UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DO SOLO

Manoeli Lupatini

Sistemas e manejos agrícolas modulam componentes multitróficos no solo

Santa Maria, RS
2016

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Tese apresentada ao Curso de Pós-Graduação em Ciência do Solo, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Ciência do Solo**. Defesa realizada por videoconferência.

Orientador: Profº Drº. Rodrigo Josemar Seminoti Jacques

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Ficha catalográfica elaborada através do Programa de Geração Automática
da Biblioteca Central da UFSM, com os dados fornecidos pelo(a) autor(a).

Lupatini, Manoeli
Sistemas e manejos agrícolas modulam componentes multitróficos no solo / Manoeli Lupatini.- 2016.
109 p.; 30 cm

Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós-Graduação em Ciência do Solo, RS, 2016

1. Microbioma 2. Agroecosistema 3. Bioindicadores 4. Sequenciamento 5. Ecologia Microbiana I. Título.

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Aprovado em 29 de Abril de 2016:

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Santa Maria, RS
2016

AGRADECIMENTOS

O meu doutorado foi um inesquecível período em minha vida pessoal e profissional. Muitas pessoas contribuíram para que o mesmo fosse realizado.

Primeiramente, eu gostaria de agradecer ao meu orientador Rodrigo J.S. Jacques o qual me forneceu todo o suporte necessário para a realização desse estudo. Além disso, gostaria de agradecer à todos que trabalham no laboratório "Biologia e Microbiologia Ambiental Prof. Marcos R. Fries" da Universidade Federal de Santa Maria. Também, gostaria de agradecer ao grupo do "Centro Interdisciplinar de Pesquisa em Biotecnologia – CIP-Biotec" da Universidade Federal do Pampa, especialmente ao Prof. Luiz F.W. Roesch pelas valiosas discussões sobre microbiologia e bioinformática. Especial agradecimento à Edicarla, Daiana, Marta, Paulo, Antônio, Natielo, Marcelo, Andressa e Daniel pelo ótimo tempo que passamos juntos.

Julgo este ser o melhor momento, por representar o final de um ciclo na minha carreira científica, para expressar meu humilde agradecimento à pessoa que julgo ser a maior responsável pelo meu caminho na ciência: Zaida I. Antoniolli. Foi um grande prazer ter escolhido (quase que aleatóriamente...) trabalhar no seu grupo desde o início da minha graduação, no longínquo 2004. Daí, são quase 12 anos em que mantemos uma ótima relação de trabalho e pessoal. Posso dizer ao longo desses anos, a professora ainda é um exemplo de profissional e pessoa, que nunca se deixa abater pelas dificuldades e que sempre mantêm os pés no chão, mesmo nas mais inesperadas situações.

Com grande prazer, eu gostaria de expressar à minha sincera gratidão à todos os mestrandos, doutorando, posdoutorandos e técnicos do "Netherlands Institute of Ecology (NIOO/KNAW)", Netherlands, pelas discussões científicas e pelas interessantes conversas sempre somadas à uma grande quantidade de café. O período do meu doutorado na Holanda foi de grandes conquistas, autoconhecimento e principalmente, de muitos desafios e fracassos, o que não poderia faltar quando grandes riscos são assumidos. Meu sincero agradecimento à Drª Eiko K. Kuramae, que me acolheu no seu grupo e abriu portas para a conclusão de dois doutorados, um na Holanda e outro no Brasil. Agradeço imensamente a ajuda e apoio que recebei do Dr. Gerard Korthals, que gentilmente cedeu todas as informações relacionados ao experimento dessa tese, e contribuições significativas na escrita dos trabalhos. Obrigada Mathias e Norico por serem colegas pacientes e pelas valiosas discussões sobre bioinformática e estatística.

Não só para o trabalho vive um doutorando. Sempre terei uma boa lembrança das pessoas com quem tive o prazer de dividir o espaço dentro e fora do NIOO. Especialmente, gostaria de agradecer à Amaranta, Viviane, Adriano, Victor, Ruth, Juan, Thiago, Julia, Paolo, Johnny, Olaf, Yan Yan e Yin Yin pelas risadas e pelos interessantes assuntos abordados no meios das tardes sonolentas de quem esta tentando escrever uma tese de doutorado. Um obrigada especial ao colega e amigo Matheus, o qual dividiu comigo grande parte do tempo em que estive na Holanda. Sem sua presença, a minha estadia não seria tão divertida e interessante. Sempre vou lembrar com carinho os nossos cafés no "*Daniel's Coffee*" após o trabalho, as muitas cervejas e o "smoky whisky nos finais de semana e tardes preguiçosas de domingo espalhados em um sofá na chuvosa Holanda.

Não apenas colegas, mas também pela grande amizade, eu gostaria de agradecer a Afnan Suleiman e Alessandro Rosa. Em diferentes períodos do meu doutorado (e da minha vida pessoal), vocês foram muito importantes para ajudar a me manter sempre em frente. Obrigado, Afnan, que desde o início do doutorado, temos trabalhado em conjunto de uma maneira espetacular e sempre nos perguntando: O que é isso frente ao Universo???. Obrigado, Alessandro, que por estranho destino ou coincidência, não suficiente nascermos na mesma cidade, cursamos Agronomia e o mestrado juntos, e acabamos dividindo a mesma casa na Holanda durante o doutorado sanduíche. Lá, continuamos nossas conversas filosóficas sobre todos os aspectos da ciência que começou anos antes.

Não posso deixar de agradecer algumas pessoas que inesperadamente apareceram (ou reapareceram) na minha vida. O último ano do doutorado não seria tão alegre e confortante se não fosse a convivência com minhas duas colegas de casa que apareceram em minha vida, Júlia e Ju, as quais se tornaram amigas para todas as horas, principalmente as horas boas! Depois de algum tempo sem contato devido aos diferentes caminhos seguidos após o mestrado, meu grande amigo Gustavo reapareceu e me mostrou que amizade não se acaba pela distância ou pelo tempo e que amizades verdadeiras devem ser cultivadas até os finais das madrugadas.

Eu especialmente agradeço à minha família, meus pais e minha irmã por todo o apoio. Às vezes não foi fácil superar a distância, mas todo o seu amor incondicional e incentivo fizeram de mim uma pessoa mais forte para superar a dificuldades.

Se eu não mencionei alguém aqui, isso não significa que eu não agradeço pela ajuda. Não posso dizer que o doutorado foi um período sempre fácil e alegre. Também foi um período de intenso trabalho, incertezas e grandes frustrações. Porém, esse período esta terminando e agora é hora de procurar novos desafios e seguir em frente.

RESUMO

SISTEMAS E MANEJOS AGRÍCOLAS MODULAM COMPONENTES MULTITRÓFICOS NO SOLO

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O microbioma e as relações com a meiofauna do solo são importantes para a estabilidade e funcionalidade dos agroecossistemas, incluindo os seus efeitos potenciais sobre a supressividade do solo. No entanto, pouco se sabe sobre como os sistemas agrícolas e métodos alternativos para o controle de patógenos de plantas determinam a comunidade microbiana, a meiofauna solo e a produtividade de plantas. Neste estudo, avaliamos a composição do microbioma do solo (bactérias, fungos e protistas), utilizando o sequenciamento de nova geração (marcadores ribossômicas 16S e 18S), a população de nematóides de vida livre e parasitas, a produtividade da planta e sua inter-relações em um experimento de longa duração dividindo sistema convencional e orgânico em métodos alternativos de controle de patógenos de plantas. Os sistemas de cultivo convencional e orgânico largamente determinaram a comunidade microbiana do solo, a meiofauna e produtividade da planta, enquanto que os efeitos dos tratamentos alternativos foram de menor magnitude. O sistema de manejo orgânico aumentou a diversidade taxonômica e filogenética das comunidades de bactérias e fungos em comparação com o sistema de agricultura convencional, enquanto não foram observados efeitos sobre a diversidade de protistas. A população de nematóides de vida livre foi favorecida no sistema orgânico, enquanto a população de nematóides parasitas e produtividade da planta foi maior no sistema convencional. A diversidade e a estrutura da comunidade microbiana parecem estar relacionadas com a diminuição de nematóides parasitas no sistema orgânico e em certos tratamentos do solo, o quais foram caracterizados por grupos microbianos conhecidos por serem envolvidos na supressão de patógenos de solo. Entender o microbioma solo e as interações multitróficas em agroecossistemas oferecem um potencial para um manejo mais sustentável por meio de microrganismos benéficos.

ABSTRACT

FARMING AND MANAGEMENT SYSTEMS MODULATE MULTITROPHIC COMPONENTS IN SOIL

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Soil microbiome and relationships with soil meiofauna are important for the stability and functionality of agroecosystems, including their potential effects on soil suppressiveness. However, little is know about how farming systems and alternative methods for controlling plant pathogens determine microbial community, soil meiofauna and plant productivity. In this study, we assessed the composition of soil microbiome (bacterial, fungal and protist) using a high-throughput sequencing approach (16S and 18S ribosomal markers), the population of parasitic and free-living nematodes, the plant productivity and its inter-relationships in a long-term experiment dividing conventional and organic systems into alternative methods for plant pathogen control. Conventional and organic farming systems had major influence on soil microbial community, meiofauna and plant productivity, while the effects of the soil health treatments were of smaller magnitude. Organically managed system increased taxonomic and phylogenetic diversity of the bacteria and fungal communities compared with conventional farming system, while no effects were observed on protist community. Organic farming increase the population of free-living nematodes and conventional increase the population of parasitic nematodes and plant productivity. Microbial diversity and community structure appear to be related with parasitic nematode suppression in system receiving organic fertilizer and certain soil health treatments, which were characterized by component microbial groups known to be involved in suppression of soil pathogens. Understand the soil microbiome and multitrophic interactions in agroecosystems offer a potential for managing the soil environment from ecology towards a more sustainable control of plant pathogens using beneficial microorganisms.

INTRODUÇÃO

Com o advento da revolução verde, houve uma rápida expansão na produção agrícola baseada no intenso uso de fertilizantes e pesticidas, aumentando a produtividade das culturas, modificando o manejo do solo e promovendo conversões de ecossistemas naturais ao redor do mundo (Tilman et al., 2001). No entanto, há uma constante preocupação que a intensificação da agricultura leve à degradação de ecossistemas, incluindo a degradação do solo, a contaminação e acumulação de pesticidas e o aumento da emissão de gases de efeito estufa (Vitousek, 1997). De fato, os sistemas agrícolas intensivos são considerados uma das grandes ameaças à biodiversidade global (Sala et al., 2000). A conversão de sistemas intensivos, ou convencionais, para sistemas orgânicos tem sido considerada uma solução potencial para diminuir a perda da diversidade e aumentar a sustentabilidade da produção agrícola em longo prazo (Gonthier et al., 2014; Clark et al., 2016). O sistema de agricultura convencional é baseado no uso de agroquímicos, como uso de fertilizantes para aumentar a produção das culturas e uso de fungicidas e herbicidas sintéticos para promover o controle de patógenos e plantas daninhas (Kremen e Miles, 2012). Contrariamente, o sistema orgânico consiste do manejo agrícola com nenhum ou mínimo uso de compostos agroquímicos, onde a produtividade e a funcionalidade do agroecossistema é baseada na adição de compostos orgânicos, disponibilidade natural de nutrientes por meio da rotação de culturas e controle biológico (Lammerts van Bueren et al., 2002).

Um dos aspectos mais importantes dos sistemas agrícolas é o correto manejo do solo (Powlson et al., 2011). O solo fornece os principais serviços em um ecossistema agrícola, como ciclagem de nutrientes, disponibilidade de água e controle de patógenos e doenças (Brussaard et al., 2007). O microbioma e a meiofauna do solo representam importantes componentes que sustentam serviços e processos do ecossistema terrestre, onde a diversidade a composição determinam a sustentabilidade e produtividade primária (Wagg et al., 2014). Bactérias representam a maior parte da biodiversidade no solo e estão envolvidas em funções chaves do ecossistema, incluindo a ciclagem de nutrientes e supressão de patógenos de plantas (Mazzola, 2004; Wakelin et al., 2013). A comunidade de fungos desempenha um papel importante como simbiontes obrigatórios de plantas, decompositores ou patógenos no solo (Schneider et al., 2010; Xu et al., 2012; Penton et al., 2014). A meiofauna do solo e os protistas cumprem diversas funções ecológicas no solo através de “food webs”, como predação de outros organismos, incluindo bactérias e nematóides, decomposição da matéria

orgânica e ciclagem de nutrientes (Paungfoo-Lonhienne et al., 2015). No entanto, os efeitos dos sistemas agrícolas e manejo do solo na comunidade microbiana e na meiofauna do solo são menos claros do que os efeitos em macroorganismos (Gonthier et al., 2014). A intensificação da agricultura tem um impacto substancial na diversidade de plantas e animais, levando à diminuição ou perda de espécies e à homogeneização de ecossistemas, com consequências negativas à funcionalidade dos ecossistemas (Gabriel et al., 2006; Jonason et al., 2011). Porém, é esperado que em sistemas orgânicos, a maior diversidade e a presença de certos grupos microbianos promovam importantes serviços de solo e melhorem a estabilidade do ecossistema através da supressão de doenças de plantas, interação com a meiofauna do solo e maior produtividade vegetal (Saleem et al., 2013; Vivant et al., 2013).

Agroecossistemas frequentemente enfrentam problemas relacionados à patógenos de plantas que limitam a produção agrícola, como nematóides (*Pratylenchidae* e *Meloidogynidae*) e fungos (*Rhizoctonia* and *Verticillium*). O método mais comum para o controle de patógenos é o uso de pesticidas (nematicidas e fungicidas). No entanto, o uso desses compostos químicos pode provocar consequências negativas para o meio ambiente por possuírem um potencial efeito tóxico em organismos não-alvo e provocarem poluição ambiental (Oka, 2010). Por isso, há a necessidade de desenvolvimento de técnicas alternativas que possam ser usadas como meio de manter a produção agrícola sem o uso intensivo de compostos químicos. Métodos alternativos incluem o uso de compostos orgânicos (Blok et al., 2000), plantas de cobertura (Pudasaini et al., 2006), uso de resíduos de produtos (Widmer e Abawi, 2002), desinfestação anaeróbica (Mowlick et al., 2012), entre outros. Apesar desses métodos serem considerados sustentáveis, é esperado que sua utilização provoque modificações na comunidade microbiana e na meiofauna no solo (Cretoiu et al., 2014). Entender os efeitos dos métodos alternativas de controle de patógenos no microbioma, na meiofauna do solo e os mecanismos ecológicos envolvidos oferecem uma promissora oportunidade para melhorar a sustentabilidade dos agroecossistemas e aumentar a produtividade agrícola (Van Bruggen e Semenov, 2000; Lazarovits et al., 2014).

