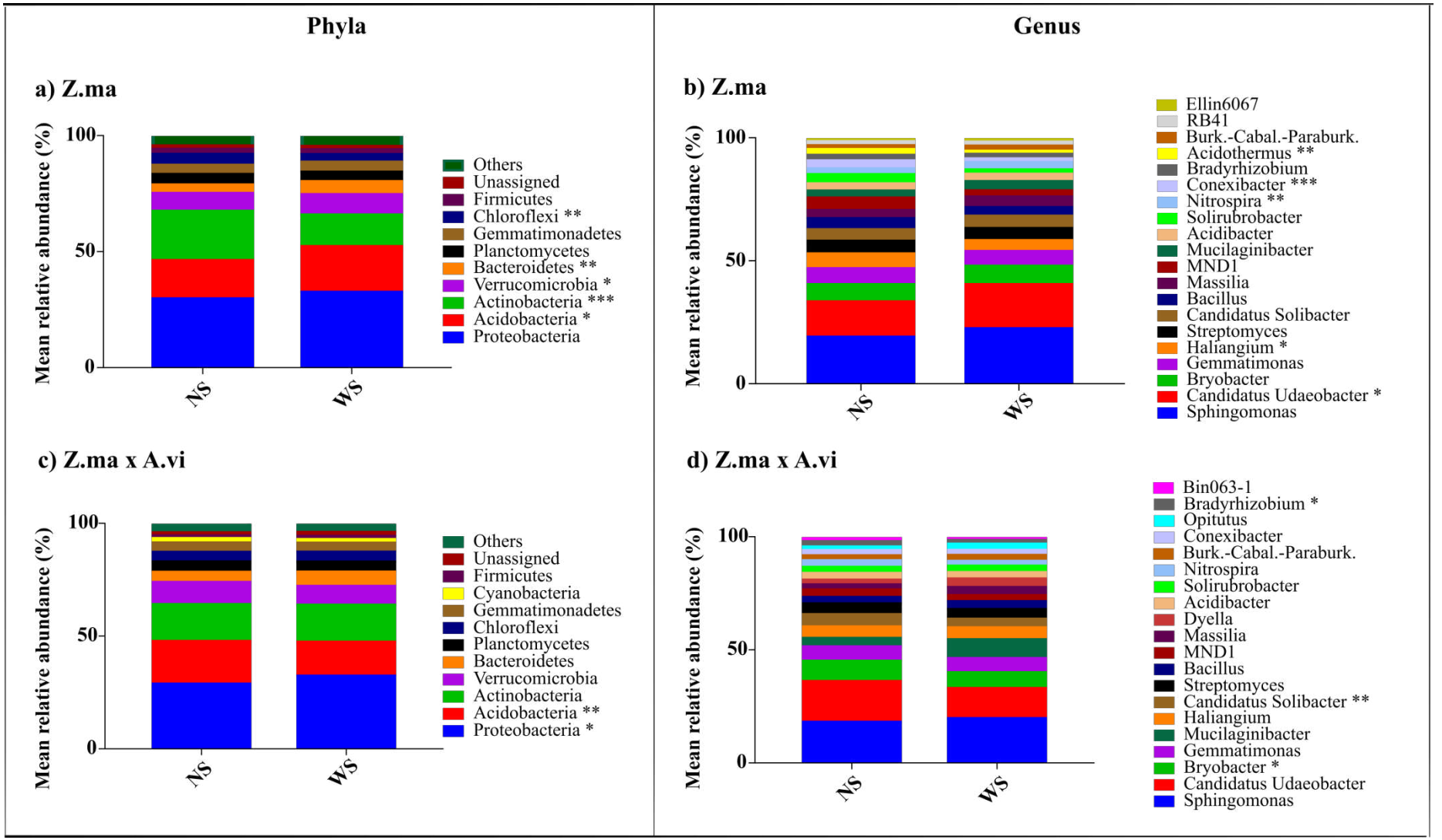


Fig. 2. Multidimensional Scaling (MDS) comparing soil microbial community as a function of electric conductivity. **(a)** Bacteria and Archaea of Zea mays in monoculture and **(b)** Fungi, **(c)** Bacteria and Archaea of Z. mays in competition with Amaranthus viridis and **(d)** Fungi, and **(e)** Bacteria and Archaea of Z. mays in competition with Bidens pilosa and **(f)** Fungi. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.


Phyla
a) Z.ma
Mean relative abundance (%)
100
50
0
NS
WS
Others
Unassigned
Firmicutes
Chloroflexi **
Gemmatimonadetes
Planctomycetes
Bacteroidetes **
Verrucomicrobia *
Actinobacteria ***
Acidobacteria *
Proteobacteria
c) Z.ma x A.vi
Mean relative abundance (%)
100
50
0
NS
WS
Others
Unassigned
Firmicutes
Cyanobacteria
Gemmatimonadetes
Chloroflexi
Planctomycetes
Bacteroidetes
Verrucomicrobia
Actinobacteria
Acidobacteria **
Proteobacteria *
Genus
b) Z.ma
Mean relative abundance (%)
100
50
0
NS
WS
Ellin6067
RB41
Burk.-Cabal.-Paraburk.
Acidothermus **
Bradyrhizobium
Conexibacter ***
Nitrospira **
Solirubrobacter
Acidibacter
Mucilaginibacter
MND1
Massilia
Bacillus
Candidatus Solibacter
Streptomyces
Haliangium *
Gemmatimonas
Bryobacter
Candidatus Udaeobacter *
Sphingomonas
d) Z.ma x A.vi
Mean relative abundance (%)
100
50
0
NS
WS
Bin063-1
Bradyrhizobium *
Opitutus
Conexibacter
Burk.-Cabal.-Paraburk.
Nitrospira
Solirubrobacter
Acidibacter
Dyella
Massilia
MND1
Bacillus
Streptomyces
Candidatus Solibacter **
Haliangium
Mucilaginibacter
Gemmatimonas
Bryobacter *
Candidatus Udaeobacter
Sphingomonas

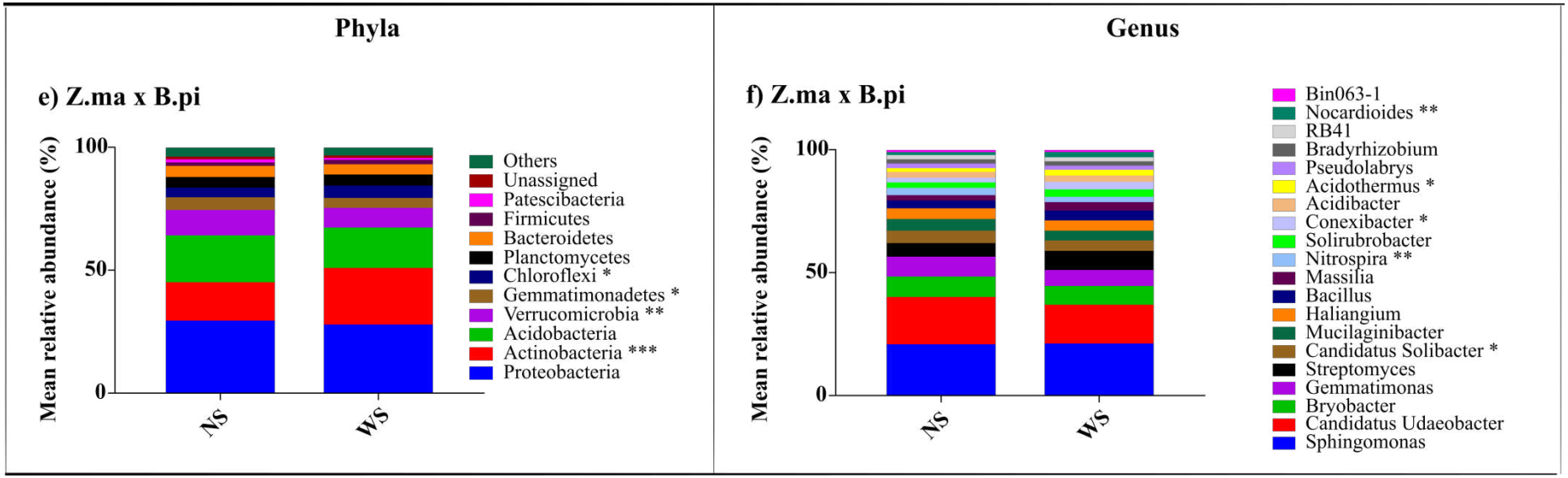
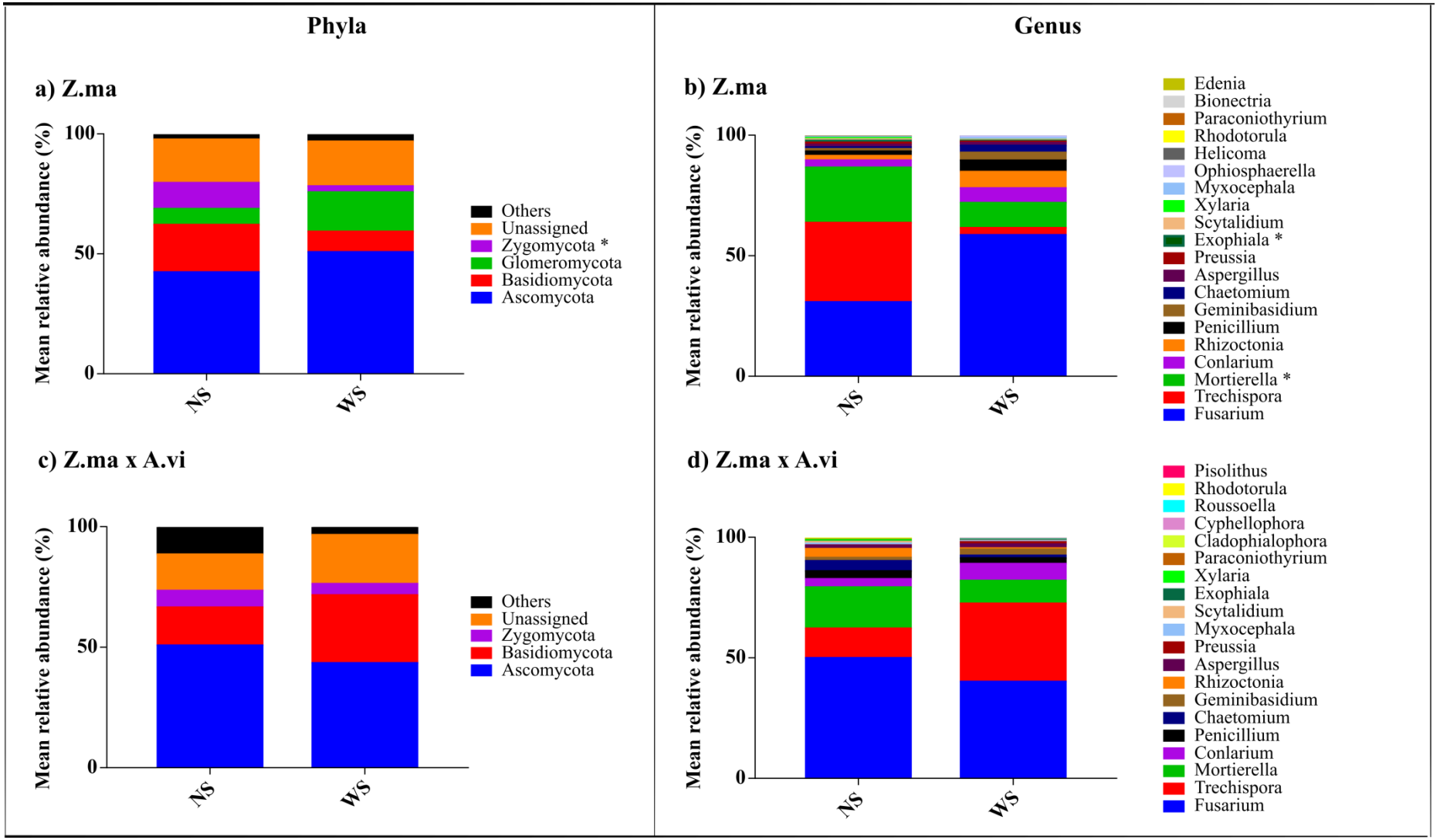