Espécies microbianas pertencentes à diferentes grupos taxonômicos apresentam significativa importância ecológica e agronômica, mas permanecem sem serem detectados ou identificados pelos métodos baseados em morfologia tradicionais (Lentendu et al., 2014). Para entender sua suas relações com outros membros da biota do solo em agroecossistemas, vários níveis tróficos microbianos devem ser avaliados simultaneamente (Luo et al., 2014). A

primeira geração de ferramentas moleculares, como técnicas de *fingerprint* (RFLP, RISA, DGGE, *Restriction Fragment Length Polymorphism*, *Ribosomal Intergenic Spacer analysis* e *Denaturing Gradient Gel Electrophoresis,* respectivamente) ou perfis de ácidos graxos (PLFA, do inglês *Phospholipid Fatty Acid)*, usadas para examinar mudanças na composição das comunidades microbiana monstraram diferenças estruturais entre os vários tipos de sistemas orgânicos e convencionais e diferenças pontuais entre tratamentos usados para o controle de patógenos foram observadas (Widmer e Abawi, 2002; Hartmann e Widmer, 2006). No entanto, recentes tecnologias de sequenciamento oferecem novas alternativas para explorar a microbiota do solo com níveis maiores de cobertura e resolução taxonômica, e tem um grande potencial de fornecer uma visão completa da comunidade, como também na identificação de grupos microbianos associados aos sistemas de manejo (van Agtmaal et al., 2015; Hartmann et al., 2015).

Existem poucos experimentos agrícolas de longo prazo comparando sistemas convencionais e orgânicos (Esperschütz et al., 2007) e faltam estudos relacionando métodos alternativos de controle de patógenos do solo manejados dentro desses sistemas. O Soil Health Experiment (SHE) representa um sistema agrícola controlado para acessar a resposta de longo prazo no microbioma, na meiofauna e na produtividade de plantas. Desde 1996, o SHE vêm sendo manejado de acordo com os sistemas convencional e orgânico; os sistemas foram divididos em componentes chamados Soil Health Treatments (SHTs, métodos alternativos para o controle de patógenos), que são: composto, quitina, *Tagetes*, g*rass-clover*, biofumigação, desinfestação anaeróbica do solo, controle físico, combinação (*Tagetes,* composto e quitina), e dois tratamentos controle (tratamento químico e testemunha). É um experimento único na literatura contemporânea com um desenho experimental factorial e parcelas experimentais repetidas, onde os mesmos SHTs, variedade de culturas e intensidades de fertilização são simultaneamente aplicadas sobre o mesmo tipo de solo - solo arenoso (Korthals et al., 2014). Desde 2006 até 2013, os SHTs foram aplicados no solo duas vezes (2006 e 2009) e as parcelas têm sido continuamente manejados de acordo com os sistemas agrícolas convencional e orgânico por mais de 8 anos.

Neste contexto, no estudo descrito nessa tese, a comunidade de bactérias, archaeas, fungos e protozoários do solo foi avaliada baseado nos marcadores ribossomais genes 16S e 18S por meio da tecnologia de sequenciamento *Ion Torrent* para examinar a resposta das comunidades microbianas, nematóides parasitas de plantas e de vida livre, e produtividade de

plantas aos sistemas agrícolas convencional e orgânico e aos tratamentos alternativos (SHTs) para controle de patógenos do solo. O estudo conduzido nessa tese foi divido em dois capítulos, cada um contendo hipóteses específicas. O **capítulo 1**, "***Response of soil microbial communities to long-term farming systems and soil health treatments for plant-pathogens control*** ", foi desenvolvido com base na comunidade de bactérias e arquaeas do solo, onde as seguintes hipóteses foram testadas: no nível dos sistemas agrícolas, (1) o sistema orgânico promove uma maior diversidade microbiana do que o sistema convencional; (2) a heterogeneidade da comunidade microbiana é maior no sistema orgânico do que no sistema convencional; (3) grupos microbianos copiotróficos dominam no sistema orgânico; no nível dos tratamentos, (4) existe um efeito de longo prazo dos tratamentos na diversidade e composição da comunidade microbiana, que podem estar relacionados à supressão de patógenos do solo. O **capítulo 2**, *"**Multitrophic responses to long-term farming systems"***, foi direcionado à comunidade de fungos e protozoários do solo, nematóides parasitas e de vida livre e produtividade de planta e suas relações ecológicas. Nesse capítulo, as seguintes hipóteses foram testadas: (1) sistema de agricultura orgânica tem um efeito positivo sobre a diversidade de comunidade microbioma; (2) o manejo orgânico aumenta a população de nematóides de vida livre e inibe a população de nematóides parasitas de plantas; e (3) componentes multitróficos mudam em concordância principalmente devido à respostas semelhantes aos sistemas agrícolas. Ao longo tempo, identificar microrganismos associados com sistemas agrícolas e monitorar suas relações ecológicas em um agroecossistema pode ser útil como um indicador de manejo sustentável e trazer uma nova visão sobre práticas agriculturas benéficas para a saúde do solo e produtividade de plantas.

Response of soil microbial communities to long-term farming systems and soil health treatments for plant-pathogens control*

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Running Head: Microbiome response to farming systems and soil health

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Keywords: Microbiome; Organic farming; Conventional farming; Agro-ecosystem; 16S rRNA; Bioindicator; Microbial Ecology

* Submitted to Applied and Environmental Microbiology

ABSTRACT

Organic farming systems and sustainable management of soil pathogens aim at reducing the use of agricultural chemicals in order to improve ecosystem health. Despite the important role of the microbial communities in agroecosystems, we still have limited understanding of the complex response of microbial diversity and composition to organic and conventional farming systems and to alternative methods for controlling plant pathogens. Here we report the structural response of the soil bacterial communities to organic and conventional farming systems and Soil Health Treatments (SHTs; sustainable alternatives for chemical control) in a long-term experiment using a high-throughput sequencing of microbial 16S ribosomal gene marker. Conventional and organic farming systems had major influence on soil microbial diversity and community composition while the effects of the soil health treatments were of smaller magnitude. Organically managed system increased taxonomic and phylogenetic richness, diversity and heterogeneity of the soil microbiota when compared with conventional farming system. Microbial communities proved to be sensitive to soil health treatments, evidencing the long-lasting effect on microbial composition, but not on diversity and heterogeneity. Soil health treatments were characterized by specific microbial taxa known to be involved with soil suppressiveness to pathogens (plant-parasitic nematodes and soil-borne fungi). Our results provide a comprehensive survey of the response of microbial communities to different agricultural systems and soil treatments for controlling plant pathogens and give novel insights to improve the sustainability of agro-ecosystems by means of beneficial microorganisms.

INTRODUCTION

Over the past decades, anthropogenic alteration of soils is causing unprecedented changes in biodiversity as species are driven to local and global extinction (Pimm et al., 1995, Barnosky et al., 2011). For terrestrial ecosystems, the dramatic declines of native species caused by the increased use of synthetic fertilizers, pesticides and land conversion to increase food production are altering the natural environmental conditions (Hole et al., 2005), leading to a growing concerning on the sustainability of intensive farming systems. The agriculture intensification has a substantial impact on plant and animal diversity (Gabriel et al., 2006, Jonason et al., 2011). However, the effects of agricultural management on below-ground diversity are poorly understood (Li et al., 2012). This lack of knowledge is a significant concerning because soil-borne microbes, especially bacteria, represent the majority of biodiversity in soil ecosystems and are involved in multiple ecosystem functions, including nutrient cycling and plant health (Mazzola, 2004, Wakelin et al., 2013).

The intensification of agriculture has led to search for conservation strategies. Converting conventional farms to organic farming system seems to be a potential solution to diminish the loss of biodiversity and increase sustainable food production (Gonthier et al., 2014). Organic farming system consists of low-input agroecosystem farms; plant productivity and ecosystem functionality are based on the natural availability of plant nutrients, use of green manure and biological pathogen control (Lammerts van Bueren et al., 2002). In contrast, conventional system relies on intensive use of agrochemicals, such as synthetic fertilizers to increase crop productivity and use of fungicides and pesticides to promote plant protection against pathogens (Kremen and Miles, 2012). Effects of farming systems on microbial communities are complex and time-dependent (Jonason et al., 2011). In general, it has been reported that management practices in organic farming systems change the microbial composition towards

a more copiotrophic community (Chaudhry et al., 2012), promote habitat diversification, increase the diversity and benefit microbial taxa involved in plant health when compared to conventional systems (Esperschütz et al., 2007, Sugiyama et al., 2010, Reilly et al., 2013). Although positive effects of organic management have been widely reported (Liu et al., 2007, Ge et al., 2008, Jonason et al., 2011, Hartmann et al., 2015), the effects of farming systems on diversity of microbial communities are complex and commonly controversial (Kleijn et al., 2001). Although an increase of diversity after manure amendment is frequently observed (Ge et al., 2008), other studies reported no differences or decrease of bacterial diversity and richness when organic systems were compared to conventional management (Sugiyama et al., 2010, Reilly et al., 2013). Bengtsson et al. (2005) argue that in most cases organic farming can be expected to benefit the biodiversity, but the effects will differ between taxonomic groups and landscapes.

Agroecosystems often face problems with plant-pathogens, such as parasitic nematodes (*e.g. Pratylenchidae* and *Meloidogynidae*), and soil-borne fungi (*e.g. Rhizoctonia solani* and *Verticillium dahliae*) affecting a large number of important crops (Back et al., 2002). A common method to control these pathogens is the use of chemical pesticides, which are under critical review due potential toxic effect on non-target organisms and environmental pollution (Oka, 2010). Therefore, the development of methods for suppression of pathogens as an alternative to chemical control is an urgent need. These methods can be applied in organic farming systems, but also enable conventional farmers to reduce the use of pesticides. Alternative approaches are organic amendments (compost) (Mehta et al., 2014), cover crops (*Asteraceae* plants) (Pudasaini et al., 2006), green manure crops (grass-clover) (Widmer and Abawi, 2002), composts or non-composted waste products (chitin) or those based on physical methods (biological soil disinfestation) (Mowlick et al., 2012). Although these management

practices are environmentally-friendly, it is expected to induce shifts on microbial diversity and composition (Mehta et al., 2014). At treatment level, the microbes play an important role in above- and below- ground processes, including their potential effects on soil suppressiveness (Cretoiu et al., 2013). In this light, the ability to understand and manage microbial community through alternative practices for pathogen control offer a promising approach to improve sustainable crop production (Postma et al., 2008).

The broad spectrum of agricultural managements and practices used for plant-pathogens control in farming systems limits comparability among different studies (Liu et al., 2007, Xue et al., 2012). Up to date, there are few long-term agroecosystems experiments comparing organic and conventional systems (Esperschütz et al., 2007), and seldom studies on these two farming systems on plant-pathogens control, what is the ultimately required for evaluating the sustainability of agricultural practices (Grünwald et al., 2000). The Soil Health Experiment (SHE) used in this study located at Wageningen University Research (WUR) station in Vredepeel, represents a system to access the response of microbial communities to a long-term conventional and organic farming systems. It is a unique experimental field reported in contemporary literature with full-factorial experimental design and replicated experimental plots, where the same soil treatments, crop varieties, crop rotations and fertilization intensities are simultaneously applied in both conventional and organic farming systems under the same sandy soil type (Korthals et al., 2014). The long-term research site was set up in 2006 by dividing conventional and organic systems into component parts, namely Soil Health Treatments (SHTs), which are: compost, chitin, marigold, grass-clover, biofumigation, anaerobic soil disinfestation, physical control, combination of marigold, compost and chitin, and two control treatments (chemical and untreated control). Since 2006 until 2013, SHTs were applied in soil two times (2006 and 2009) and the plots have been continuously managed

according to conventional and organic farming systems for more than 8 years. In a previous study, the SHTs were evaluated about the their potential effects on plant-parasitic nematode *Pratylenchus penetrans*, and soil-borne pathogenic fungus *V. dahliae* (Korthals et al., 2014). However**,** the responses of the soil microbial community to different managements and the potential role of microbial community in soil suppressiveness were not studied. In this context, we assessed the bacterial and archaeal communities based on 16S rRNA gene marker by next generation sequencing technology to examine the response of microbial communities to long-term conventional and organic farming systems and soil health treatments. For farming systems, we addressed the following hypotheses: (1) organic farming system promotes higher microbial diversity than conventional system; (2) the microbial community variability is higher in organic than in conventional farming system; (3) the copiotrophic taxa dominate the microbial community in organic system. For SHTs, we hypothesized that there is a legacy effects of the treatments on the diversity and composition of the microbial communities, which could be related with plant-pathogen suppression. Based on microbial community assessment, we aimed to detect specific structural shifts and identify microbial taxa associated with specific farming system or soil health treatments, which might be useful as a bioindicator of sustainable management of agroecosystems and might bring novel insight on soil beneficial agriculture practices for soil health and plant productivity.

MATERIAL AND METHODS

The Soil Health Experiment, experimental design and historical management. The Soil Health Experiment (SHE) is located at Wageningen Univeristy Research (WUR) station in Vredepeel, in the southeast of the Netherlands (51º 32’ 27.10” N and 5º 51’14.86” E). The site has been in agricultural cultivation since 1955, and has a mean annual air temperature of 10.2

ºC and mean annual precipitation of 766 mm. On SHE, all crops and alternative treatments for pathogen control applied were compared simultaneously on the same soil type (sandy soil; 1.1% clay, 3.7% silt and 94.9% fine sand) and in which conventional and organic farming systems differ only in fertilization and plant protection methods. In spring 2006, the experimental field was divided into 160 plots, each 6 m x 6 m and arranged in a randomized block design with four replicates. Within each block, two agricultural farming systems, conventional and organic, were randomized. Each year between 2006 and 2013, a crop was grown on the entire experimental field: 2006: Wheat (Conv) or barley (Org); 2007: potato (Conv, Org); 2008: lily (Conv, Org); 2009: Wheat (Conv) or barley (Org); 2010: potato (Conv, Org), 2011: carrot (Conv, Org), 2012: maize (Conv, Org), 2013: maize (Conv, Org). Both systems received approximately the same amount of nutrients per hectare and year according to fertilizer recommendations for the crops. The organic system exclusively received organic fertilizers, whereas conventional system was based in a fertilization scheme combining organic and mineral fertilizers. In April 2013, initial fertilization was carried out with cattle slurry. One month later, mineral fertilizers were applied in the conventional system, and farm yard manure was applied in 17th of April in the organic system (details on nutrients inputs for the conventional system are in Korthals et al. (2014)). In conventional system the plant protection was performed using herbicides, fungicides and insecticides according to the thresholds for each crop (following the rules of European Union). In the organic system, the mechanical weeding was performed. For a complete description of the experimental field and the main conclusion of the previous study, see Korthals et al. (2014).