Fig. 3. Taxonomic distribution of OTUs of Bacteria and Archaea in rhizospheric soil treatments. **(a)** At the phylum level of monoculture of Zea mays, **(b)** at the genus level (95 % of similarity) of monoculture of Z. mays, **(c)** at the phylum level of competition between Z. mays and Amaranthus viriris, **(d)** at the genus level (95 % of similarity) of competition between Z. mays and A. viriris, **(e)** at the phylum level of competition between Z. mays and Bidens pilosa, and **(d)** at the genus level (95 % of similarity) of competition between Z. mays and B. pilosa. The asterisks indicate that this OTUs differs significantly relative abundance between treatments by the t test (*, ** and ***, significant to 5, 1 and 0,1 %, respectively). The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by “x” between the name of two species.


Phyla
Genus
a) Z.ma
b) Z.ma
c) Z.ma x A.vi
d) Z.ma x A.vi
Mean relative abundance (%)
100
50
0
NS
WS
Others
Unassigned
Zygomycota *
Glomeromycota
Basidiomycota
Ascomycota
Zygomycota
Edenia
Bionectria
Paraconiothyrium
Rhodotorula
Helicoma
Ophiosphaerella
Myxocephala
Xylaria
Scytalidium
Exophiala *
Preussia
Aspergillus
Chaetomium
Geminibasidium
Penicillium
Rhizoctonia
Conlarium
Mortierella *
Trechispora
Fusarium
Pisolithus
Rhodotorula
Roussoella
Cyphellophora
Cladophialophora
Paraconiothyrium
Xylaria
Exophiala
Scytalidium
Myxocephala
Preussia
Aspergillus
Rhizoctonia
Geminibasidium
Chaetomium
Penicillium
Conlarium
Mortierella
Trechispora
Fusarium

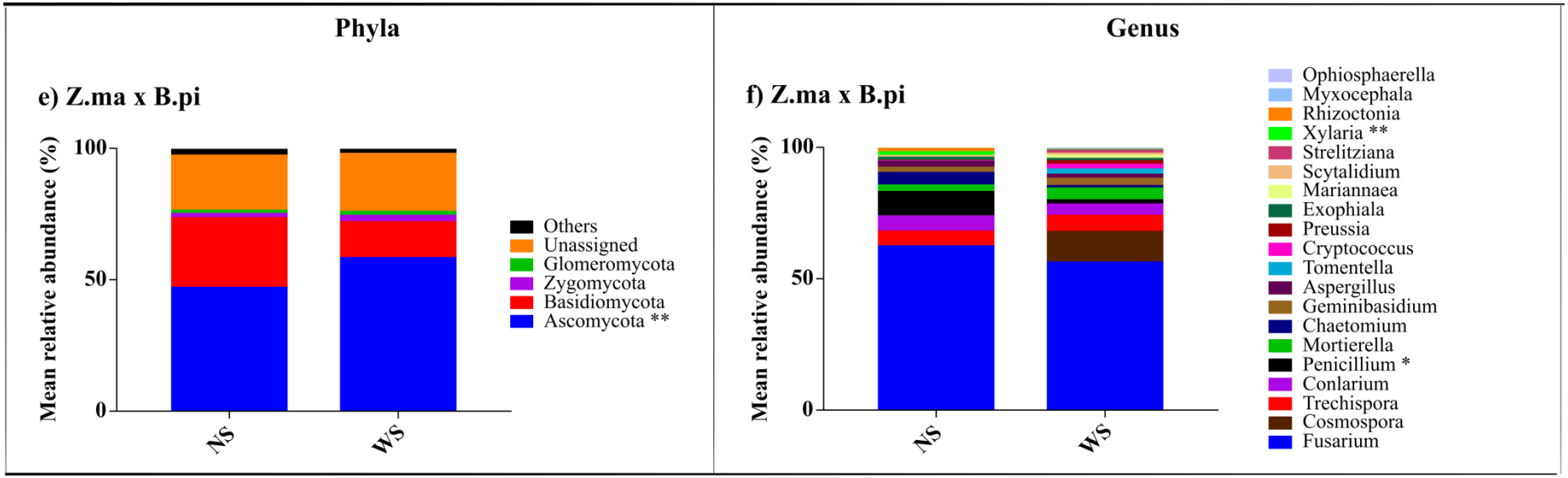


Fig. 4. Taxonomic distribution of OTUs of Fungi in rhizospheric soil treatments. **(a)** At the phylum level of monoculture of Zea mays, **(b)** at the genus level (95 % of similarity) of monoculture of Z. mays, **(c)** at the phylum level of competition between Z. mays and Amaranthus viriris, **(d)** at the genus level (95 % of similarity) of competition between Z. mays and A. viriris, **(e)** at the phylum level of competition between Z. mays and Bidens pilosa, and **(d)** at the genus level (95 % of similarity) of competition between Z. mays and B. pilosa. The asterisks indicate that this OTUs differs significantly relative abundance between treatments by the t test (*, ** and ***, significant to 5, 1 and 0,1 %, respectively). The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by “x” between the name of two species.

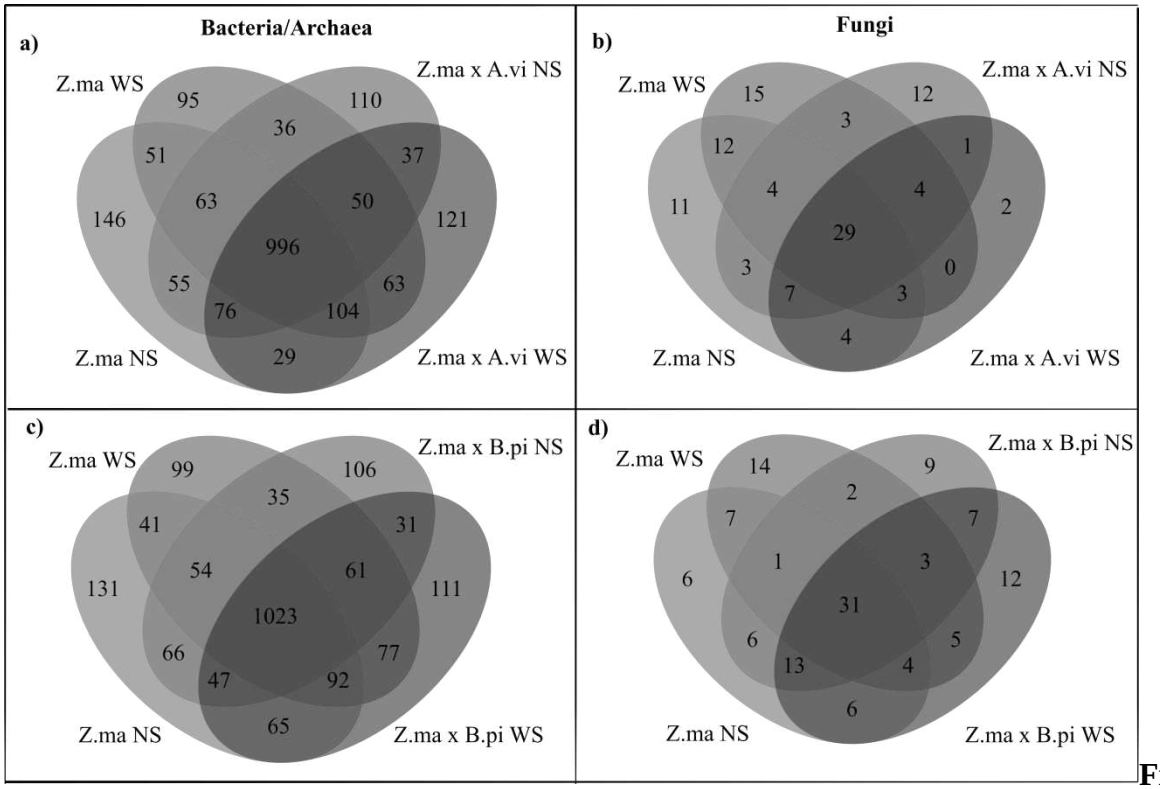
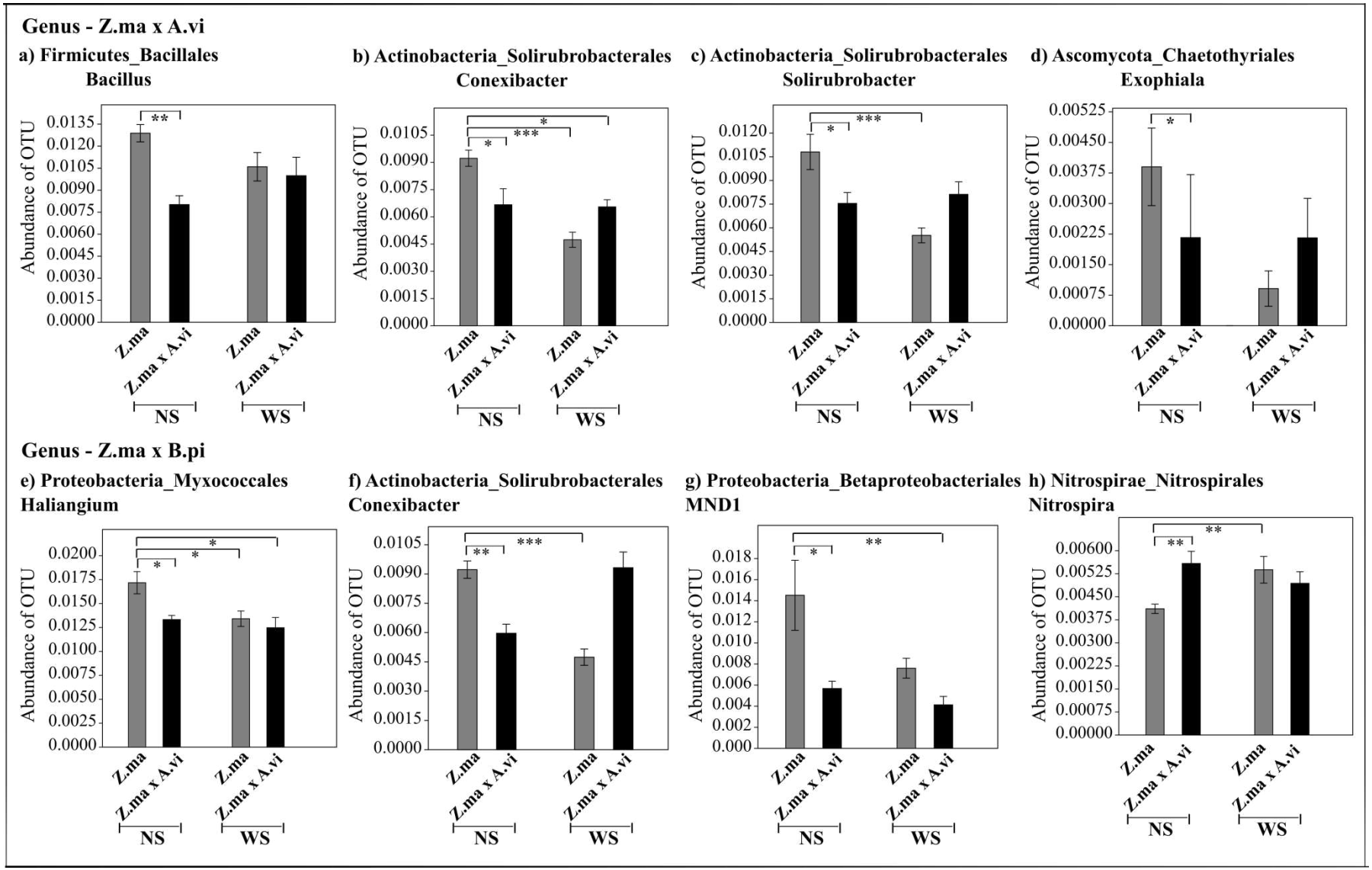