Since the beginning of the experiment (2006), the soil health treatments were applied two times until 2013, the year in which the soil sampling for this study was performed. From the end of July 2006 till May 2007, nine different SHTs were applied: Compost (CO): 50t/ha

mature, certified compost (65% wood, 10% leaves and 25% grass and inoculated with *Trichoderma harzianum* provided by Orgapower) was incorporated in the 20 cm soil surface; Chitin (CH): 20 t/ha of chitin-rich material based on shrimp debris (Gembri provided by Ecoline) was incorporated in the 20 cm soil surface; Marigold (MA): *Tagetes patula* (cv. Ground Control, seed density of 10 kg/ha) was grown from July 2006 till January 2007 and then incorporated in the upper 0-20 cm soil layer; Grass-clover (GC): a mixture of four different rye-grass species (4 kg/ha cv. Tetraflorum, 7 kg/ha cv. Miracle, 2 kg/ha cv. Pomposo, 1 kg/ha cv. Tomaso) and two clover species (1 kg/ha cv. Riesling, 7 kg/ha cv. Maro) were grown from 27 July 2006 till 12 March 2007 and then incorporated in the 20 cm soil surface; Biofumigation (BF): biofumigation is a technique where plants containing glucosinolates (often *Brassicaceae*) are incorporated and degraded in the soil into plant metabolites (*i.e.*, isothiocyanates, nitriles and thiocyanates) with biocidal properties. In this study *Brassica juncea* (cv. Energy) was grown from 27 July till 20 September 2006, replenished with 117 t/ha Broccoli (cv. Montop) and then incorporated in the top 20 cm soil surface. Soil Anaerobic Disinfestation (AD): in August 2006, 50 t/ha fresh organic matter (a mixture of different rye-grass species) was incorporated in the top 20 cm soil surface, irrigated with 20 mm water per plot and covered with a virtually impermeable film (VIF) of plastic (0.035 mm thick HyTibarrier delivered by Hyplast); in November 2006 the plastic was removed; Physical Control (PH): the soil was treated with hot air (Cultivit), what is based on blowing extremely hot air (720-780 ºC) into rotavating humid soil; Combination (CB): three different treatments (MA, CO and CH) were subsequently combined on the same plot. Two control treatments were also applied: Chemical control (CC): on September 2006 the soil was treated with 300 L/ha Metam sodium (Monam 510 g a.i./L), applied with a rotary spading injector, a common technique allowed by the Dutch ministry. CC was only applied in conventional system;

Caliente (CL): a byproduct of mustard production (70 L/ha). This treatment was applied only in organic system for comparative purpose to CC in conventional system (no chemical inputs are allowed in organic system). Control treatment (CT): the soil was given no extra treatment and left fallow after wheat harvest till next growing season. The SHTs were applied for the second time from the end of July 2009 till December 2009 as described for 2006.

Soil sampling, DNA isolation and 16S rRNA gene sequencing. Soil sampling was performed in May 2013. Three soil cores (top-layer 0 to 10 cm) from each SHT plot were sampled and pooled to make a single composite sample, resulting in 60 independent samples. Soil sampling was performed in three blocks and in both conventional and organic systems during the initial stage of maize crop. This sampling scheme was chosen since it reflects the long-term effects of conventional and organic farming systems and the legacy effects of SHTs on microbial communities. Samples were stored at -80 ºC until DNA isolation process. From each sample 2g of soil was used for total DNA isolation using the DNA PowerSoil kit (MoBio laboratories, Inc.) and the yield and quality were determined using NanoDrop 1000 spectrophotometer (Thermo scientific, USA). Bacterial and Archaeal communities were determined based on the hypervariable region V4 of 16S rRNA gene using the primers 515F/806R (Caporaso et al., 2012) designed for Ion Torrent™ semiconductor technology. The barcodes of 8 bases were added to primer 515F and unidirectional sequencing was performed from the A-key adapter. A two-base linker sequence was inserted between the adapter and primers to reduce the possible effects of composite primer on PCR amplification. A 25 uL reaction was prepared containing 5 uL *Taq* FastStart High Fidelity Enzyme Blend, 10x FastStart High Fidelity Buffer with 18 mM $MgCl_2$ (Roche Diagnostics Ltd., Burgess Hill, UK), 0.2 mM of each dNTP (Promega UK Ltd. Southampton, UK) with each primer used at

0.1 M. For each reaction 1 uL of DNA template was used. The conditions used were a hot start of 95 ºC for 5 min, followed by 35 cycles of 95 ºC for 30 s, 50 ºC for 30 s and 72 ºC for 1 min with a final extension at 72 ºC for 10 min. The PCR reactions were conducted in triplicate. Reactions were amplified in a C1000 Touch thermal cycler (Bio-Rad, Hemel Hempstead, UK). Resultant amplicons were visualized on a 1% (w/v) TBE agarose gel to assess quality of amplification before pooling the triplicate reactions. The PCR pooled samples were recovered from agarose gel and purified using a QIAquick gel extraction kit (Qiagen). The purified samples were quantified with Quant-iT Broad-Range DNA Assay Kit (Invitrogen) in conjunction with the BioTek Synergy HT microplate reader and combined in equimolar ratios. The template preparation of 16S rRNA gene library was performed using Ion OneTouch 2 System and Ion PGM Template OT2 400 Kit, and sequenced using Ion PGM Sequencing 400 on Ion PGM System using Ion 318 Chip v2.

Sequence data processing. The 16S rRNA partial gene reads were analyzed using MOTHUR version 1.33.2 (Schloss et al., 2009) combined with workflow engines Snakemake (Koster and Rahmann, 2012). Briefly, to reduce sequencing errors, multiplexed reads were filtered for quality and assigned to samples by matching to barcode sequences. Reads were trimmed including 1 mismatch to the barcode and 2 mismatches to the primer, 8 maximum homopolymer, minimum length of 250 bp, maximun length of 290 and quality score >25. After trimming, the sequences were aligned using the Silva template (Quast et al., 2013), preclustered and potentially chimeric sequences were removed using the UCHIME (Edgar et al., 2011). Sequences were classified using Silva rRNA database (release SSU_Ref_119) with a confidence threshold of 80% (Quast et al., 2013) and sequences classified as chloroplasts and mitochondria were removed. To build an Operational Taxonomic Unit (OTU) table of

each sample and taxonomic assignments for each OTU from 16S rRNA gene, a distance matrix was calculated and sequences obtained were clustered with average neighbor algorithm at a 0.03 dissimilarity threshold. Details about the commands used for sequence processing are available on https://gitlab.bioinf.nioo.knaw.nl/amplicon-metagenomics/iontorrent-vsearch/commits/vredepeel/. The sequences are available at the European Nucleotide Archive (ENA) (https://www.ebi.ac.uk/ena/data/view/PRJEB10907) under the study Accession no. PRJEB10907 (ERP012206).

Statistical analysis

Coverage and taxonomic composition. The biom file created on MOTHUR was imported to R (R Core Team, 2008) using "phyloseq" package (McMurdie and Holmes, 2013). To estimate limited sampling relates to entire sampled population, a Good's coverage estimator was calculated at 97% similarity cutoff (Good, 1953). Microbial communities at phyla level were compared using two-way ANOVA after plotting the residuals and confirming the normality of the data by Shapiro-Wilk W test ($P > 0.05$) using *shapiro.test* or by Kolmogorov-Smirnov test ($P < 0.05$) using *ks.test*, both tests in "stats" package. Non-normally distributed data were transformed using Box-Cox using *boxcox* function in "MASS" package (Venables and Ripley, 2002) or square root transformation using *sqrt* in "base" package (R Core Team, 2008). When the differences were significant, they were further analyzed using a post-hoc test by the *HSD.test* (pairwise comparison between treatments, *i.e.,* more than two groups) in "agricolae" package (40) and *pairwise.t.test* (pairwise comparison between systems, *i.e.,* two groups) in "stats" package. A heatmap was used to visualize the differences in abundance using *heatmap.2* in "gplots" package (Warnes et al., 2015).

Alpha-diversity. For the estimation of alpha diversity and richness, the data set was rarefied to 1,691 sequences per sample and three different approaches were employed: (a) community richness was calculated by Observed OTU and ACE estimator, (b) compositional diversity was assessed by applying the exponential of Shannon diversity index ($e^{H'}/S$; (Hill et al., 2003)) considering the number and abundance of species using *estimate_richness* function in "phyloseq" package; and (c) phylogenetic diversity was calculated by Faith's phylogenetic diversity index (Faith's PD, (Faith, 1992)) incorporating phylogenetic *distances* between species (*pd* function in "picante" package (Kembel et al., 2010). The diversity index was analyzed using two-way analysis of variance (ANOVA) after plotting the residuals and confirming the normality of the data using the Shapiro-Wilk W test. When the differences were significant, they were further analyzed using a post-hoc *pairwise.t.test* in "stats" package.

Community variability (beta-diversity). For further analyses, OTUs with less than 10 sequences were removed. To assess community variability, the absolute number of sequences was transformed to relative abundance and the permuted analysis of betadispersion of pairwise Bray-Curtis and unweighted UniFrac similarities using function *betadisper* in "vegan" package (Anderson et al., 2006, Oksanen, 2013). The permutation-based hypothesis tests for differences in dispersion of each sample to the group centroid and then tested for differences in these distances between groups. The pairwise comparisons of group mean dispersion were performed by t-test using *permutest* in "vegan" package. To visualize significant results, we explored the dissimilarities based on the distance to the centroids determined from the mean positions of the respective samples of conventional and organic systems and plotted in a boxplot.

Identification of strict habitat specialists. As higher taxonomic levels provide little information to infer the ecological preferences of microbial taxa, we decided to identify the *strict habitat specialists* based on OTU level. To test whether a single OTU was associated with farming systems (conventional or organic) or soil health treatments, representing habitat types within farming systems, we conducted a species indicator analysis with the *multipatt* function in "indicspecies" package (de Caceres and Legendre, 2009) in R. This analysis identifies *habitat specialists* based on OTU fidelity (the degree to which an OTU is present at all sites of a defined sample group or habitat) and specificity (the degree to which an OTU is found only in a given sample group or habitat) (Legendre and Legendre, 1998). Because low abundance of individual OTU is prone to erroneously indicate a taxa as *strict habitat specialist* (Pandit et al., 2009), we used the same previous data set where OTUs with less than 10 sequences were excluded. Further, a randomized strategy (permutation) was applied to test the probability that an association between an OTU and a habitat (that is, farming system or SHT) was not at random. The statistical significance was tested using 999 permutations. A circular maximum likelihood phylogenetic tree was constructed based on representative sequences for each OTU selected as *strict habitat specialists* between farming systems (conventional x organic) and among SHTs within farming systems (that is, SHT within Conventional and Organic). The phylogenetic tree was constructed using a distance matrix with MUSCLE algorithm (Edgar, 2004) available in QIIME and displayed using iTOL (Letunic and Bork, 2006).

RESULTS

Number of 16S rRNA sequences and coverage

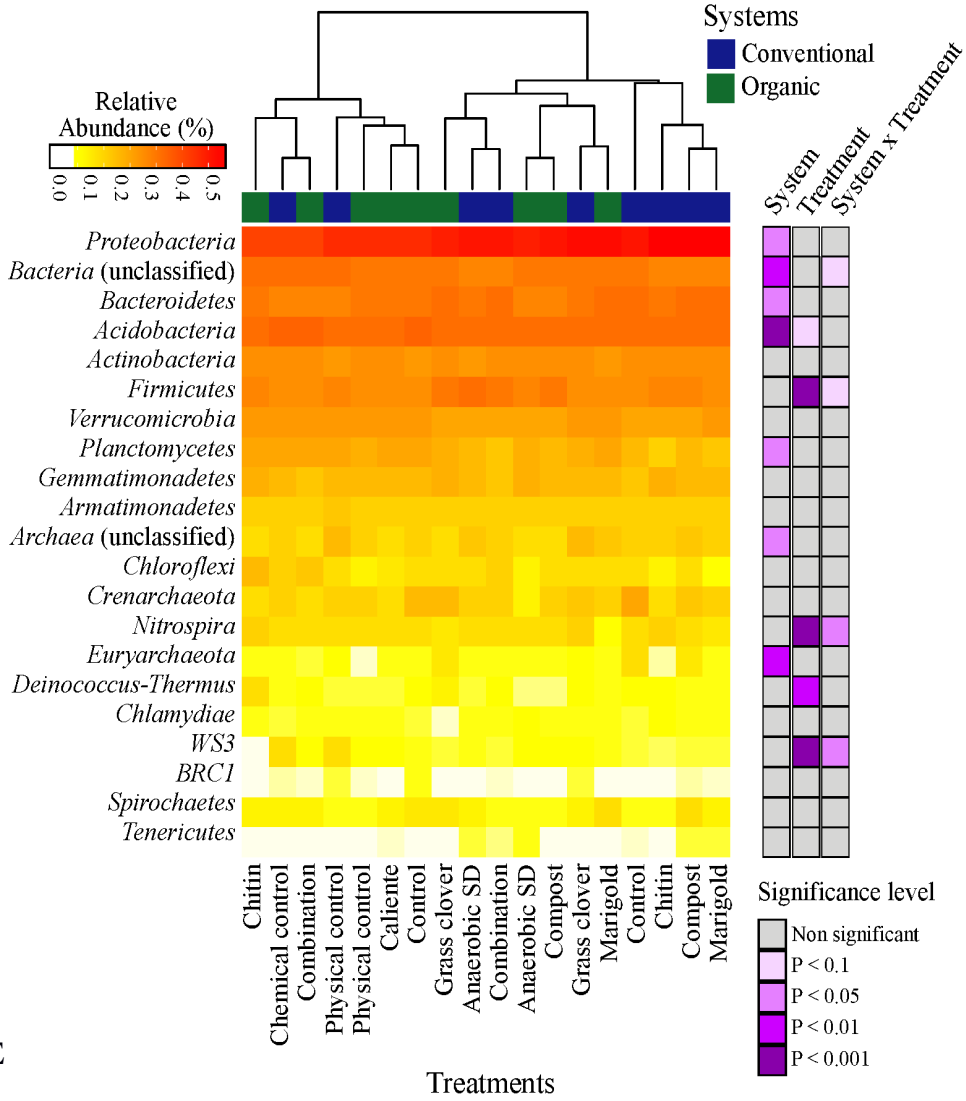
Microbial communities were assessed by sequencing 16S rRNA gene sequences from a long-term experiment with eight Soil Health Treatments (SHTs) under conventional and organic farming systems. After quality filtering, a total of 625,298 sequences were obtained from 56 samples with an average of 11,579 sequences (minimum length of 250 bp, maximum length of 290 and quality score >25) (Table S1 in Supplemental material 1). Biofumigation treatment was not considered because less than 300 sequences were recovered per sample (Table S1). A total of 3,507 OTUs (more than 10 sequences/sample) were obtained using a 97% identity cut-off. According to Good's coverage estimator, more than 80% (80-93%) of the OTUs in most of the samples, 77% in one replicate of control organic treatment and 79% in one replicate of caliente treatment in organic system were captured (Table S1).

Effects of farming system and SHT on taxonomic phylum composition

The taxonomic composition of different farming systems and SHTs summarized at phyla level is shown in Fig. 1. Overall, a total of 19 phyla (*Archaea* and *Bacteria* domains), 54 classes, 74 orders, 140 families and 230 genera were found within the soil samples. The complete list of all detected bacteria taxa (from Phylum to OTU level) is shown in Supplemental material 2 (It will became available on online verion of this study). Overall, irrespectively of systems or treatments, bacterial communities were dominated by *Proteobacteria* (33.8%), *Bacteroidetes* (11.4%), *Acidobacteria* (9.55%), *Actinobacteria* (5.8%), *Firmicutes* (4.3%), *Verrucomicrobia* (2.9%), *Planctomycetes* (2.4%), *Gemmatimonadetes* (1.4%) and *Armatimonadetes* (1.1%). Other phyla were represented by a

relative abundance less than 1%. A fraction of 24.9% and 0.8% of the 16S rRNA reads remained as unclassified at phylum level for Bacteria and Archaea domains, respectively. The relative abundance of each phylum is shown in Fig. 1. from highest to lowest abundances.

The abundances of most bacterial phyla were not statistically different between systems, treatments or the interaction 'system x treatment' (Fig. 1; Table S2). Only *Proteobacteria* (ANOVA, $P < 0.05$), *Euryarchaeota* ($P < 0.01$), *Acidobacteria* ($P < 0.001$) and *Planctomycetes* ($P < 0.05$) were significantly affected by farming systems (Fig. 1; Table S2). The relative abundances of *Proteobacteria* (t-test, $P < 0.1$) and *Euryarchaeota* (t-test, $P < 0.05$) were higher in conventional system, while the abundances of *Acidobacteria* (t-test, $P < 0.05$) and *Planctomycetes* (t-test, $P < 0.05$) increased in organic system. *Firmicutes*, *Nitrospira* and *WS3* showed no farming system effect, but *Firmicutes* and *Nitrospira* were more frequent in Anaerobic soil disinfestation and *WS3* was more frequent in physical control, both of them in conventional system (Table S2). The effects of farming systems on *Bacteroidetes* ($P < 0.05$) and of treatments on *Deinococcus-Thermus* ($P < 0.01$) were statistically supported by ANOVA, but not by the pairwise comparison. The interaction 'system x treatment' on relative abundances of Bacterial unclassified, *Nitrospira* and *WS3* was statistically significant and supported by ANOVA ($P < 0.1$, $P < 0.05$). *Actinobacteria*, *Verrucomicrobia*, *Gemmatimonadetes, Armatimonadetes*, *Crenarchaeota*, *Chloroflexi*, *BRC1, Spirochaetes* and *Tenericutes* abundances were not affect by farming systems, treatments nor 'system x treatment' interaction ($P > 0.1$).



FIGURE

1: Heatmap of the response of bacterial community structure at phyla level to farming systems (organic and conventional farming) and Soil Health Treatments (SHTs; AD: anaerobic soil disinfestation, CC: chemical control, CL: caliente, CH: chitin, CB: combination, CO: compost, CT: control treatment, GC: grass-clover, MA: Marigold, PH: physical control). The left panel show the significance levels (ANOVA test) for systems, SHTs or the interaction between farming systems and SHTs.

Farming systems and SHTs effects on α-diversity

To investigate changes in microbial diversity in different farming systems and soil treatments, we used taxonomic and phylogenetic metrics approaches. The farming system was a significant driver of microbial taxonomic and phylogenetic α-diversities (ANOVA; Observed OTU and Shannon, $P < 0.001$; Faith's PD, $P < 0.05$). The α-diversity of microbial community in organic system was significantly higher than in conventional system (Fig. 2). This result was true for taxonomic observed richness (Observed OTU; 798.5 for organic *vs.* 754 for conventional, t-test, $P < 0.001$), taxonomic diversity (Shannon; 6.0 in organic *vs.* 5.8 in conventional, t-test, $P < 0.001$) and phylogenetic diversity (Faith's PD; 59.3 in organic *vs.* 55.2 in conventional, t-test, $P < 0.05$). The system effect on α-diversity of bacterial community based on ACE estimator was statistically less robust (ANOVA; $P < 0.1$), but a significant pairwise comparison was detected (2250.5 in organic *vs.* 2121.0 in conventional, t-test, $P < 0.05$). In contrast to the significant effects of farming system, differences in α-diversity among treatments and the interaction 'system x treatment' were small and not significant ($P > 0.1$).

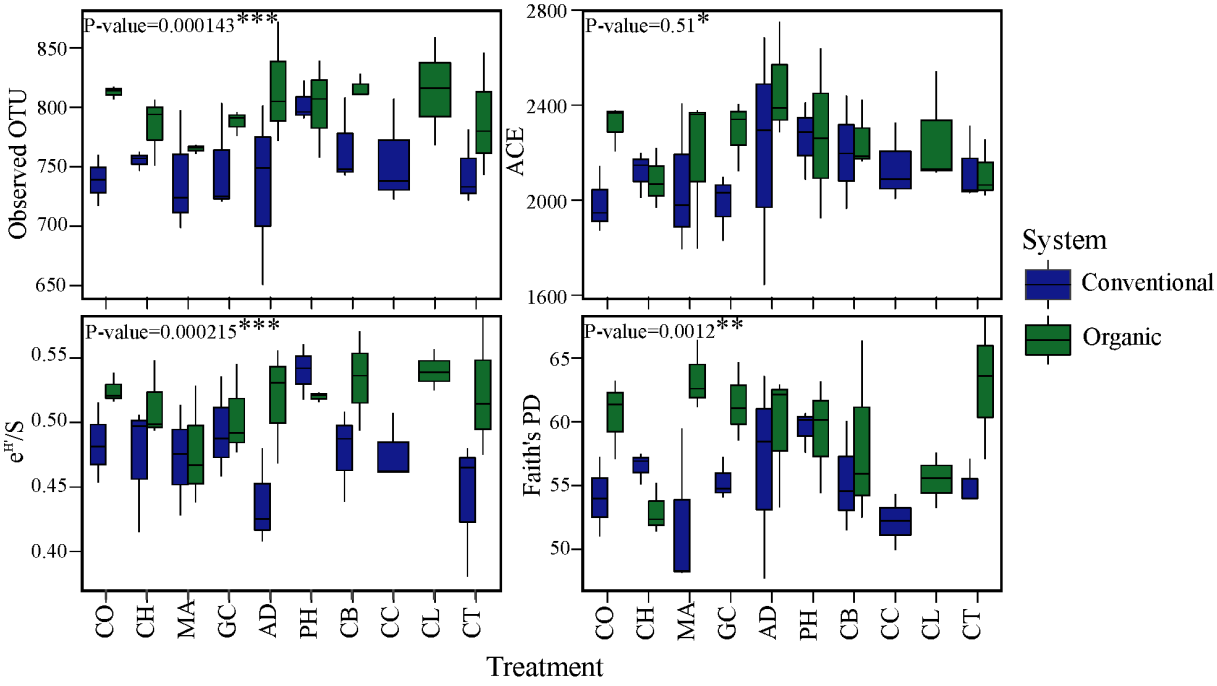


FIGURE 2: Effects of farming systems and Soil Health Treatments (SHTs; AD: anaerobic soil disinfestation, CC: chemical control, CL: caliente, CH: chitin, CB: combination, CO: compost, CT: control treatment, GC: grass-clover, MA: Marigold, PH: physical control) on bacterial community α-diversities. On the boxplots, the center lines show the medians, the bottom and upper limits indicates the 25th and 75th percentiles and the whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. The values for each diversity index are showed on y-axis and STHs on x-axis. The significance of the effect of farming systems based on two-way ANOVA on α-diversities are represented by *** ($P < 0.001$), ** ($P < 0.05$) and * ($P < 0.1$).

Community variability and farming systems

To determine whether microbial community variability (estimated by beta-diversity based on taxonomic and phylogenetic dispersions) were altered by farming systems and/or by SHTs, we used Bray-Curtis and unweighted UniFrac metric associated with permutest and pairwise comparison. The farming system was a significant driver of microbial taxonomic and

phylogenetic variabilities (Fig. 3 and Fig. S1), but no significant effects in community dispersion were observed among the treatments within organic and conventional farming systems ($p > 0.1$) (data not shown). The organic farming system had higher effect on community variability than conventional farming, with higher effect on phylogenetic (permutest, $F = 24.4$, $P < 0.001$) than on taxonomic dispersion (permutest; $F = 3.3$, $P > 0.05$) (Fig. 3 and Fig. S1).

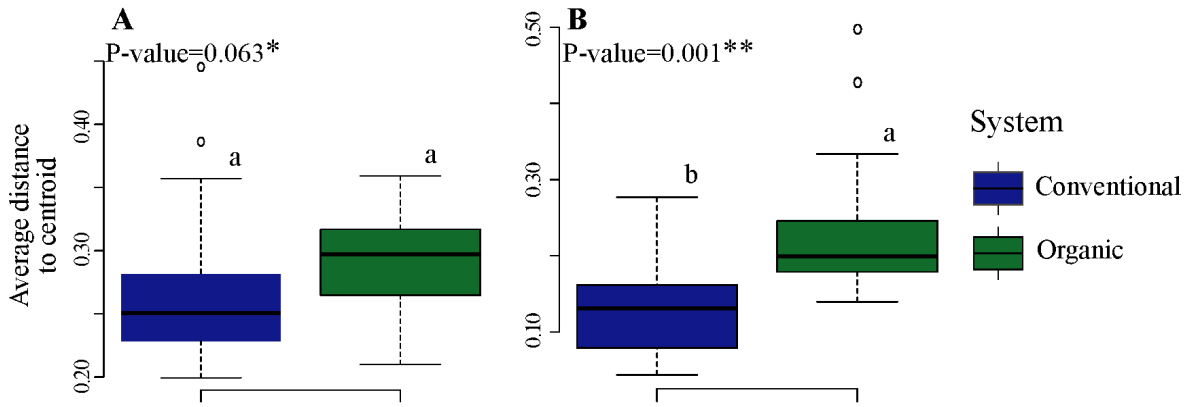


FIGURE 3: Taxonomic (A) and phylogenetic (B) variability in bacterial community structure (assessed by analysis of beta-diversity, a metric of variability based on Bray-Curtis and unweighted UniFrac, respectively) in conventional and organic farming systems. Because the soil health treatments (SHTs) did not show significant effect on community variability ($P > 0.1$), the samples from SHTs were pooled to represent each farming system and the result of beta dispersion was summarized to show only the effects of farming systems. On the boxplots, the center lines show the medians, the bottom and upper limits indicates the 25th and 75th percentiles and the whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Different letters on each box represent significant differences in variance homogeneity between farming systems as determined by HDS-test (* $P<0.1$ and ** $P<0.1$).

Habitat specialist taxa of farming system and SHT

In order to find the legacy effects of either farming system or SHT, we carried out an indicator species analysis at OTU level, which identify potential *strict habit specialists* for habitat. The indicator species is based on the relative frequency and relative average abundance, identify a given OTU that tend to be present mostly in a single habitat type (that is, only in one farming system or SHT) and in most of the samples from that habitat, suggesting the preference for a given environmental condition. For every OTU identified as specialist, the information on relative abundance of OTUs in each treatment group and taxonomic classification are provided in Supplemental material 2. Most of the OTUs did not show significant differences in relative abundance and frequency (that is, potential specialist behavior) between either farming systems or SHTs, but we detected 1,001 OTUs strict specialists to farming systems or to SHTs (*multipatt*; the significance levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered), representing 28.5% of the total OTU data set (3,507 OTUs). The taxonomic dendrograms (Fig. 4; Fig. S2) illustrate the associations between OTUs and the farming systems and between OTUs and SHTs. Among 1,001 OTUs identified as habitat specialists, 836 OTUs (83.4%) were associated with conventional system (Fig. S2), 48 OTUs (4.8%) with organic system, 92 OTUs (9.2%) with a specific SHT and 25 OTUs (2.5%) with either farming system or SHT (Fig. 4). The OTUs associated to farming systems or SHTs were broadly distributed across the phylogenetic tree with no deep or shallow taxonomic clades responding regularly to a specific management (Fig. S2; Fig. 4). However, abundant phyla *Proteobacteria* and *Acidobacteria* showed an accumulation of these habitat specialist OTUs. Notably, the Anaerobic soil disinfestation treatment constituted a contrast to the heterogeneous distributions of the taxonomic clades across soil treatments. On this

treatment, habitat specific OTUs belonging to *Bacillales* and *Clostridialles* (*Firmicutes*) dominated the community (Fig. 4).

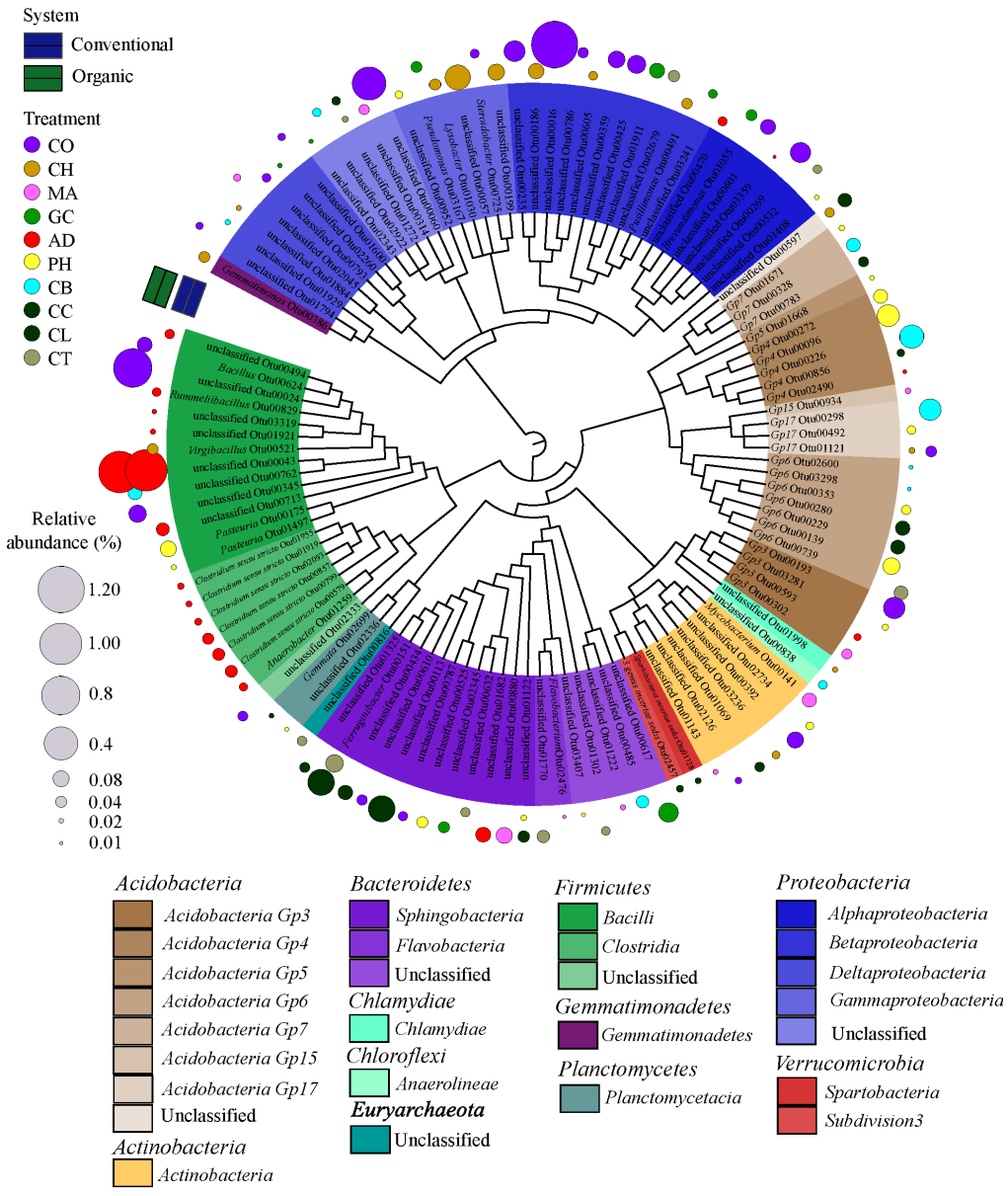


FIGURE 4: Dendrogram showing the the habitat specialists and taxonomy information (at genus level) associated with soil health treatments (SHTs; AD: anaerobic soil disinfestation, CC: chemical control, CL: caliente, CH: chitin, CB: combination, CO: compost, CT: control treatment, GC: grass-clover, MA: Marigold, PH: physical control). Only the strict specialist

OTUs - cut-off 97% - (9.2% of the total OTU data) with statistical significance of the association were considered. The taxonomic affiliation at class level of each specialist OTU is identified by the colors range in the below panel and within the tree. The habits preference for a given OTU is indicated in circles outside of the tree. The SHTs within farming systems (conventional is represented by blue and organic by green colors). Detailed information on abundance of each OTU is provided in supplemental material 2.