Fig. 5. Venn diagram of OTUs from **(a)** Bacteria and Archaea of Zea mays in monoculture and the competition between Z. mays and Amaranthus viridis, with and without salt, and **(b)** Fungi in the same conditions. **(c)** Bacteria and Archaea of Zea mays in monoculture and the competition between Z. mays and Bidens pilosa, with and without fertilizer, and **(d)** Fungi in the same conditions. Exclusive and shared OTUs among treatments are based on 97 % similarity. The numbers inside the diagram indicate the numbers of OTUs. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.


Genus - Z.ma x A.vi
a) Firmicutes_Bacillales
Bacillus
b) Actinobacteria_Solirubrobacterales
Conexibacter
c) Actinobacteria_Solirubrobacterales
Solirubrobacter
d) Ascomycota_Chaetothyriales
Exophiala
Genus - Z.ma x B.pi
e) Proteobacteria_Myxococcales
Haliangium
f) Actinobacteria_Solirubrobacterales
Conexibacter
g) Proteobacteria_Betaproteobacteriales
MND1
h) Nitrospirae_Nitrospirales
Nitrospira
Abundance of OTU
Z.ma
Z.ma x A.vi
NS
WS

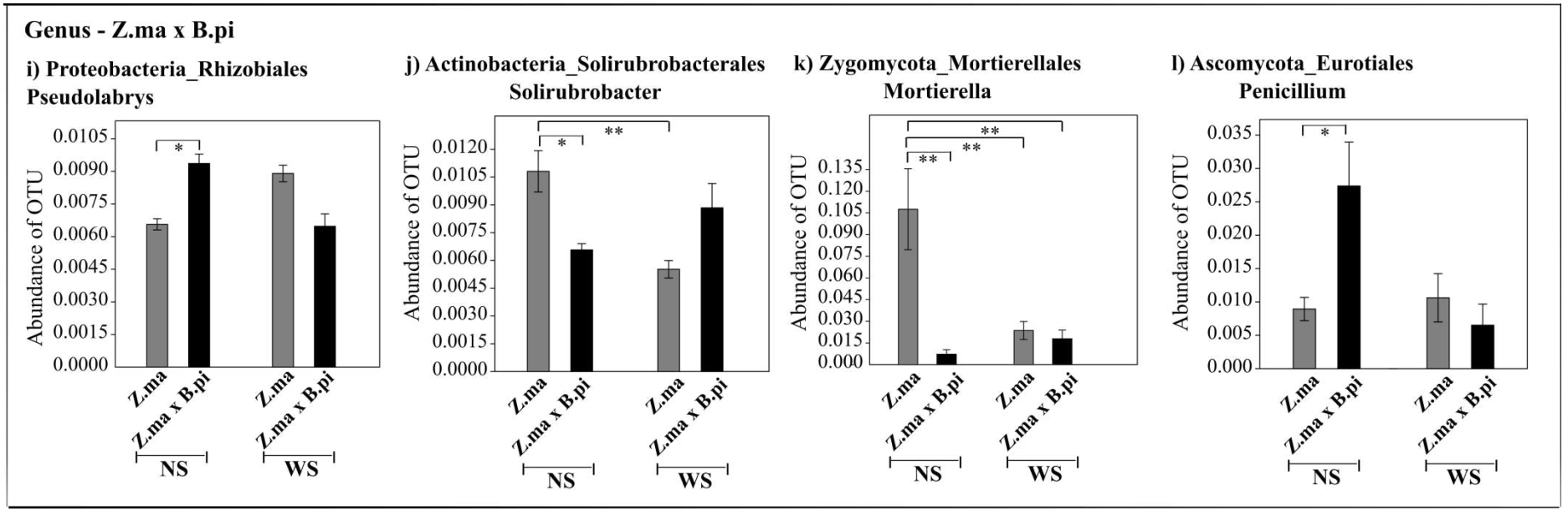


Fig. 6. Important genera of Bacteria and Fungi present in the rizhospheric soil that showed significant differences in their relative abundances when comparing the competitions between Zea mays and weeds, with and without salt, using maize monoculture as a reference. OTUs of Bacteria of competition between Z. mays and A. viridis **(a)** genus Bacillus, belonging to order Bacillales and to phyla Firmicutes, **(b)** genus Conexibacter, belonging to order Solirubrobacterales and to phyla Actinobacteria, **(c)** genus Solirubrobacter, belonging to order Solirubrobacterales and to phyla Actinobacteria and OTU of Fungi **(d)** genus Exophiala belonging to order Chaetothyriales and to phyla Ascomycota. OTUs of Bacteria of competition between Z. mays and B. pilosa **(e)** genus Haliangium belonging to order Myxococcales and to phyla Proteobacteria, **(f)** genus Conexibacter belonging to order Solirubrobacterales and to phyla Actinobacteria, **(g)** genus MND1 belonging to order Betaproteobacteriales and to phyla Proteobacteria, **(h)** genus Nitrospira belonging to order Nitrospirales and to phyla Nitrospirae, **(i)** genus Pseudolabrys belonging to order Rhizobiales and to phyla Proteobacteria, **(j)** genus Solirubrobacter belonging to order Solirubrobacterales and to phyla Actinobacteria, and OTUs of Fungi **(k)** genus Mortierella belonging to order Mortierellales and to phyla Zygomycota and **(l)** genus Penicillium belonging to order Eurotiales and to phyla Ascomycota. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

Tables

Table 1. Indexes of diversity for each treatment of rhizosphere in soils cultivated with and without added salt. Chao, Shannon and Simpson indexes for Bacteria, Archaea, and Fungi. The results for each treatment comprises the four replicates and the asterisks indicate that this index differs significantly between treatments by the t test (*, ** and ***, significant to 5, 1 and 0,1 %) and the ns means that there was no significant difference between means (p>0.05) of all indexes in all salt treatments. The plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

Bacteria/Archaea	**Eletric condition**	**Chao** $^{+\ ns}$	**Shannon** $^{\pm}$	**Simpson** $^{\S\ ns}$
Z. mays	NS	5195.2431 ± 307.2880	9.7828 ± 0.1079 *	0.9960 ± 0.0009
	WS	5093.2103 ± 287.4717	9.5576 ± 0.1191	0.9950 ± 0.0006
A. viridis	NS	4636.8329 ± 445.0034	9.6368 ± 0.1379	0.9956 ± 0.0009
	WS	5086.3894 ± 180.3623	9.6894 ± 0.0961	0.9956 ± 0.0006
B. pilosa	NS	4961.1695 ± 296.0216	9.4086 ± 0.0917	0.9935 ± 0.0012
	WS	4898.4518 ± 246.6021	9.4518 ± 0.1705	0.9940 ± 0.0012
Z. mays x A. viridis	NS	5099.8294 ± 242.0216	9.6512 ± 0.1148	0.9954 ± 0.0006
	WS	5300.7363 ± 417.0106	9.7482 ± 0.2007	0.9959 ± 0.0008
Z. mays x B. pilosa	NS	5194.0844 ± 201.1774	9.6171 ± 0.1482	0.9951 ± 0.0011
	WS	5153.7984 ± 86.2519	9.7380 ± 0.1378	0.9955 ± 0.0009
Fungi	**Eletric condition**	**Chao** $^{+\ ns}$	**Shannon** $^{\pm}$	**Simpson** $^{\S\ ns}$
Z. mays	NS	416.7630 ± 62.7906	5.3894 ± 0.6544	0.9287 ± 0.0332
	WS	377.7358 ± 25.4669	5.5617 ± 0.2738	0.9431 ± 0.0145
A. viridis	NS	315.6301 ± 44.2553	5.6142 ± 0.1613 *	0.9336 ± 0.0125
	WS	274.5759 ± 73.7789	5.0898 ± 0.3789	0.9185 ± 0.0318
B. pilosa	NS	315.0498 ± 69.7866	5.2229 ± 0.5824	0.8910 ± 0.0822
	WS	241.4849 ± 42.4828	5.1477 ± 0.6351	0.9163 ± 0.0629
Z. mays x A. viridis	NS	322.7808 ± 80.3205	5.2563 ± 0.2137	0.9199 ± 0.0146
	WS	313.7334 ± 48.9412	5.5253 ± 0.2486	0.9361 ± 0.0210