DISCUSSION

The Soil Health Experiment (SHE) represents a unique experiment to assess the response of microbial communities to a long-term farming systems (conventional and organic) and Soil Health Treatments (SHTs). This study was limited to temporal sampling (single time point) and spatial extent (local scale) and should not be generalized for the farming systems performed in all ecosystems. Although the consistent results in this study provide novel ecological insights into microbial ecology in agro-ecosystems, concrete conclusions is still difficult and need to be confirmed by long-term experiments over distinct environmental conditions, management practices and larger geographic scales. Besides, the complexity of microbial communities and the technical constraints so far limited our understanding of the relationship between soil microbiota and agricultural managements. However, using novel approaches based on high-throughput sequencing, we could explore the soil microorganisms at higher coverage and resolution. This approach enable us to identify potential microbial groups and individual microbial taxon associated with specific management practices.

Regarding the diversity of the soil microbial community, our hypothesis on the increase of microbial diversity in organic farming system as compared to conventional farming system

was supported. It is difficult to draw robust and generalized conclusions on the effect of systems management on microbial diversity, but an increase in microbial diversity has been repeatedly observed in organic in comparison with conventional system (Maeder et al., 2002, Hartmann et al., 2015). The increase of microbial diversity in organic systems is strongly associated with the management applied, including the organic amendments and practices related with reduction or absence of chemical inputs and biological plant protection (Sun et al., 2004, Chaudhry et al., 2012). The enhancement of microbial diversity also benefits the functional activities and an even distribution of species within the microbial assemblages, which implies in a stable and functional redundant community leading to an ecosystem functionality built around healthier interactions between microbial-microbial and microbial-soil-plant (Brussaard et al., 2007, Postma et al., 2008, Crowder et al., 2010, Wagg et al., 2014). The decrease of microbial diversity in the conventional system may be explained by the direct or indirect long-term stresses caused by the use of pesticides, fungicides and herbicides used for plant protection. These agrochemicals reduce the total microbial diversity because of the potential to inhibit or eliminate certain groups of microbes and select members adapted or able to growth under conventional farming practices (el Fantroussi et al., 1999, Liu et al., 2007, Stagnari et al., 2014).

Our study revealed consistent farming system effects on microbial community variability, suggesting a more heterogeneous community in organic than conventional system. We suggest that the availability of rich substrate in soil through the introduction of cattle farm yard manure, the biological practices without the interference of synthetic compounds and the presence of weed species provide heterogeneous habitat niches, which can be occupied by a highly variable microbial community leading to an increase of the beta-diversity (Wander et al., 1995). The lower heterogeneity (that is, the lower beta diversity) in microbial community

in conventional system is an indication of biotic homogenization, the process of increasing similarity in the composition of communities across an array of taxonomic or functional groups (Olden et al., 2004). Biotic homogenization is a common pattern of the above-ground community in conventional systems (Gabriel et al., 2006), and recently was reported for microbial communities as a response to long-term cultivation (Montecchia et al., 2015). When poor agricultural practices are applied, such as uniformly crop monocultures, fertilization and intensive use of agrochemicals, the chain-reaction of (bio)diversity loss reduce the ecological niches leading to a homogenization of the microbial community and their functional gene pool, altering the ecosystem functioning and reducing the ecosystem resilience (Olden et al., 2004, Constancias et al., 2013, Guan et al., 2013, Figuerola et al., 2014).

Besides the effects of farming systems on microbial community, we hypothesized that there is a legacy effects of the SHTs on diversity. It is expected that the differences between SHTs (*e.g.* organic matter composition, C/N, physical disturbances) may alter the physical, chemical and biological properties of the soil with consequent shifts in microbial diversity (Jacquiod et al., 2013). However, this study does not support evidence for the occurrence of long-term effects of SHTs on microbial diversity and richness. The first possible explanation is that different SHTs affects microbial diversity only in short-term and this effect may not be observed three years after the last application of the different treatments in this study (Sarathchandra et al., 1996). Some studies suggest a strong and fast resilience of the microbial diversity after a pronounced disturbance on soil community caused by management practices (Ding et al., 2014, van Agtmaal et al., 2015). Second, the continuous long-term farming system can counteract the effects of the soil health treatments, which were applied only twice. It has been suggested that long-term management practices are more likely to greatly influence the microbial community than temporal disturbances in soil (Buckley and Schmidt,

2003). Finally, we believe that the legacy effect of the SHTs occurs in specific microbial groups and cannot be resolved by determining the diversity and heterogeneity of entire microbial community, because shifts in some groups might be compensated by shifts in others (Hartmann and Widmer, 2006).

It has been proposed that due to larger availability of organic carbon and nitrogen, organic system should favor copiotrophic bacteria, while oligotrophic should predominate in conventional systems, where the organic carbon quality is low (Ding et al., 2014, Hartmann et al., 2015). In this study, we observed that the differences in the structure of microbial communities between conventional and organic farming systems were mainly related to a large fraction of habitat specialist OTUs broadly dispersed across the phylogenetic groups belonging to almost all phyla found in soil. Only few taxonomic groups revealed to responding more uniformly to farming systems. For example, most of habitat specialists assigned to *Proteobacteria* and *Euryarchaeota* were associated with conventional system and an increase of *Acidobacteria* and *Planctomycetes* was detected in organic system. These findings are not necessarily surprising, but are an opposite trend towards the copiotrophic-oligotrophic categories expected. However, the rather dispersed OTU association between conventional and organic systems within these taxonomic groups are in agreement with the contrasting behavior of individual members within phyla reported previously (Rousk et al., 2010). Not all members in a taxonomic clade demonstrate the same ecological characteristics, implying that the general lifestyle classification might not be applied for all members in a phylum (Fierer et al., 2007, Navarrete et al., 2013), and responses to management will occur at lower taxonomic levels rather than at major groups (Hartmann et al., 2015). *Proteobacteria* have been suggested to be a primarily copiotrophic phylum in soil (Li et al., 2012), while the lifestyle of microbial groups belonging to *Euryarchaeota,* which are predominately

methanogens, are largely unknown (Angel et al., 2011). However, the increased abundance of taxa belonging to these two Phyla in conventional farming system may be promoted by the input of fertilizers, which create copiotrophic environment in nutrient-rich microhabitats and stimulate plant growth, enhancing carbon availability and favoring the growth rate of members of these phyla (Fierer et al., 2011, Fröhlich-Nowoisky et al., 2014). Members of *Acidobacteria* and *Planctomyces* have been suggested to be adapted to nutrient-poor soils, and the input of organic amendments is expected to inhibit their activity (Jenkins et al., 2002, Fierer et al., 2007, Chaudhry et al., 2012). However, *Acidobacteria* and *Planctomyces* are involved in the turnover of soil organic carbon and nutrient availability, pointing out that the manure addition in soil might promote the proliferation of these groups (Buckley et al., 2006, Ng et al., 2012, Rawat et al., 2012).

Microbial communities proved to be sensitive to soil health treatments, supporting the hypothesis that there is a long-lasting effect of SHTs on soil microbial composition. This is an important finding, because microbial taxa strongly associated with management practices may help to elucidate the process behind soil suppressiveness (Hartmann et al., 2015). In previous study in the SHE (Korthals et al., 2014), the SHTs were evaluated within conventional system on the potential effects on plant-parasitic nematode *P. penetrans* and soil-borne fungi *V. dahliae*. The combination, chitin, anaerobic soil disinfestation and marigold treatments were more effective in controlling *P. penetrans* and *V. dahliae* when compared with chemical control. In contrast, grass-clover, biofumigation, cultivit and compost were not effective alternatives. However, in that study, the bacterial community was not assessed. In this study, we revealed several taxa associated with SHTs distributed among major taxonomic groups, for which we have little or no information about the ecological roles. Therefore, we can only speculate the ecological importance of the detected taxa based on what has been described in

previous studies and compare with findings on pathogen control (Korthals et al., 2014). A complete description of the results is beyond the scope of this study and we only focus on some consistent findings and their potential as soil microbe indicators for sustainable practices.

In anaerobic soil disinfestation treatment most of habitat specific OTUs were represented by taxa belonging to *Bacillales* and *Clostridialles* (*Firmicutes*), whose dominance is linked to their spore-forming capability, a competitive advantage under anaerobic conditions. Members belonging to family *Bacillales* have been described to be responsible for suppression of soil-borne disease-causing fungi (*Verticillium*, *Rhizoctonia* and *Fusarium*) and plant-parasitic nematodes (*Meloidogyne* and *Pratylenchus*) through production of antimicrobial compounds, pore-forming toxins (crystal proteins) and plant resistance induction (van Loon et al., 1998, Wei et al., 2003, Mowlick et al., 2012). Thus, this treatment selected *Firmicutes* taxa that might be involved in suppression of fungi and nematodes. In addition, habitat specific OTUs belonging to phylum *Nitrospira*, nitrite-oxidizing bacteria, were also associated with this treatment. This may be an indication of previous accumulation of ammonia (NH_3) and production of nitrite (NO_2), both nitrogenous compounds released due to decomposing of organic material known to play an important role in the suppression of fungi and nematodes (Tenuta and Lazarovits, 2002, Oka, 2010).

The genus *Lysobacter*, chitinolytic bacteria, was found to be associated with chitin treatment and have been described to have an important role in soil suppressiveness, with a potential antagonistic property against *Rhizoctonia* and nematodes plant pathogens (Nour et al., 2003, Postma et al., 2008). The genus *Virgibacillus,* another chitinolytic bacteria (Essghaier et al., 2011, Cretoiu et al., 2014), was also found to be associated with chitin treatment, but its role in soil suppressiveness is not described yet. Chitin is a major component

of nematode egg shells and cell wall of most plant-pathogenic fungi (Cretoiu et al., 2014), and it is assumed that chitin amendments increase the number of chitinolytic microorganisms and chitinase activity, which in turn suppress nematodes and fungi (Oka, 2010). Members of *Flavobacteriales* and *Chitinophagaceae* associated with marigold may also suppress soil nematodes by their chitinase activity (Tian et al., 2007, Glavina Del Rio et al., 2010, Kharade and McBride, 2013), suggesting that besides its ability to produce nematicidal compounds, marigold can also recruit nematode-antagonistic microorganisms (Hooks et al., 2010).

The previous taxa described to be associated with treatments with potential suppressive effects were also detected in compost, grass-clover and cultivit treatments, which were not effective alternatives against nematodes and fungi in SHE (Korthals et al., 2014). The potential plant pathogens antagonists *Pasteuria*, *Pseudomonas* and *Burkholderiales* were associated with cultivit and grass-clover treatments. Bacterial taxa belonging to these groups have been described to be potential against plant-parasitic nematodes and fungi (Tian et al., 2007). However, our results suggest that multiple mechanisms may accounted for an effective soil suppressiveness and the simple presence of taxa with antagonistic behavior against plant pathogens is not a sufficient proof for successful suppression of a pathogen in soil (Weller et al., 2002). Thereafter, the alternative methods to control plant pathogens require more detailed studies in combination with molecular and traditional approaches used in plant pathology and microbiology.

Altogether our results indicated that conventional and organic farming systems had a major influence on soil diversity and composition of microbial communities while the effects of the soil health treatments were of smaller magnitude. Organic farming system promoted beneficial effects on biotic aspects regarding to microbial diversities, richness and community heterogeneity. However, the response of microbial community to farming systems is diverse

and complex, and simple conclusions like “organic systems increased the soil biodiversity” may not be directly synonymous with concomitant increase in soil health and plant productivity. Furthermore, impact of the diversity losses in conventional system is not yet known; it is not clear how microbial diversity is related to ecosystem function and whether the changes in diversity we observed are reversible and the long-term consequences remain to be unexplored. Moreover, we detected that there is a long-lasting legacy of the SHT which selects for treatment-specific microbial members that are consistent with the existing knowledge, but the limited phylogenetic and functional information precludes more definite conclusions about the beneficial impact of individual taxonomic groups with soil suppressiveness. However, the observed shifts in microbial diversity, community structure and individual taxon bring novel insights into the potential of managing the microbial community for sustainable agricultural productivity.

ACKNOWLEDGEMENTS

The authors acknowledge Johnny Visser and colleagues of Vredepeel experimental farm, Leonardo Pitombo for the soil sampling, Agata Pijl and Victor Carrion for laboratory assistance. This study was funded by BE-BASIC Foundation (F08.002.05) and The Netherlands Organization for Scientific Research (NWO) and FAPESP grant number 729.004.003. The scholarship to the first author was granted by FAPERGS/CAPES (Research Agency of the State of Rio Grande do Sul/Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil). Publication number xxxxx of the Netherlands Institute of Ecology, NIOO-KNAW.

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SUPPLEMENTAL MATERIAL 1

Table S1. Sample ID, Soil Health Treatments (SHTs) in conventional and organic systems, number of 16S rRNA sequences and Good's coverage used in this study.

SampleID	SHT	System	Number of sequences*	Good's coverage**
Sample74	Anaerobic-soil-disinfestation	Conventional	11673	0.87
Sample104	Anaerobic-soil-disinfestation	Conventional	18377	0.92
Sample15	Anaerobic-soil-disinfestation	Conventional	19021	0.90
Sample109	Chemical-control	Conventional	7532	0.86
Sample73	Chemical-control	Conventional	11911	0.88
Sample13	Chemical-control	Conventional	22875	0.93
Sample80	Chitin	Conventional	9944	0.89
Sample101	Chitin	Conventional	12378	0.88
Sample11	Chitin	Conventional	22918	0.91
Sample108	Combination	Conventional	9182	0.86
Sample79	Combination	Conventional	15386	0.92
Sample16	Combination	Conventional	16453	0.88
Sample103	Compost	Conventional	12120	0.90
Sample17	Compost	Conventional	13211	0.89
Sample75	Compost	Conventional	14592	0.90
Sample71	Control	Conventional	11228	0.88
Sample19	Control	Conventional	15708	0.91
Sample110	Control	Conventional	16538	0.91
Sample102	Grass-clover	Conventional	10643	0.88
Sample76	Grass-clover	Conventional	11217	0.88
Sample12	Grass-clover	Conventional	24389	0.92
Sample72	Marigold	Conventional	13467	0.91
Sample106	Marigold	Conventional	18208	0.89
Sample18	Marigold	Conventional	23461	0.93
Sample20	PhysicalControl	Conventional	10820	0.88
Sample77	PhysicalControl	Conventional	16404	0.90
Sample107	PhysicalControl	Conventional	17731	0.89
Sample14	Biofumigation	Conventional	0	-
Sample78	Biofumigation	Conventional	0	-
Sample105	Biofumigation	Conventional	287	-
Sample65	Anaerobic-soil-disinfestation	Organic	1884	0.68
Sample113	Anaerobic-soil-disinfestation	Organic	7607	0.85
Sample7	Anaerobic-soil-disinfestation	Organic	9184	0.85
Sample68	Caliente	Organic	5133	0.79
Sample111	Caliente	Organic	10845	0.88

SampleID	SHT	System	Number of sequences*	Good's coverage**
Sample9	Caliente	Organic	14916	0.89
Sample64	Chitin	Organic	4322	0.80
Sample119	Chitin	Organic	4880	0.82
Sample2	Chitin	Organic	9098	0.87
Sample67	Combination	Organic	6262	0.83
Sample120	Combination	Organic	8886	0.83
Sample8	Combination	Organic	9276	0.87
Sample10	Compost	Organic	12273	0.87
Sample66	Compost	Organic	8651	0.84
Sample116	Compost	Organic	11144	0.87
Sample114	Control	Organic	2633	0.77
Sample62	Control	Organic	7306	0.83
Sample1	Control	Organic	13739	0.88
Sample118	Grass-clover	Organic	9432	0.85
Sample61	Grass-clover	Organic	5159	0.83
Sample5	Grass-clover	Organic	8076	0.86
Sample63	Marigold	Organic	4269	0.80
Sample6	Marigold	Organic	8217	0.85
Sample112	Marigold	Organic	8787	0.87
Sample69	PhysicalControl	Organic	6240	0.80
Sample4	PhysicalControl	Organic	9010	0.87
Sample115	PhysicalControl	Organic	10682	0.86
Sample3	Biofumigation	Organic	20	-
Sample70	Biofumigation	Organic	212	-
Sample117	Biofumigation	Organic	139	-

* The number after reads processing and removal of Cyanobacteria_Chloroplast, Mitochondria_genus_incertae_sedis, unknown and unclassified.
** Good's estimator of coverage was calculated using the formula: (1−(singletons/individuals)) × 100 only for Bacteria and Archaea Domain.

Table S2. Relative abundance of soil bacterial and archaeal phyla in Soil Health Treatments (SHTs) and conventional and organic farming systems

	Farming System																			
	Conventional									**Organic**									**Statistics**	
Phylum/SHT	**Anaerobic-soil-disinfestation**	**Chemical-control**	**Chitin**	**Combination**	**Compost**	**Control**	**Grass-clover**	**Marigold**	**Physical Control**	**Anaerobic-soil-disinfestation**	**Caliente**	**Chitin**	**Combination**	**Compost**	**Control**	**Grass-clover**	**Marigold**	**Physical Control**	**System**	**Treatment**	**System * Treatment**	**Block**
Proteobacteria	0.51[a] (0.03) **A**[c]	0.42 (0.13)	0.56 (0.30)	0.51 (0.01)	0.54 (0.03)	0.50 (0.10)	0.52 (0.04)	0.55 (0.08)	0.46 (0.02)	0.50 (0.03) **B**	0.47 (0.08)	0.43 (0.11)	0.42 (0.12)	0.51 (0.03)	0.46 (0.07)	0.48 (0.06)	0.52 (0.07)	0.48 (0.08)	*[b]	ns	ns	*
Firmicutes	0.11 (0.04) **a**	0.05 (0.01) **b**	0.07 (0.04) **ab**	0.09 (0.02) **ab**	0.06 (0.03) **ab**	0.05 (0.01) **b**	0.05 (0.01) **ab**	0.05 (0.01) **b**	0.06 (0.01) **ab**	0.08 (0.01)	0.04 (0.00)	0.07 (0.01)	0.06 (0.01)	0.08 (0.02)	0.04 (0.01)	0.09 (0.03)	0.05 (0.01)	0.06 (0.00)	ns	***	•	ns
Acidobacteria	0.12 (0.04) **B**	0.21 (0.1)	0.10 (0.01)	0.12 (0.02)	0.12 (0.01)	0.12 (0.03)	0.15 (0.03)	0.12 (0.06)	0.19 (0.03)	0.16 (0.03) **A**	0.19 (0.07)	0.19 (0.07)	0.21 (0.07)	0.16 (0.05)	0.21 (0.02)	0.13 (0.04)	0.15 (0.03)	0.19 (0.06)	***	•	ns	**
Bacteroidetes[d]	0.10 (0.03)	0.08 (0.02)	0.10 (0.03)	0.11 (0.03)	0.11 (0.02)	0.14 (0.07)	0.08 (0.02)	0.11 (0.02)	0.07 (0.00)	0.08 (0.00)	0.09 (0.01)	0.08 (0.01)	0.08 (0.01)	0.07 (0.00)	0.09 (0.02)	0.10 (0.00)	0.10 (0.03)	0.08 (0.02)	*	ns	ns	*
Actinobacteria	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.05 (0.00)	0.04 (0.01)	0.05 (0.00)	0.04 (0.01)	0.05 (0.00)	0.04 (0.01)	0.05 (0.01)	0.04 (0.02)	0.05 (0.03)	0.04 (0.02)	0.05 (0.02)	0.04 (0.01)	0.05 (0.01)	0.04 (0.01)	0.05 (0.03)	ns	ns	ns	ns
Verrucomicrobia	0.02 (0.01)	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.00)	0.02 (0.01)	0.02 (0.01)	0.02 (0.02)	0.03 (0.00)	0.02 (0.00)	0.03 (0.01)	0.02 (0.01)	0.03 (0.01)	0.02 (0.01)	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.03 (0.02)	ns	ns	ns	ns
unclassified_A	4.47 (2.10) x 10^{-3} **A**	3.37 (2.03) x 10^{-3}	2.95 x 10^{-3} (9.36 x 10^{-4})	2.38 (2.36) x 10^{-3}	4.04 (2.34) x 10^{-3}	2.11 (1.92) x 10^{-3}	6.51 (5.89) x 10^{-3}	3.52 (4.45) x 10^{-3}	6.88 (7.60) x 10^{-3}	1.48 (1.42) x 10^{-3} **B**	1.50 x 10^{-3} (9.23 x 10^{-4})	1.17 x 10^{-3} (8.21 x 10^{-4})	1.57 (1.12) x 10^{-3}	1.47 (1.98) x 10^{-3}	2.75 (2.99) x 10^{-3}	1.34 (2.14) x 10^{-3}	4.60 (2.87) x 10^{-3}	2.97 (2.30) x 10^{-3}	*	ns	ns	*

	Farming System																			
	Conventional									Organic								Statistics		
Crenarchaeota	2.78 (1.15) x 10^{-3}	2.38 (1.98) x 10^{-3}	1.96 x 10^{-3} (9.07 x 10^{-4})	2.76 (1.98) x 10^{-3}	4.30 (1.99) x 10^{-3}	1.71 x 10^{-2} (0.02)	4.17 (2.25) x 10^{-3}	3.36 (4.08) x 10^{-3}	3.35 (2.87) x 10^{-3}	6.21 x 10^{-3} (5.78 x $10^{-4)}$	1.62 x 10^{-3} (8.96 x $10^{-4)}$	1.44 x 10^{-3} (8.32 x 10^{-4})	1.89 x 10^{-3} (2.58 x $10^{-4)}$	2.02 (1.72) x 10^{-3}	6.11 (5.79) x 10^{-3}	7.93 x 10^{-3} (0.01)	2.13 (1.22) x 10^{-3}	2.29 (1.93) x 10^{-3}	ns	ns	ns	ns
unclassified_B	0.08 (0.01)	0.13 (0.07)	0.07 (2.94 x 10^{-3})	0.07 (0.01)	0.08 (4.30 x 10^{-3})	0.08 (0.02)	0.10 (0.01)	0.07 (0.01)	0.12 (0.03)	0.09 (0.01)	0.10 (0.01)	0.11 (0.02)	0.13 (0.03)	0.09 (3.80 x 10^{-3})	0.09 (0.02)	0.09 (0.02)	0.08 (0.01)	0.09 (0.01)	**	ns	*	ns
	B									**A**												
Gemmatimonadetes	7.27 (1.04) x 10^{-3}	7.41 (1.24) x 10^{-3}	9.28 x 10^{-3} (7.60 x 10^{-4})	5.03 (2.56) x 10^{-3}	6.38 (2.10) x 10^{-3}	5.01 (3.16) x 10^{-3}	7.02 x 10^{-3} (8.39 x 10^{-4})	6.53 x 10^{-3} (2.07 x 10^{-4})	6.33 (1.50) x 10^{-3}	8.39 (1.25) x 10^{-3}	7.32 (3.54) x 10^{-3}	9.13 (3.92) x 10^{-3}	5.66 (4.00) x 10^{-3}	7.55 (2.77) x 10^{-3}	6.38 (4.43) x 10^{-3}	8.73 x 10^{-3} (9.70 x 10^{-4})	7.67 (3.44) x 10^{-3}	7.52 (1.91) x 10^{-3}	ns	ns	ns	*
Planctomycetes	6.47 (1.70) x 10^{-3}	0.01 (6.81 x 10^{-3})	3.45 x 10^{-3} (6.21 x 10^{-4})	5.31 (3.34) x 10^{-3}	7.30 (3.02) x 10^{-3}	7.92E (6.14) x 10^{-3}	9.04 (4.03) x 10^{-3}	5.64 (5.43) x 10^{-3}	0.01 (2.15 x 10^{-3})	0.01 (4.47 x 10^{-3})	0.01 (4.36 x 10^{-3})	0.01 (8.78 x 10^{-3})	0.01 (7.05 x 10^{-3})	6.95 x 10^{-3} (1.92 x 10^{-3})	0.01 (5.74 x 10^{-3})	8.00 x 10^{-3} (3.59 x 10^{-3})	0.01 (7.94 x 10^{-3})	8.66 x 10^{-3} (2.84 x 10^{-3})	*	ns	ns	•
	B									**A**												
Nitrospira	1.28 x 10^{-3} (6.42 x 10^{-4})	1.23 x 10^{-3} (4.41 x $10^{-4)}$	2.87 (1.30) x 10^{-3}	1.12 x 10^{-3} (1.95 x $10^{-4)}$	1.54 (1.00) x 10^{-3}	1.27 x 10^{-3} (4.96 x $10^{-4)}$	2.13 x 10^{-3} (1.03 x $10^{-4)}$	9.53 x 10^{-4} (2.19 x $10^{-4)}$	1.15 x 10^{-3} (3.86 x $10^{-4)}$	1.24 (1.00) x 10^{-3}	1.68 x 10^{-3} (2.58 x $10^{-4)}$	2.70 x 10^{-3} (6.09 x $10^{-4)}$	1.91 x 10^{-3} (7.63 x $10^{-4)}$	1.47 x 10^{-3} (2.93 x $10^{-4)}$	1.14 x 10^{-3} (1.61 x $10^{-4)}$	8.25x 10^{-4} (5.40 x $10^{-4)}$	5.55 x 10^{-4} (2.62 x $10^{-4)}$	1.98 x 10^{-3} (4.80 x $10^{-4)}$	ns	***	*	*
	a	**b**	**ab**	**ab**	**ab**	**b**	**b**	**b**	**ab**													
Chloroflexi	1.10 x 10^{-3} (4.29 x 10^{-4})	2.67 (3.20) x 10^{-3}	6.17 x 10^{-4} (6.55 x 10^{-4})	2.12 (1.60) x 10^{-3}	1.63 (1.44) x 10^{-3}	1.41 x 10^{-3} (6.66 x 10^{-4})	1.49 x 10^{-3} (7.20 x 10^{-4})	4.02x 10^{-4} (4.69 x 10^{-5})	1.84 x 10^{-3} (1.31 x 10^{-4})	6.40 (5.57) x 10^{-4}	9.02 (6.97) x 10^{-4}	6.13 (7.13) x 10^{-3}	4.10 (2.68) x 10^{-3}	1.93 (1.75) x 10^{-3}	1.92 (1.73) x 10^{-3}	1.95 (1.96) x 10^{-3}	1.22 x 10^{-3} (8.67 x 10^{-4})	7.48 x 10^{-4} (1.66 x 10^{-4})	ns	ns	ns	ns
Spirochaetes	6.40 (4.91) x 10^{-4}	6.57 (3.13) x 10^{-4}	2.26 (1.18) x 10^{-4}	3.24 (1.17) x 10^{-4}	1.07 (1.13) x 10^{-3}	3.69 (1.56) x 10^{-4}	6.51 (5.76) x 10^{-4}	6.57 (4.57) x 10^{-4}	3.39 (1.56) x 10^{-4}	2.85 (3.09) x 10^{-4}	6.07 (2.86) x 10^{-4}	7.42 (2.17) x 10^{-4}	7.99 (3.92) x 10^{-4}	3.35x 10^{-4} (6.82 x 10^{-5})	8.34 (6.13) x 10^{-4}	8.29 (5.94) x 10^{-4}	1.48 x 10^{-3} (5.19 x 10^{-4})	3.71 (1.38) x 10^{-4}	ns	ns	ns	ns
Armatimonadetes	2.83 x 10^{-3} (9.51 x 10^{-4})	3.80 x 10^{-3} (7.82 x 10^{-4})	3.16 x 10^{-3} (8.46 x 10^{-4})	2.37 x 10^{-3} (3.59 x 10^{-4})	3.20 (1.00) x 10^{-3}	3.69 x 10^{-3} (2.29 x 10^{-4})	2.98 x 10^{-3} (5.28 x 10^{-4})	3.50 x 10^{-3} (5.38 x 10^{-4})	4.37 x 10^{-3} (5.15 x 10^{-4})	2.68 x 10^{-3} (8.68 x 10^{-4})	3.52 (1.03) x 10^{-3}	3.87 x 10^{-3} (3.32 x 10^{-4})	3.61 (1.24) x 10^{-3}	3.36 (1.03) x 10^{-3}	2.78 x 10^{-3} (1.45 x 10^{-4})	3.12 x 10^{-3} (5.49 x 10^{-4})	3.36 x 10^{-3} (6.94 x 10^{-4})	3.85 (1.35) x 10^{-3}	ns	ns	ns	ns