Z. mays x B. pilosa	NS	290.8204 ± 88.3957	5.4087 ± 0.4371	0.9285 ± 0.0143
	WS	383.0308 ± 101.8972	5.5151 ± 0.3351	0.9138 ± 0.0277

[+] Non-parametric estimator used to predict species richness (total number of OTUs presents), based in singletons and doubletons (Chao1).
[±] Diversity index that considers the evenness of species more than richness, considering the common species. The 1-D index was calculated, the value ranges from 0 to 1 and increases as the diversity increases.
[§] Diversity index that indicates species richness, considering the abundance of individual taxa. A higher number indicate more diversity (H).
(Chiarucci et al. 2011; Kim et al. 2017; Lemos et al. 2011).
* The asterisks means that there was significant difference between means with and without added salt ($p<0.05$).
** The asterisks means that there was significant difference between means with and without added salt ($p<0.01$).
*** The asterisks means that there was significant difference between means with and without added salt ($p<0.001$).

Supplementary Data

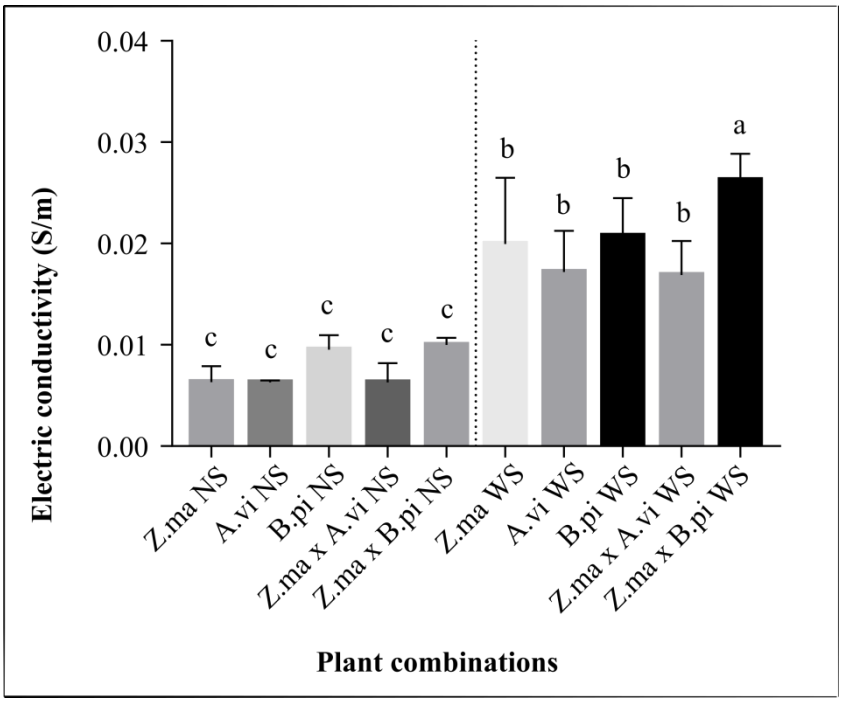


Fig. S1. Electric conductivity values in siemens per meter (S/m) measured in soils after 40 days of growth of plants in a greenhouse. The bars represent the standard deviation and different letters indicate significant differences between means based on one-way ANOVAs followed by the Tukey's test ($p<0.05$), when comparing all treatments. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

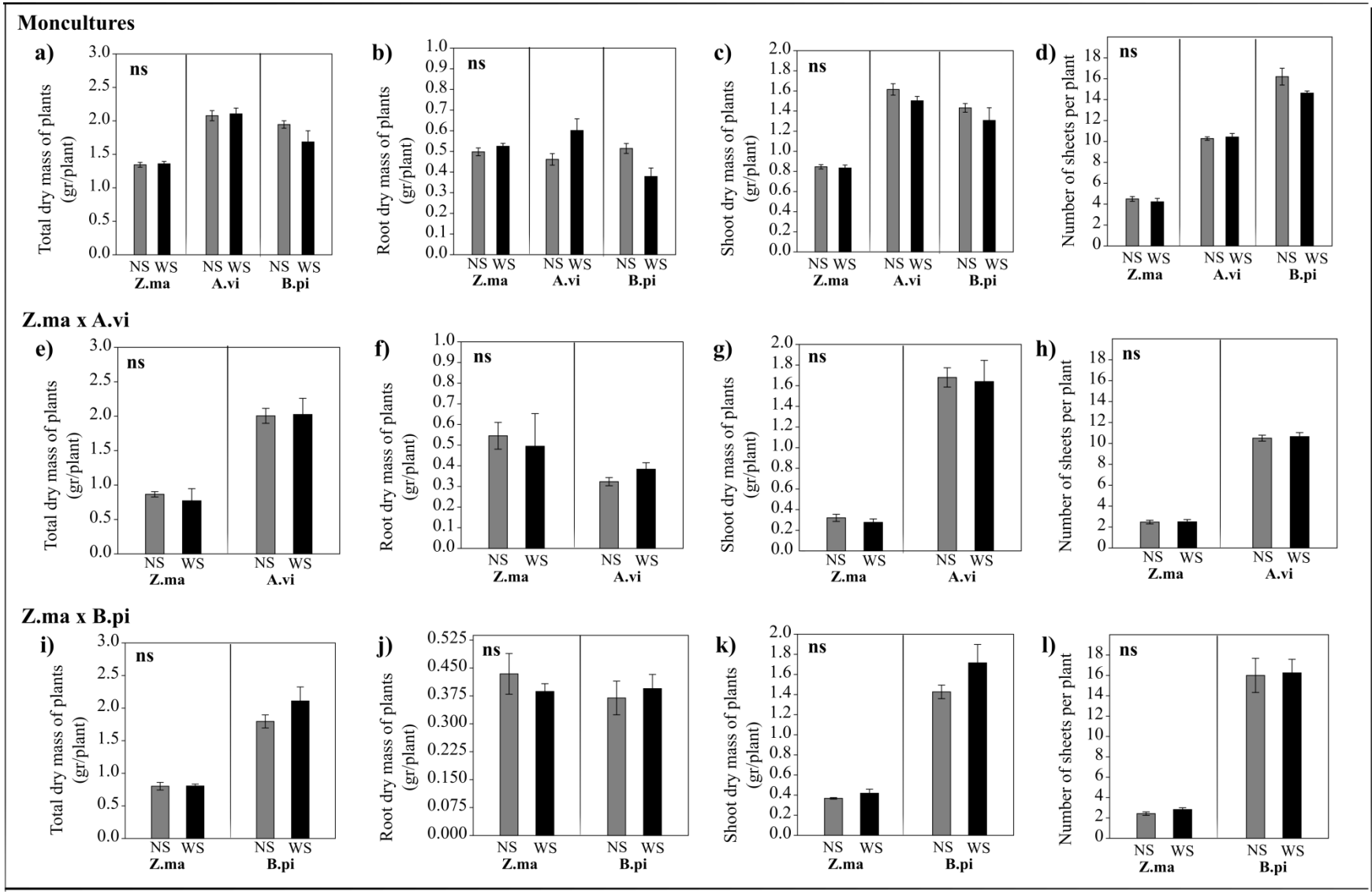


Fig. S2. Morphological characteristics of Zea mays and weeds in monoculture and in competition under to two different salt levels. The fist, analysis of monocultures of Zea mays, Amaranthus viridis and Bidens pilosa for **(a)** total dry matter, **(b)** root dry matter, **(c)** shoot dry matter and

(d) number of sheets. Analyzes of each plant in competition between Z. mays and A. viridis **(e)** total dry matter, **(f)** root dry matter, **(g)** shoot dry matter and **(h)** number of sheets, and analyzes of each plant in competition between Z. mays and B. pilosa **(i)** total dry matter, **(j)** root dry matter, **(k)** shoot dry matter and **(l)** number of sheets. The bars represent the standard deviation and ns indicate no difference between paired bars by the t test. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

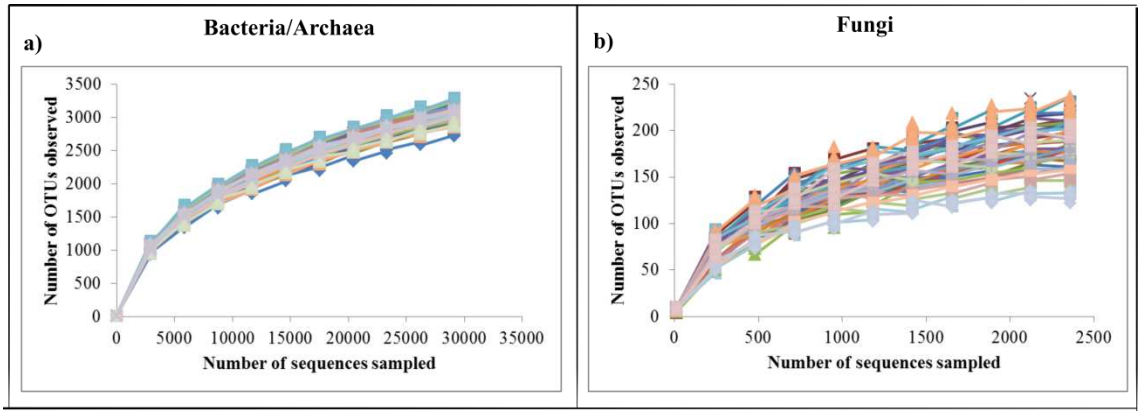


Fig. S3. Rarefaction curves for different treatments calculated based on OTUs defined as 97 % similarity level for **(a)** Bacteria and Archaea, and **(b)** Fungi.