	Farming System																				
	Conventional									Organic								Statistics			
WS3	3.31 (2.83) x 10^{-4}	1.12 x 10^{-3} (9.17 x 10^{-4})	8.07 (3.06) x 10^{-5}	1.68 (1.46) x 10^{-4}	1.39 (1.08) x 10^{-4}	1.33 (1.15) x 10^{-4}	5.38 (4.18) x 10^{-4}	1.15 (1.62) x 10^{-4}	1.09 x 10^{-3} (3.44) x 10^{-4})	4.35 (2.74) x 10^{-4}	5.25 (4.31) x 10^{-4}	0.00 (0.00)	5.90 (4.92) x 10^{-4}	5.09 (2.53) x 10^{-4}	2.88 (2.78) x 10^{-4}	1.20 (2.07) x 10^{-4}	2.94 (1.83) x 10^{-4}	4.53 (3.31) x 10^{-4}	ns	***	*	•
	abc	**ab**	**bc**	**abc**	**abc**	**bc**	**abc**	**c**	**a**												
Euryarchaeota	3.55 (2.44) x 10^{-4}	3.76 (1.98) x 10^{-4}	4.50 (4.38) x 10^{-5}	3.39 (1.34) x 10^{-4}	8.82 x 10^{-5} (1.06 x 10^{-3})	1.06 x 10^{-3} (5.45 x 10^{-4})	4.50 (5.79) x 10^{-4}	2.34 (7.04) x 10^{-4}	4.29 (1.15) x 10^{-4}	2.05 (3.54) x 10^{-4}	2.35 x 10^{-4} (4.67 x 10^{-5})	2.48 (4.29) x 10^{-4}	1.96 (1.31) x 10^{-4}	2.37 (1.31) x 10^{-4}	2.07 (2.34) x 10^{-4}	8.55 (9.13) x 10^{-4}	2.97 (4.12) x 10^{-4}	3.42 (5.93) x 10^{-5}	**	ns	ns	ns
	A									**B**											
Deinococcus-Thermus[d]	1.76 x 10^{-4} (5.70 x 10^{-4})	3.27 (2.58) x 10^{-4}	5.77 (2.59) x 10^{-4}	5.68 (2.28) x 10^{-4}	2.25 (1.91) x 10^{-4}	5.77 (4.36) x 10^{-4}	4.01 (1.79) x 10^{-4}	2.11 (1.69) x 10^{-4}	1.55 (2.13) x 10^{-4}	7.99 x 10^{-5} (1.38 x 10^{-4})	1.54 x 10^{-4} (6.08 x 10^{-5})	1.08 x 10^{-3} (8.30 x 10^{-4})	4.89 (2.24) x 10^{-4}	6.47 x 10^{-5} (1.12 x 10^{-4})	4.18 (3.98) x 10^{-4}	7.83 (8.09) x 10^{-4}	2.13 (1.91) x 10^{-4}	1.14 (1.19) x 10^{-4}	ns	**	ns	ns
Chlamydiae	2.77 (1.42) x 10^{-4}	1.91 (2.21) x 10^{-4}	4.83 x 10^{-4} (4.99 x 10^{-5})	2.42 (1.44) x 10^{-4}	2.07 (1.45) x 10^{-4}	1.52 (1.61) x 10^{-4}	3.00 (3.06) x 10^{-4}	2.08 x 10^{-4} (4.42 x 10^{-5})	2.71 (1.85) x 10^{-4}	4.38 (3.84) x 10^{-4}	2.11 x 10^{-4} (1.12 x 10^{-5})	2.40 (2.37) x 10^{-4}	3.54 (1.06) x 10^{-4}	2.02 (2.76) x 10^{-4}	1.32 (2.29) x 10^{-4}	3.98 x 10^{-5} (6.90 x 10^{-5})	2.16 x 10^{-4} (8.03 x 10^{-5})	2.00 (1.84) x 10^{-4}	ns	ns	ns	ns
Tenericutes[e]	1.15 (1.99) x 10^{-4}	0.00 (0.00)	0.00 (0.00)	6.19 (5.95) x 10^{-5}	1.13 (9.79) x 10^{-4}	3.26 (5.65) x 10^{-5}	0.00 (0.00)	1.06 (1.83) x 10^{-4}	0.00 (0.00)	2.05 (3.54) x 10^{-4}	2.40 (4.16) x 10^{-5}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	ns	ns	ns	ns
BRC1[e]	0.00 (0.00)	4.57 (4.54) x 10^{-5}	1.58 (2.73) x 10^{-5}	2.30 (3.98) x 10^{-5}	5.37 (4.71) x 10^{-5}	0.00 (0.00)	1.27 (5.70) x 10^{-5}	4.00 (6.92) x 10^{-5}	1.11 (1.93) x 10^{-4}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.87 (6.70) x 10^{-5}	0.00 (0.00)	2.07 (2.34) x 10^{-4}	0.00 (0.00)	0.00 (0.00)	3.96 (6.86) x 10^{-5}	ns	ns	ns	ns

[a] The average based on three triplicate samples in each SHT within conventional and organic systems. The values between brackets are the standard deviation (n=3).

[b] Significance levels for ANOVA test: ns = not significant ($P > 0.1$);'•' $P < 0.1$; '*' $P < 0.05$; '**' $P < 0.01$ and '***' $P < 0.001$.

[c] The uppercase letters indicate significant differences within a phylum between conventional and organic system; the uppercase letters are only showed on first treatment from each system. Lowercase letters indicate significant differences within a phylum between SHT within conventional and organic systems. Values with the different letters were significantly different ($P < 0.05$) based on upon a Tukey's HSD test.

[d] For *Bacteroidetes* and *Deinococcus-Thermus*, ANOVA show significance ($p < 0.01$), but pairwise comparison using Tukey's HSD test was not significant ($p > 0.05$).

[e] It was not possible to reach the normal distribution of the residual for Tenericutes and BRC. So, the effects of the variables were tested by the Kruskal-Wallis test analysis using *kruskal.test* in the "stats" package.

Figure S1

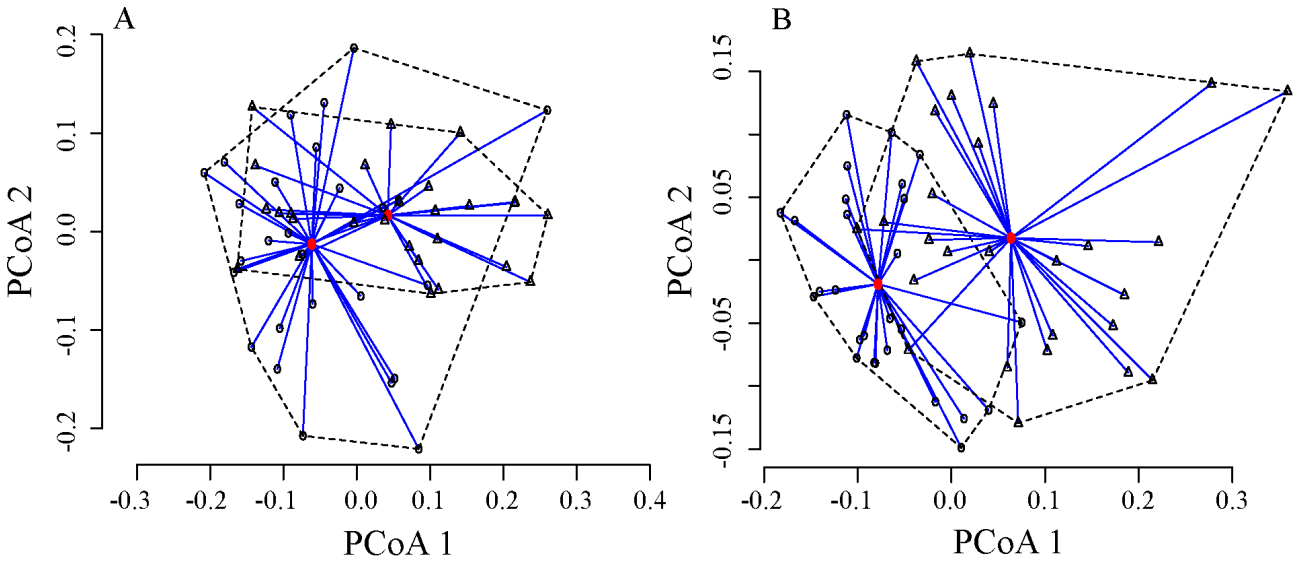

Fig. S1. Effects of farming systems on bacterial community beta diversity. The communities were analyzed at the 97% OTU level. The turnover of the microbial community is represented using Principal coordinates analysis (PCoA) ordination based on Bray-Curtis (A) and unwheight UniFrac (B) dissimilarities. Each point represents an individual sample from conventional (indicated by circles) and organic (indicated by triangle) farming systems. The centroid (indicated in red circle) is defined by the average position of all samples withing a farming system in all coordinate directions.

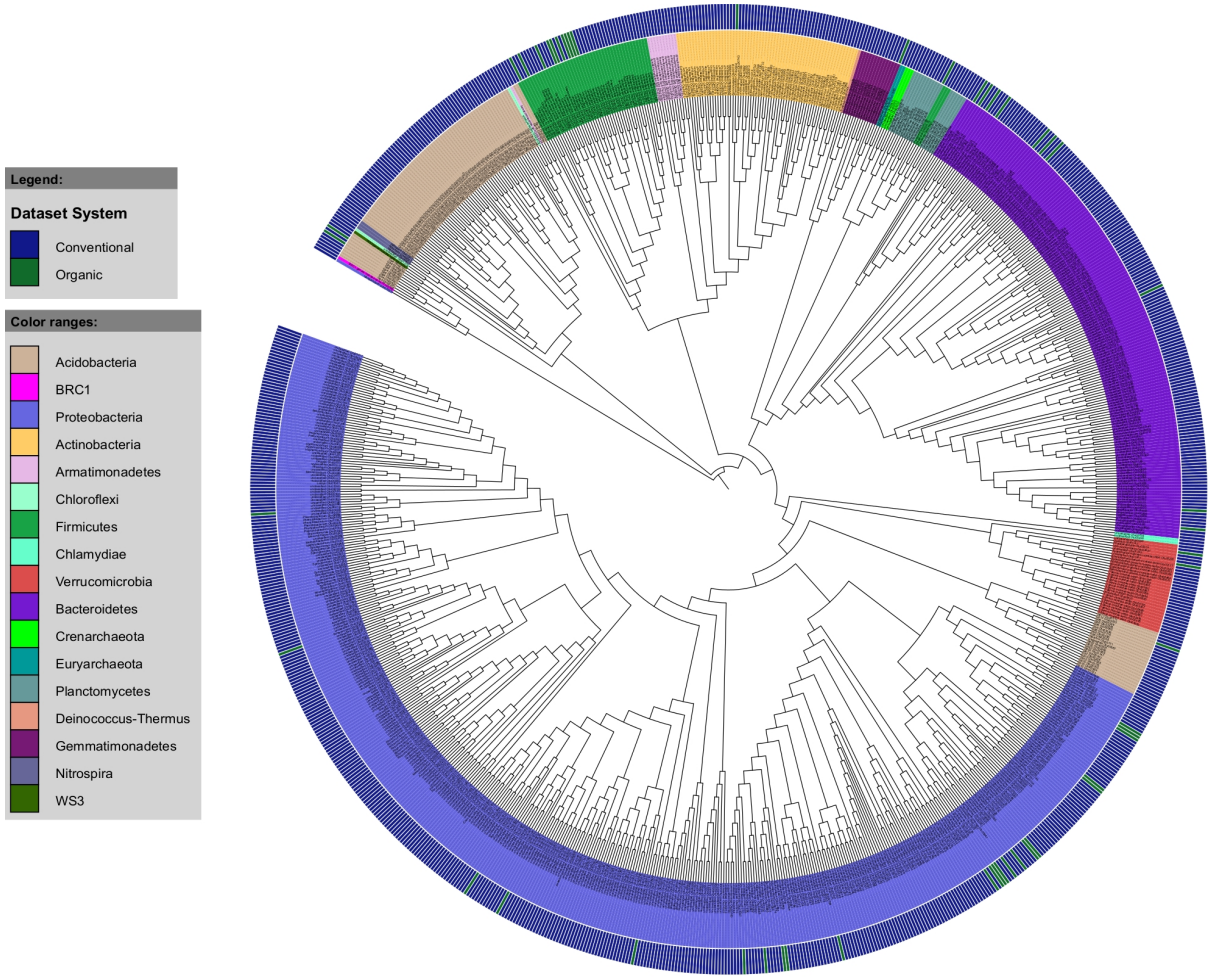

Fig. S2. Dendrogram showing the taxonomy and the habitat specialists associated with conventional and organic farming systems. Only the strict specialists OTUs - cut-off 97% - (28.5% of the total OTU data) with statistical significance of the association ($P < 0.05$, $P < 0.01$ and $P < 0.001$) were considered. The taxonomic affiliation at phylum level of each specialist OTU is identified by the colors range in the left panel and within the tree. The habits preference for a given OTU is indicated in the bars outside of the tree. The conventional is represented by blue and organic by green on the left legend. More information about abundance of each OTU is provided as supplementary information.

Multi-trophic responses to long-term farming systems*

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*It will be submitted to Scientific Nature Reports

Abstract

Soil microbiome and multi-trophic relationships are essential for the stability and functionality of agroecosystems. However, little is known about how farming systems and alternative methods for controlling plant pathogens modulate microbial community, soil mesofauna and plant productivity. In this study, we assessed the composition of eukaryotic microbial groups using a high-throughput sequencing approach (18S rRNA gene marker), the population of parasitic and free-living nematodes, the plant productivity and their inter-relationships in long-term conventional and organic farming systems. The diversity of fungal community increased in organic farming, while the diversity of protist community remained stable at both farming systems. Organic farming increased the population of free-living nematodes and suppressed the plant parasitic nematodes compared to conventional farming. Fungal diversity and community structure appeared to be related with nematode suppression in system receiving organic fertilizer, which were characterized by component microbial groups known to be involved in suppression of soil pathogens. Unraveling the microbiome and multi-trophic interaction in different farming systems offers a potential for managing the soil environment towards a more sustainable control of plant-pathogen.

Keywords: protist community; nematode;18S rRNA; fungi; low-input management; soil web; soil health

Running title: Multi-trophic interactions in agroecosystems

Introduction

Microbiome interaction and feedbacks with soil biota regulate ecosystem functioning and primary productivity in soil agroecosystems[1, 2]. Fungi play an important role in soil ecosystems as obligate root symbionts, decomposers or soil-borne pathogens[3-5]. Soil mesofauna and protist fulfill diverse functions in soil ecosystems as well by grazing living organisms, decomposing organic matter and determining nutrient cycling[6]. Top-down relationships may also regulate soil ecosystem processes and functioning. More diverse communities and presence of certain component species may promote soil services and enhance ecosystem stability through the suppression of plant pathogens, interspecific competitions and promoting higher plant productivity[7, 8].

Soils have been managed with organic and inorganic fertilizers over the past decades to increase crop yields[9]. Although positive effects of the conventional farming systems on nutrient availability and plant productivity are well described[10], there is an increase concerning that the intensive agricultural management leads to ecosystem degradation, causing soil pollution and a loss in diversity[11]. Compared with conventional systems, organic farming reduces the use of synthetic fertilizers and pesticides, and mitigates the negative impacts of intensive management in order to promote sustainable production[12]. Positive effects of organic farming system include increase of microbial diversity[13], promotion of beneficial microorganisms[14], enhance of nutrient cycling[15], and reduction of plant pathogens[16]. However, we still have an incomplete understanding on long-term benefits and limitations of organic farming systems[17].

Together with organic farming, non-chemical alternatives to inhibit plant pathogens are gaining importance[14, 16]. Methods based on biofumigation, marigold treatment, chitin and compost have been demonstrated to be highly efficient in disease suppression caused by

plant-parasitic nematodes[18-21]. Nevertheless the disease suppression induced by such alternative methods has been linked to factors other than direct effects on target organisms[22]. Those methods can modulate the soil microbiome and promote the presence of microbial groups able to interact to each other and control the pathogens in soil[23, 24]. Moreover, soil free-living nematodes, which also play an important role in soil functioning through food webs, by regulating decomposition and mineralization processess, might also be affected by soil management[25]. A broadly employment of alternative methods for pathogen control in the future includes a better understanding of its impacts on soil microbiome, soil mesofauna and mechanisms involved in specific interactions[26-28].

Cultivation-based studies have shown that fungi and protists are abundant and ecologically important for soil processes[7]. Many taxonomic groups are comprised of species that potentially exhibit different ecological importance, but remain undetected by traditional morphology-based methods applied to determine the community composition[29]. To elucidate their relationships and functioning in agroecosystems, multi-trophic levels should be assessed simultaneously for documenting the responses to agricultural management[30]. Next generation sequencing approach allows harvesting the soil microbiome at different taxonomic resolution, allowing the identification of microbial taxa associated with specific management occurring in agricultural soils[31].