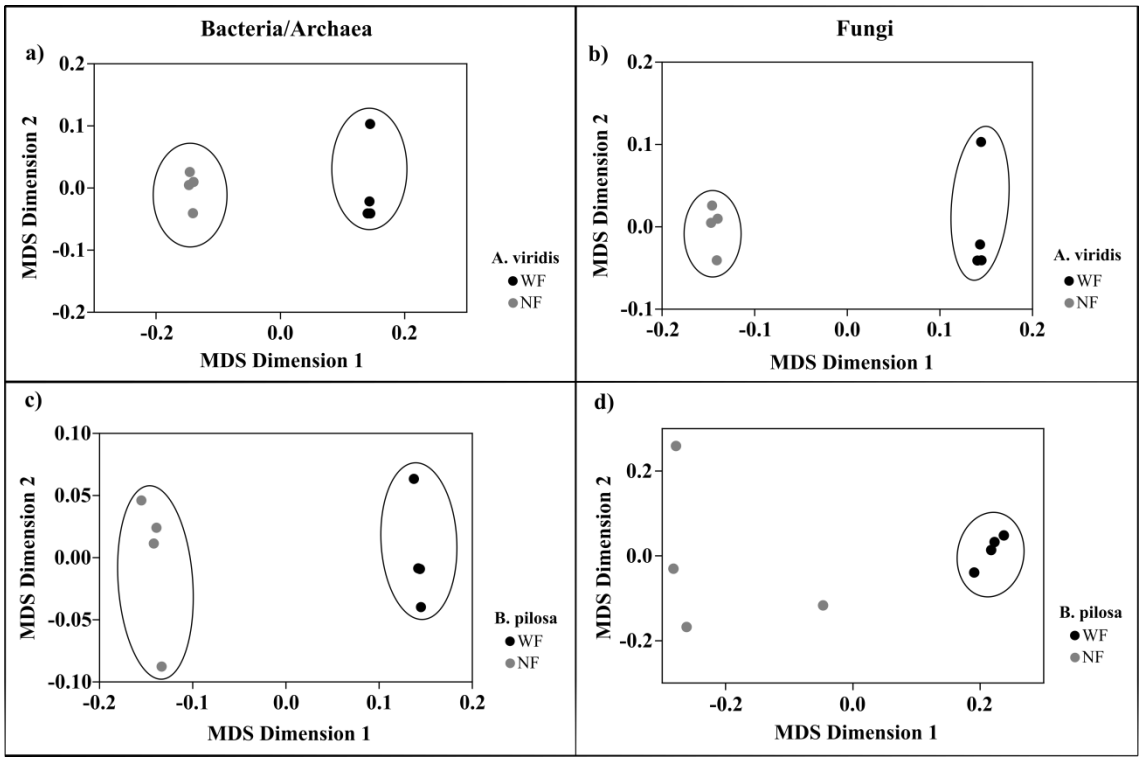
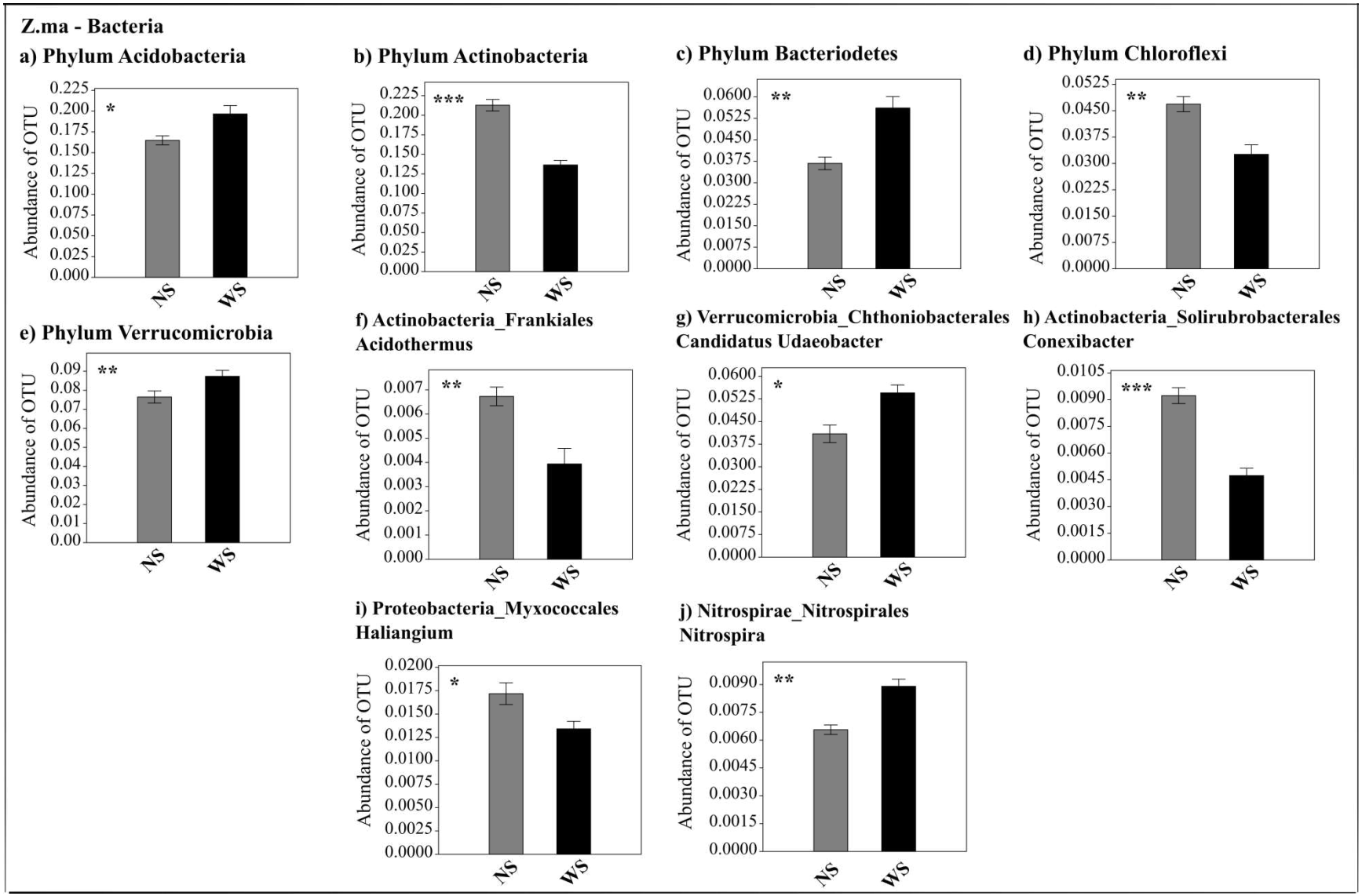


Fig. S4. Multidimensional Scaling (MDS) comparing soil microbial community as a function of electric conductivity. **(a)** Bacteria and Archaea of Amaranthus viridis in monoculture and **(b)** Fungi, **(c)** Bacteria and Archaea of Bidens pilosa in monoculture and **(d)** Fungi. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: A.vi – Amaranthus viridis and B.pi – Bidens pilosa.


Z.ma - Bacteria
a) Phylum Acidobacteria
b) Phylum Actinobacteria
c) Phylum Bacteriodetes
d) Phylum Chloroflexi
e) Phylum Verrucomicrobia
f) Actinobacteria_Frankiales
Acidothermus
g) Verrucomicrobia_Chthoniobacterales
Candidatus Udaeobacter
h) Actinobacteria_Solirubrobacterales
Conexibacter
i) Proteobacteria_Myxococcales
Haliangium
j) Nitrospirae_Nitrospirales
Nitrospira
Abundance of OTU
NS
WS

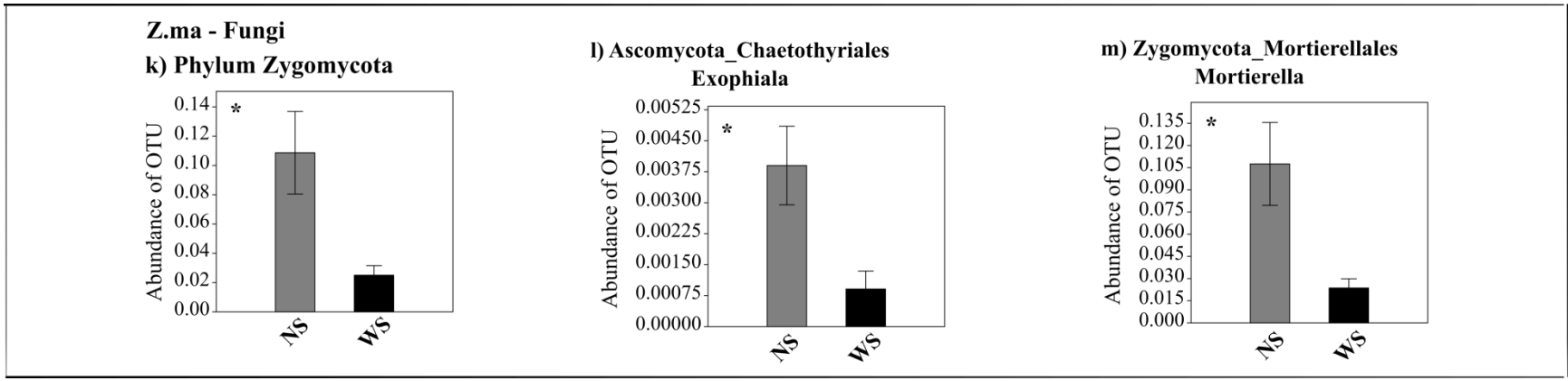


Fig. S5. Comparison of OTUs of Bacteria that significantly differed the relative abundance in rhizospheric soil with or without added salt in Zea mays in monoculture. Phyla **(a)** Acidobacteria, **(b)** Actinobacteria, **(c)** Bacteriodetes, **(d)** Chloroflexi and **(e)** Verrucomicrobia. Genera **(f)** Acidothermus, **(g)** Candidatus Udaedobacter, **(h)** Conexibacter, **(i)** Haliangium and **(j)** Nitrospira. OTUs of Fungi, phylum **(k)** Zygomycota, genera **(l)** Exophiala and **(m)** Mortierella. Asterisks indicate difference between paired bars by the t test (*,** and *** significant to 5, 1 and 0.1 %, respectively). The subtitle means: WS – with added salt and NS – no added salt and the plant species is: Z.ma – Zea mays.

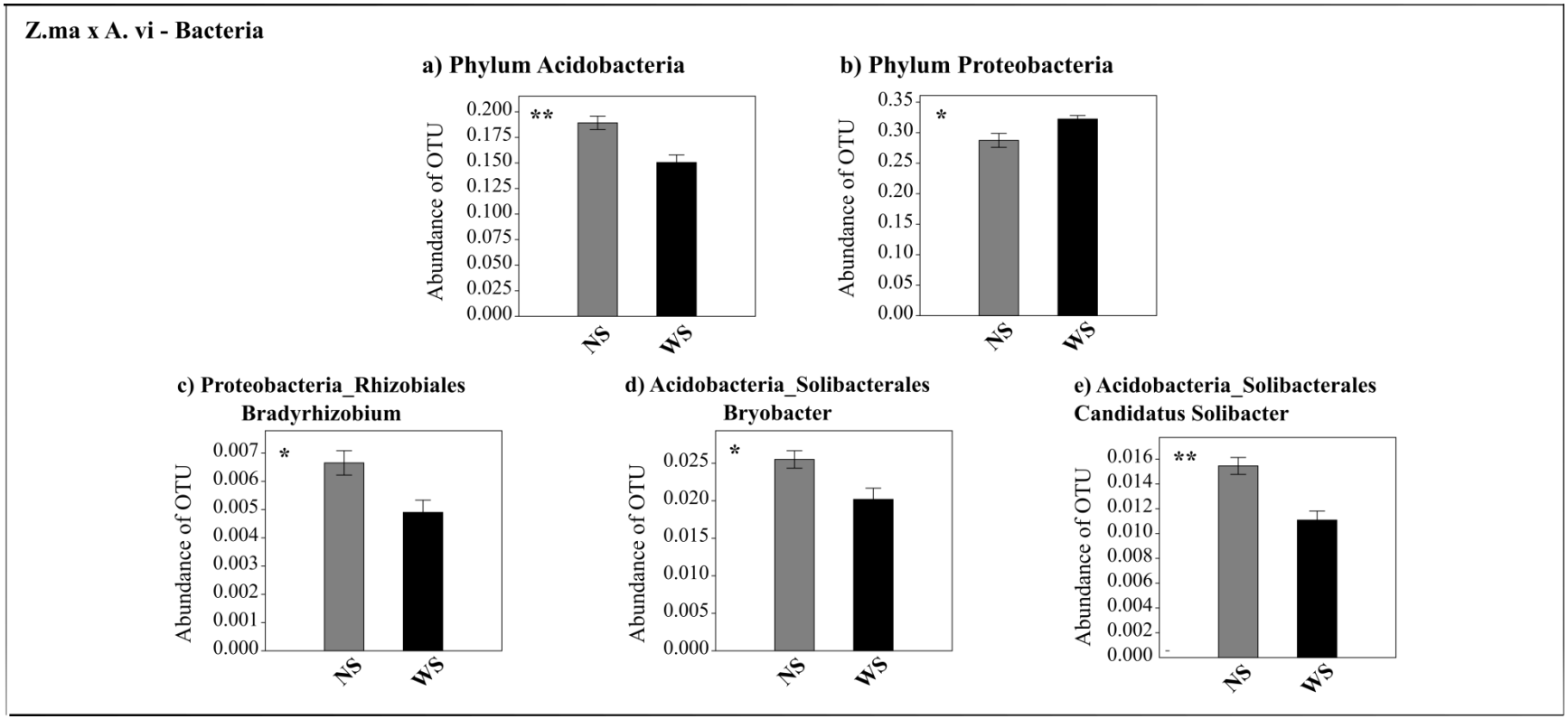


Fig. S6. Comparison of OTUs of Bacteria that significantly differed the relative abundance in rhizospheric soil with or without added salt in competition between Zea mays and Amaranthus viridis. Phyla **(a)** Acidobacteria and **(b)** Proteobacteria. Genera **(c)** Bradyrhizobimu, **(d)** Bryobacter and **(e)** Candidatus Solibacter. Asterisks indicate difference between paired bars by the t test (*,** and *** significant to 5, 1 and 0.1 %, respectively). The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays and A.vi – Amaranthus viridis. Coexistence treatments are indicated by "x" between the name of two species.

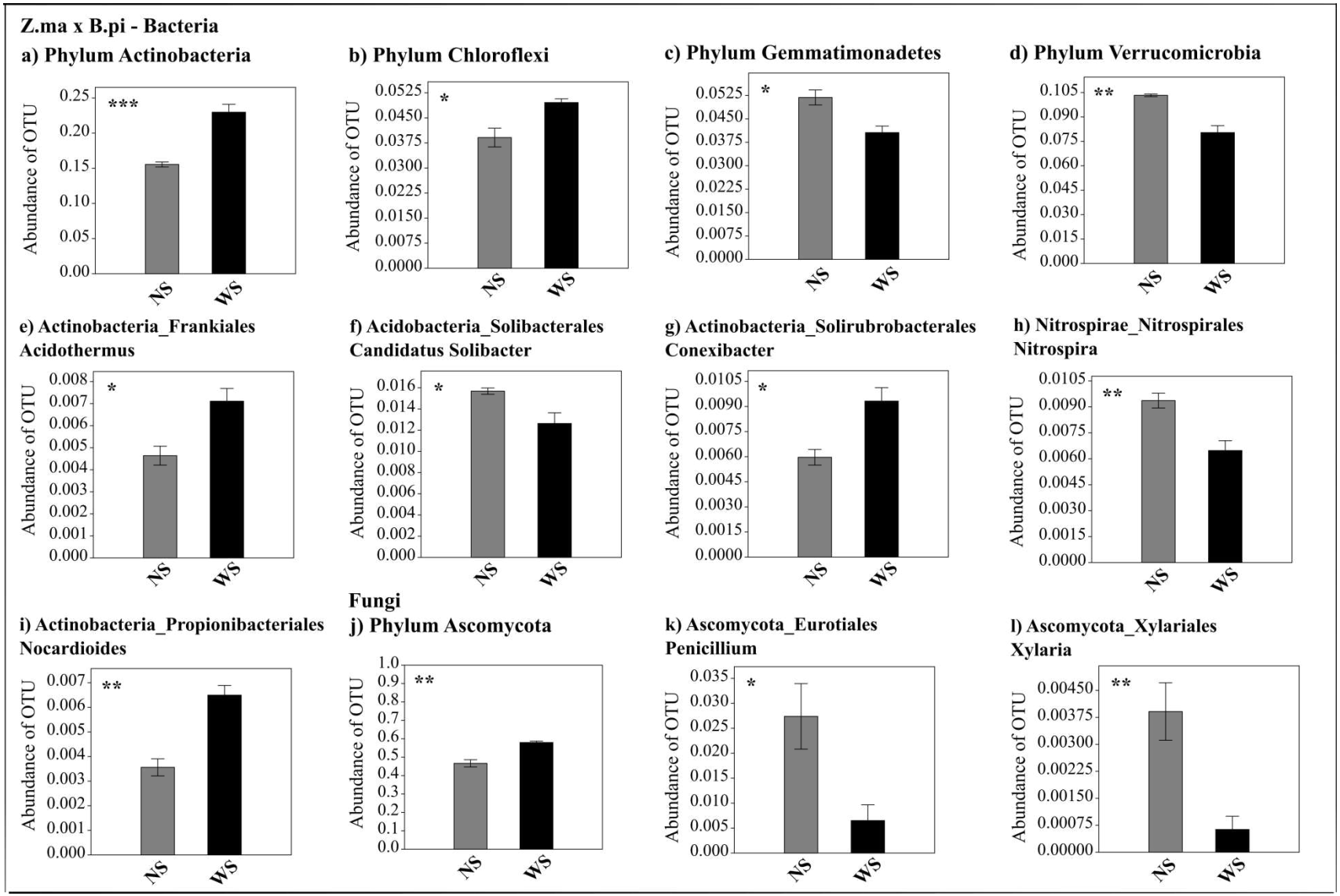


Fig. S7. Comparison of OTUs of Bacteria that significantly differed the relative abundance in rhizospheric soil with or without added salt in competition between Zea mays and Bidens pilosa. Phyla **(a)** Actinobacteria, **(b)** Chloroflexi, **(c)** Gemmatimonadetes and **(d)** Verrucomicrobia.

Genera **(e)** Acidothermus, **(f)** Candidatus Solibacter, **(g)** Conexibacter, **(h)** Nitrospira and **(i)** Nocardioides. OTUs of Fungi, phylum **(j)** Ascomycota, genera **(k)** Penicillium and **(l)** Xylaria. Asterisks indicate difference between paired bars by the t test (*,** and *** significant to 5, 1 and 0.1 %, respectively). The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

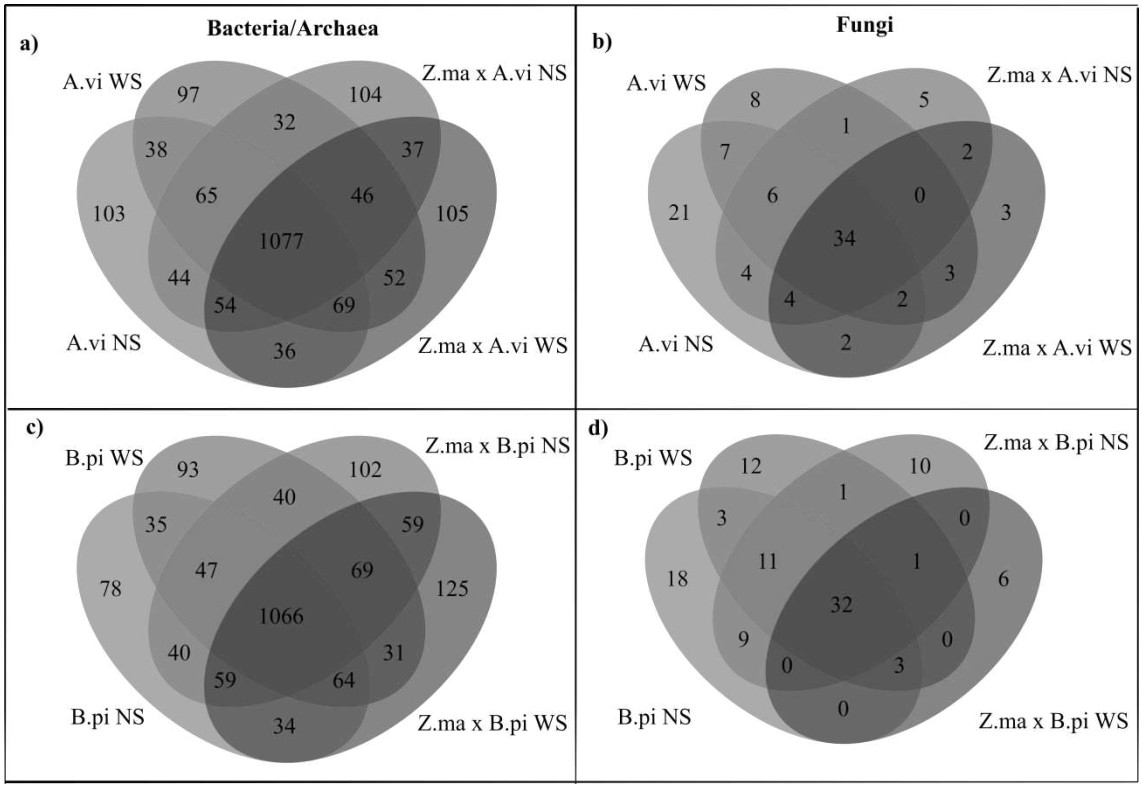


Fig. S8. Venn diagram of OTUs from **(a)** Bacteria and Archaea of Amaranthus viridis in monoculture and the competition between Zea mays and A. viridis, with and without added salt, and **(b)** Fungi in the same conditions. **(c)** Bacteria and Archaea of Bidens pilosa in monoculture and the competition between Z. mays and B. pilosa, with and without added salt, and **(d)** Fungi in the same conditions. Exclusive and shared OTUs among treatments are based on 97 % similarity. The numbers inside the diagram indicate the numbers of OTUs. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

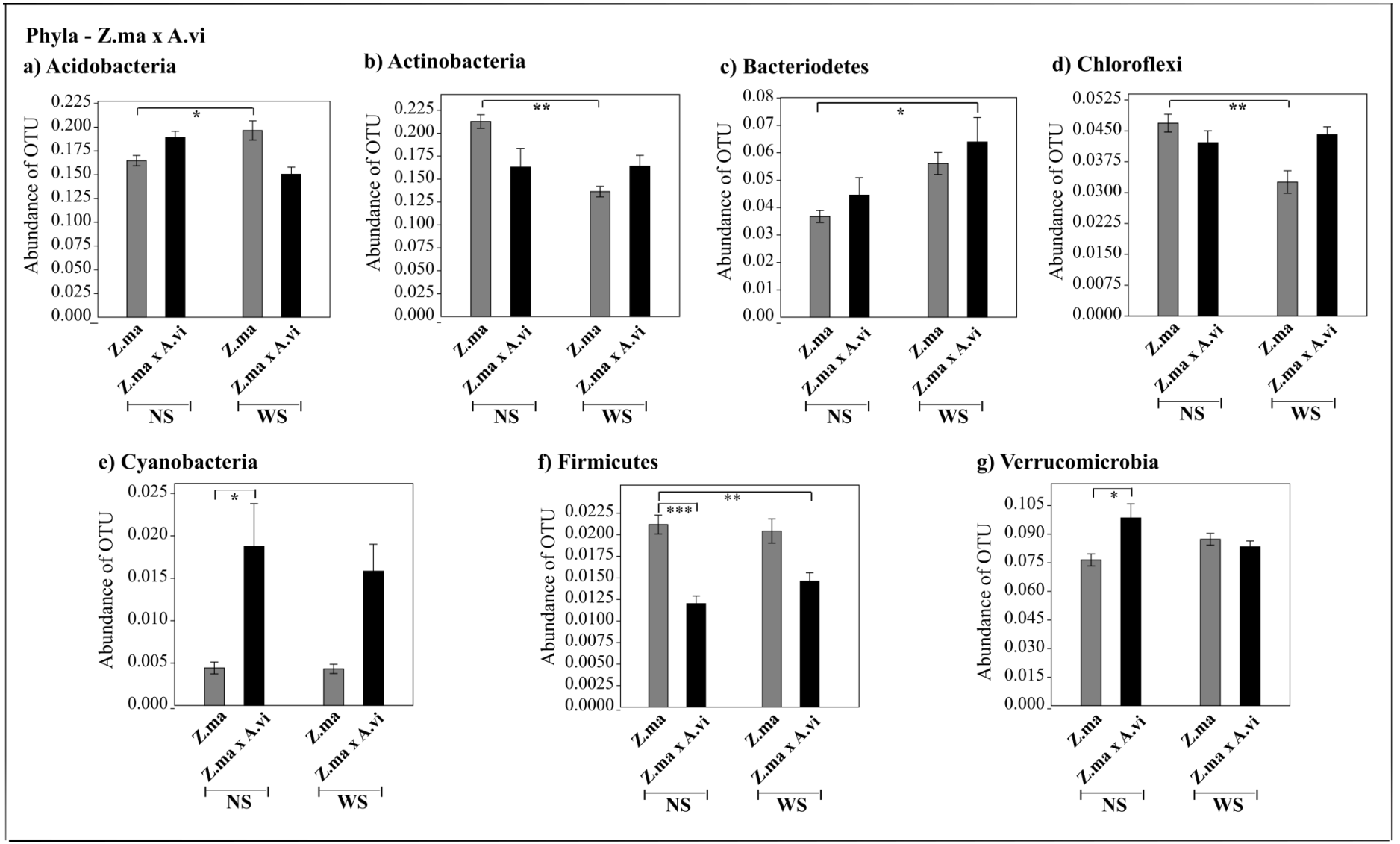


Fig. S9. Important phyla of Bacteria present in the rizhospheric soil that showed significant differences in their relative abundances when comparing the competition between Zea mays and A. viridis, with and without salt, using maize monoculture as a reference. OTUs of Bacteria **(a)**

phylum Acidobacteria, **(b)** Actinobacteria **(c)** Bacteriodetes, **(d)** Chloroflexi, **(e)** Cyanobacteria, **(f)** Firmicutes and **(g)** Verrucomicrobia. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

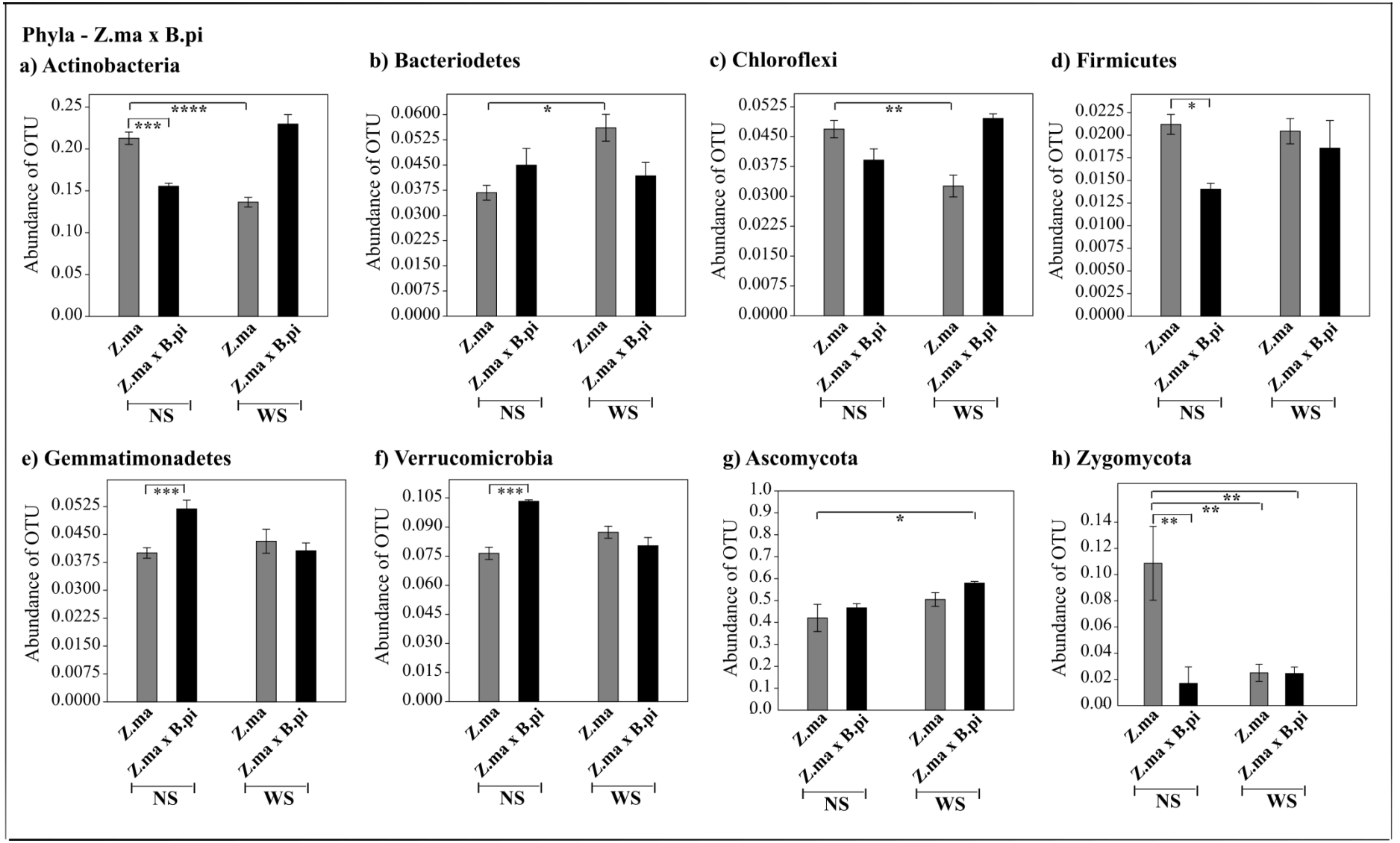


Fig. S10. Important phyla of Bacteria and Fungi present in the rizhospheric soil that showed significant differences in their relative abundances when comparing the competition between Zea mays and B. pilosa, with and without salt, using maize monoculture as a reference. OTUs of

Bacteria **(a)** phylum Actinobacteri, **(b)** Bacteriodetes, **(c)** Chloroflexi, **(d)** Firmicutes, **(e)** Gemmatimonadetes, **(f)** Verrucomicrobia, and OTUs of Fungi **(g)** phylum Ascomycota and **(h)** Zygomycota. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

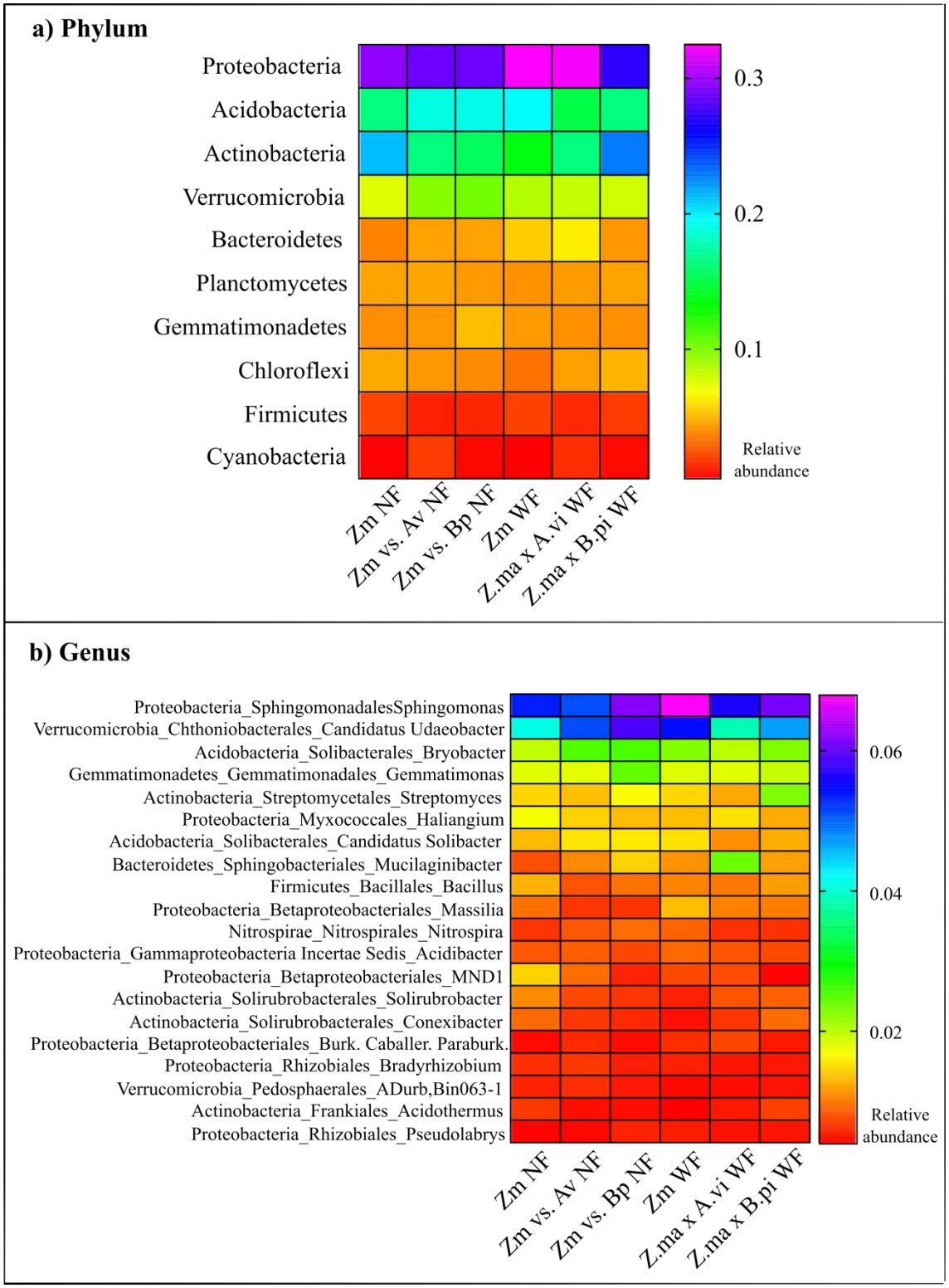


Fig. S11. Heatmap demonstrating by a color scale the changes in the abundance of the main Bacteria taxa as a function of increased soil EC and competition between plants. **(a)** Phyla of Bacteria with relative abundance >1. **(b)** The 20 most abundant genera of Bacteria. The relative abundances represent the average abundance of each taxon in each repetition per treatment. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

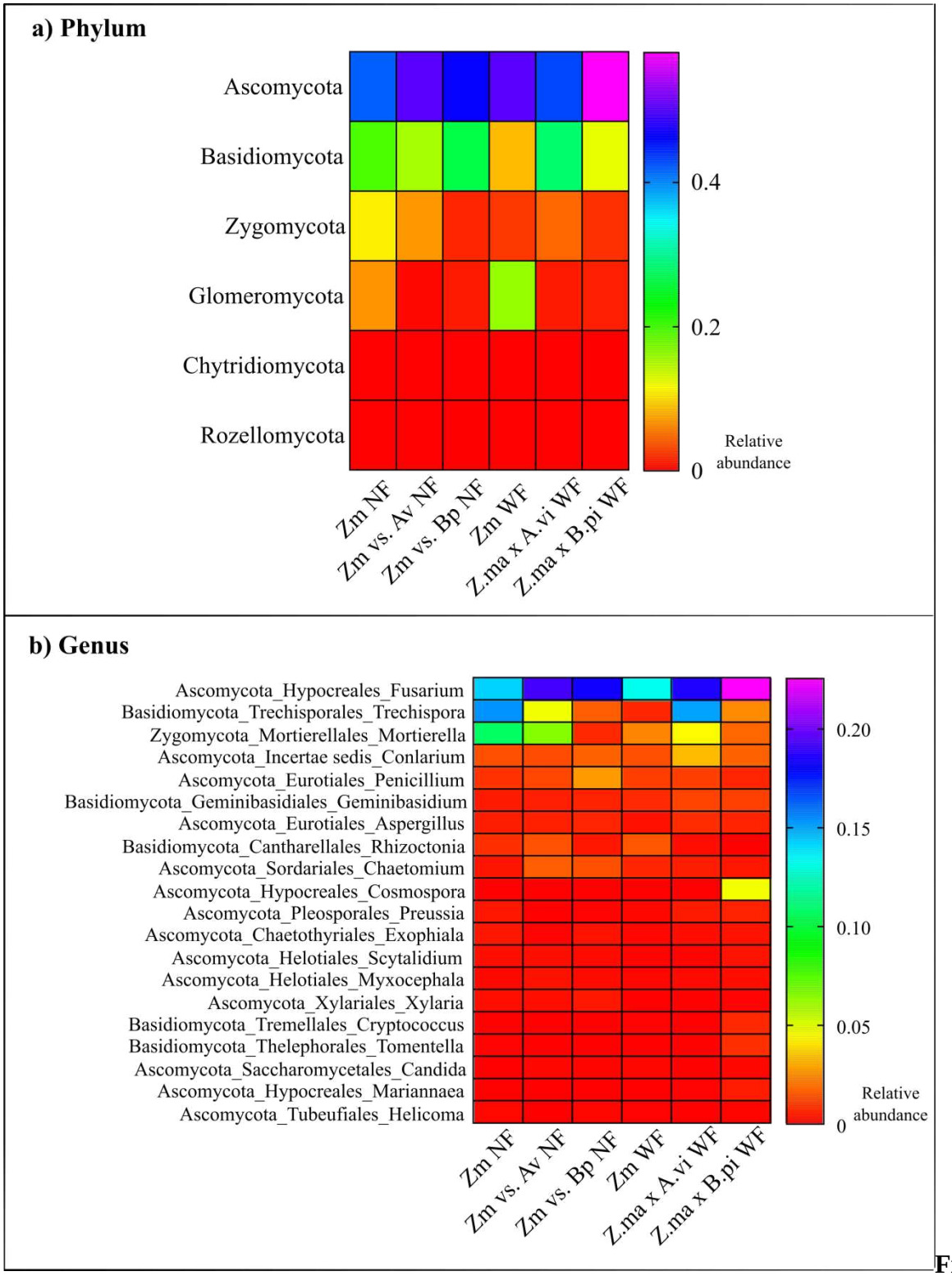


Fig. S12. Heatmap demonstrating by a color scale the changes in the abundance of the main Fungi taxa as a function of increased soil EC and competition between plants. **(a)** All phyla of Fung. **(b)** The 20 most abundant genera of Fungi. The relative abundances represent the average abundance of each taxon in each repetition per treatment. The subtitle means: WS – with added salt and NS – no added salt and the plant species are: Z.ma – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.

Table S1. Good's coverage index

Tratament	Eletric condition	Archaea	Bacteria	Fungi
Z.ma	NF	0.9887 ± 0.0031 **	0.9805 ± 0.0032	0.9949 ± 0.0049
	WF	0.9960 ± 0.0011	0.9836 ± 0.0025	0.9957 ± 0.0017
A.vi	NF	0.9901 ± 0.0120	0.9735 ± 0.0077	0.9935 ± 0.0025
	WF	0.9955 ± 0.0035	0.9817 ± 0.0025	0.9867 ± 0.0067
B.pi	NF	0.9971 ± 0.0012 *	0.9805 ± 0.0031	0.9969 ± 0.0006
	WF	0.9928 ± 0.0028	0.9805 ± 0.0027	0.9925 ± 0.0082
Z.ma x A.vi	NF	0.9927 ± 0.0029	0.9811 ± 0.0024	0.9949 ± 0.0026
	WF	0.9945 ± 0.0041	0.9831 v 0.0014	0.9935 ± 0.0020
Z.ma x B.pi	NF	0.9921 ± 0.0018	0.9833 ± 0.0007 *	0.9969 ± 0.0006
	WF	0.9919 ± 0.0042	0.9798 ± 0.0027	0.9965 ± 0.0010

* The asterisks means that there was significant difference between means with and without added salt ($p<0.05$).
** The asterisks means that there was significant difference between means with and without added salt ($p<0.01$).
The plant species are: Z. – Zea mays, A.vi – Amaranthus viridis and B.pi – Bidens pilosa. Coexistence treatments are indicated by "x" between the name of two species.