In this context, we performed a simultaneous identification of multiple organism groups in a long-term Soil Health Experiment (SHE) model system which is divided into conventional and organic farming systems into component parts, namely Soil Health Treatments (SHTs; non-chemical methods for plant pathogen control). We disentangled the effects of farming systems and soil health treatments on multiple taxonomic groups and their relationships by using high-throughput sequencing of the 18S rRNA gene marker to determine

the fungal and protist microbiomes and assessing the soil mesofauna and plant biomass. We postulated the following hypotheses: (i) organic farming system has a positive effect on the diversity of fungal and protist microbiome community; (ii) organically managed systems increase the population of free-living nematodes and suppress the population of plant-parasitic nematode; and (iii) multi-trophic components shift in concordance mainly owing to similar responses to the agricultural management. In the long-term, identify microbial taxa and monitor the collection of components in an agroecosystems will help to manage agricultural soils in a sustainable perspective to improve ecosystem health.

RESULTS

Sequencing and coverage

We analyzed 60 samples yielding a total of 2,983,755 sequences (average of 49,729 ± 502.52 per sample) after quality filtering and chimera removal. This corresponds to 2,299,226 fungal (average of 38,320 ± 798.86 per sample), 675,428 protist (average of 11,257 ± 103.60 per sample) and 9,101 other eukaryotic taxa (average of 151 ± 14.11 per sample) sequences with average of 302 bp (± 38.17) remained for community analysis. Sequence clustering yielded a total of 1,074 (average of 510 ± 98.38 per sample) OTUs, corresponding to 422 (average of 225 ± 41.48 per sample) fungal, 611 (average of 270 ± 55.39 per samples) protist and 41 (average of 15 ± 4.46) other eukaryotic taxa OTUs. This represented an average Good's coverage of 0.99 ± 0.04, 0.99 ± 0.03, 0.98 ± 0.016 and 0.93 ± 0.07 for the total community of fungal, protist and other eukaryotic taxa, respectively. The number of quality-filtered sequences and coverage are provided on Table S1, supplemental material File S1.

Clustering and taxonomic compositions

Fungal and protist OTUs were classified and clustered at each taxonomic level (*i.e.,* Kingdom,

Supergroup, Division, Class, Order, Family, Genus and Specie) based on RP2 database[32], which provide a curated taxonomy reference to access to eukaryotic small subunit (SSU) ribosomal sequences. Full list of the eukaryotic taxa, from Kingdom to species (OTU level), *e-value* accession number (PR2 and NCBI) and number of sequences information are provided in supplemental material File S2 (online version of this paper). A total of 90% of the fungal OTUs were classified at the kingdom and division levels, 89.8% of the fungal OTUs were classified at the division level, 55% were classified at the genus level and 39% were classified at the species level, accounting for 98%, 70% and 61.5% of the total fungal sequences, respectively. Overall, the fungal community was dominated by phyla Ascomycota (48%), Mucoromycota (15%) and Basidiomycota (8%), while Zoopagomycota, Kickxellomycota and Entomophthoromycota had a relative abundance of <0.01% (Fig. 1). From OTUs belonging to protist community, 100% were classified at the division level, 41% were classified at the genus level (accounting for 58% of the total protist sequences) and 18% were classified at the specie level (accounting for 31% of the total protist sequences). The protist community was almost exclusively comprised of Cercozoa (21%), with dominance of flagellates belonging to the class Filosa-Sarcomonade (62%) and Filosa-Thecofilose (25%). Dinophyta (0.9%) Choanoflagellida (0.08%), Mesomycetozoa (0.05%) and Lobosa (0.03%) also occurred in high abundance. Apicomplexa, Ciliophora, Hilomonadea, Radiolaria had a relative abundance of <0.01% (Fig.1). A total of 73% of the OTUs belonging to other eukaryotic taxa were assigned to division level, 63% were classified at genus level and 9% classified at specie level, accounting for 75.5%, 75.3% and 17.6% of the total ‘others’ eukaryotic sequences, respectively. Other eukaryotic taxa are shown in supplemental material File S2 (online version of this paper).

Diversity and structure of fungal and protist communities

The farming system significantly impacted the fungal richness (Observed OTU; P-value=0.0353), fungal taxonomic diversity (Shannon P-value=0.000379; Pielou P-value=0.000211), but not fungal phylogenetic diversity (Fig. 2). When compared with conventional system, organic management had a positive effect on both fungal diversity (Observed OTU; 95.8 for organic *vs.* 90.8 for conventional, t-test, P-value=0.026; Shannon; 2.9 for organic *vs.* 2.6 for conventional, t-test, P-value=9.3e-05) and fungal evenness (Pielou; 0.64 for organic *vs.* 0.59 for conventional, t-test, P-value=4.2e-05). In contrast, the richness, diversity and evenness of protist community remained stable at the two different farming systems (no significant effect was detected) (Fig. 1). Differences in richness, diversity and evenness among SHTs and the interaction 'farming system x SHT' were not significant for both fungal and protist communities.

Eukaryotic microbial structure responded to farming systems and soil health treatments (Fig. 3). Farming systems significantly altered the microbial structure, with most pronounced effect on fungal community (Fig. 3). Fungal community was also affected by soil health treatments, in opposite to protist community, which was not influenced by treatments (Fig.3)

Nematode composition and maize yield

The abundance of parasitic nematodes belonging to Meloidogyne and Pratylenchidae were higher in conventional system than in organic system (Meloidogyne, 64.2 in conventional *vs.* 8.4 in organic, t-test, P-value=0.00011; Pratylenchidae 1263.7 in conventional *vs.* 647.1 in organic, t-test, P-value=4e-07). The population of Tylenchorynchus and Trichodoridae, also plant parasitic nematodes, were not affected by farming systems or soil health treatments (Fig. 4). Only the population of nematodes of Pratylenchidae were affected by soil treatments; a higher abundance was observed in Chitin and smaller abundance on Marigold treatment

(1347.0 *vs.* 430.0, respectively, t-test, P-value=0.0116). On the other hand, free-living nematodes had higher abundance in organic system than conventional system (2383.8 in organic *vs.*1876.8 in conventional, t-test, P-value=0.0015). Difference in maize biomass was largely due the influence of farming systems, and no effect of soil health treatments was observed; the maize biomass was higher in conventional than in organic system (18684.10 kg/ha in conventional *vs.* 15406.0 kg/ha in organic, t-test, P-value=5.7e-08) (Fig. 4).

Inter-relationships between fungal and protist microbiomes and soil mesofauna

In order to assess the dependent effects between the fungal and protist diversities, structures and soil mesofauna, we performed a coinertia and Mantel test analyses. Our results suggest that farming system drives the community composition of different taxonomic levels convergently with no causal relationship. A link between fungal and protist communities was observed, but no relationship was detected between protist and soil mesofauna (Table 1). Fungal diversity and structure were significantly correlated with soil mesofauna population (Table 1).

Microbial taxa associated with farming system and soil health treatment

Given the effects of farming system and soil health treatment on soil fungal and protist microbiomes, we used indicator species analysis to examine which taxonomic groups (OTU level) differed between farming systems or soil health treatments. A total of 119 (29%) fungal and 105 (17%) protist OTUs were significantly ($P<0.05$) associated with specific farming system or soil health treatment. The main findings among fungal and protist OTUs significantly influenced by farming systems or soil health treatments are presented in Figures 5 and 6, respectively. Fungal and protist indicators were identified at species level when possible; otherwise OTUs were identified at lowest taxonomic possible level. Fungal and

protist OTUs were broadly distributed across the taxonomic phylogenetic trees and none of the higher taxonomic groups (*i.e.* phyla, order) had a consistent response to farming system or soil health treatment.

Overall, most of the indicator fungal OTUs were identified as putative parasitic species (52%) with the remaining being putative saprobes (23%), putative mycorrhizal (9%) or of unknown (16%). The majority of the parasitic and decomposer species (for example, members belonging to *Rhizophydium*, *Hyaloraphidium*, *Zoopagomycotina* and *Cryptomycotina*) were associated with organic farming system and were consistently reduced in conventional system (Figure 5). Among the significantly associated mycorrhizal species, there were only one putative ectomycorrhizal species and seven arbuscular mycorrhizal species (belonging to *Glomeromycota* order), which were mainly increased in conventional system (Figure 5) and associated with Control and physical control treatment. Several protist taxa significantly responded to farming system or soil health treatment (Figure 5 and 6). However, most of protist OTUs was only classified at higher taxonomic levels, thus carrying little information to infer the putative ecological role of the taxa. The majority of protist OTUs associated with farming system or soil health treatment belongs to orders Filosa-Sarcomonadea, Filosa-Thecofilosea and Filosa-Granofilosea, all belonging to phyla Cercozoa, the most abundant phylum.

DISCUSSION

Straightforward conclusions about the effects of agricultural management on multi-trophic components and their responses to farming systems are difficult[3, 33]. Single components of an ecosystem, such as diversity or soil biota, are modified by agricultural management[34, 35]. However, the relationship between them is highly scarcely explored with few studies comparing agricultural management strategies over an extended period of time[28]. The long-term Soil Health Experiment (SHE) has been continuously managed according to conventional and organic farming systems (> 8 years) and represents a suitable model system to compare the long-term effects of farming systems and non-chemical treatments (Soil Health Treatments, SHT) on multi-trophic components in an agroecosystem. Understanding whether composition of organisms in one trophic level affect the surrounding components provides new opportunities to learn how farming systems shape the community of interacting organisms and to exploit agroecosystems scenarios that favor the control of plant pathogens[1].

Farming system was the best predictor of soil fungal and protist microbiomes, with the strongest effect on diversity and structure of fungal community. Organic system increase fungal richness, diversity and evenness compared with conventional farming. In contrast, protist diversity did not show a response to farming system management. Although the fungal community appeared to be strongly structured by farming system, it had a minor effect on protist community. It has been reported an increase of microbial diversity in organically managed systems through resource availability and niche differentiation[13]. However, the response of microbial diversity and structure to farming systems is not completely clear[17], and different phylogenetic groups might respond in different ways[11]. Basal differences in physiology and ecology of fungal and protist communities suggest that their patterns of diversity and structure are controlled by distinct conditions[36]. For example, the largest

differences in fungal community structure and low diversity in conventional system may be explained by the high spatial variability of fungal populations make them more susceptible to disturbances than protozoa[37].

We found that soil mesofauna was driven by conventional and organic systems over the long term, but no effect of soil health treatments was detected on soil mesofauna. The decline in plant-parasitic nematodes in organic system may steam from detrimental relationships with microbial groups and nematicidal compounds released during the degradation of organic material[21, 28]. Free-living nematodes are important for regulation the population of other organisms[38], and their increase in organic system suggest that they may benefit from conditions promoted by organic practices[25]. In previous study realized on the SHE, a suppression of plant parasitic nematode during the first years of the experiment was observed among the soil health treatments[39]. However, we detected a later recover (after 2 years of last application of treatments) of nematode population, indicating the soil health treatments may have a short-effect on pathogen control[40]. These results are in accordance with other studies suggesting that soil mesofauna possess a high potential for resilience or tolerance following intensive disturbances[41]. Another important question on farming system is if the plant biomass increase on long-term organic management and whether or not is linked with other ecosystem parameters[42]. In our study, we observed that organic farming produced lower maize biomass compared with conventional agriculture. Although expected that less sustainable practices applied in conventional system and greater population of pathogens may reduce the plant biomass[10], the responses of plant productivity may depend on several interacting factors[43].

Consistent with our hypothesis, the results suggest that farming system determines the community composition of multiple taxonomic levels in context-dependent relationships. The effects on multi-trophic levels are in agreement with studies in agroecosystems, in which the

conditions promoted by different farming systems determine the abundance of individuals or taxonomic richness of groups of mesofauna both aboveground and belowground[44]. The exhibited dependent effects of fungal community and nematode population might be one factor helping the lower presence of pathogenic population on this system[18]. The increase of fungal diversity might promote a barrier against soil-borne pathogen proliferation in soil and invasion in plant root[8]. Furthermore, the structure of fungal communities in organic system may favor detrimental relationships with plant-parasitic nematodes by the increase abundance or presence/absence of certain taxa[6]. A link between fungal and protist communies was observed by co-inertia, but no relationship was detected between protist and mesofauna, contradicting the expectation that protozoa community will play an important role on nematode population because their broad nematode-feeding lifestyle[45].

High-throughput DNA sequencing offer new ways to explore the soil microbiota at higher taxonomic resolution and speed up our understanding of the microbial functioning in agroecosystems[5]. Sequencing circumventing major biases associated with cultivation studies, but still significant gaps in the composition and taxonomic information exist in all microbial groups, especially in protist community[46]. The lack of knowledge on soil protist communities is mainly caused by the problematic to cultivate them[47], difficult microscopic observation[48] and lack of SSU rRNA reference sequences[49]. Although 18S gene sequences enabled us to recover several groups of eukaryotes and identify them at different taxonomic levels using the most recent taxonomy reference database for protist community[32], the assignment success at lower taxonomic levels such as genus or species was lower for protist then fungal sequences.

In addition to diversity and structure, certain component microbial species may determine key soil ecosystem processes[33]. Although several OTUs associated with farming systems and soil health treatments were only classified at higher taxonomic level, thus carrying limited

biological information about their ecology rules, we based our conclusions on putative lifestyle based on closest ancestral and literature information. Symbiotic, parasitic and saprobic fungal species appear to be most susceptible microbial groups to farming systems and are potential indicators for monitoring the relationship with other trophic levels[47]. The mechanisms by which microbial taxa regulate soil mesofauna are not completely understood, and some studies suggest that the magnitude and directionality of component microbial species effects are system specific[2]. For example, the increase of microbial groups able to suppress soil pathogens, such as taxa belonging to *Cryptomycotina*[50] and *Zoopagomycotina*[51] might explain the reduction of the plant parasitic nematodes in organic system. Microbial taxa belonging to Basidiomycota and Ascomycota, the most abundant phyla, appeared to be associated with farming systems, supporting the information that saprobic fungi are among the most susceptible group to soil disturbances[52]. Contrary to expected, more mycorrhizal fungi taxa were associated with conventional than organic farming system. More diverse communities of mycorrhizal fungi could promote plant productivity, as they are the mainly responsible by nutrient uptake[33, 53] and mediate the plant defense against soil-borne pathogens[54].

Although several protist taxa were associated with farming systems and soil health treatments, the majority of taxa were identified at shallow taxonomic level and we could little conclude about their specific ecology rules. Cercozoa, the most abundant eukaryotic group, showed a clear accumulation of taxa associated with farming systems and soil health treatments. Although we can not exclude the possibility that some groups will be misrepresented because preferential PCR amplification[55] and the primers used in this study, ours results are in line with other studies, which show that Cercozoa represent the numerically dominant eukaryotic microorganisms[56]. Cercozoa include a variety of groups mainly

specialized on predation of other taxonomic groups, such as fungi and nematode, suggesting a potentially role in structuring soil food webs in agroecosystems[57]. Other protist groups not necessarily associated with farming systems but generally present in all soil conditions, such as Apicomplexa and Ichthyosporea, common parasites of soil mesofauna, may also potentially play an important role in multi-trophic interactions[2, 58].

Our results outline that long-term farming systems exhibited context-dependent effects on diversity and structure of fungal and protist microbiomes, soil mesofauna and plant biomass, and far exceed any differences observed among soil health treatments. Nonetheless, organic system promotes higher fungal diversity and presence of free-living nematodes, while the population of plant parasitic nematodes and plant biomass increase in conventional system. Although fungal diversity appears to be related with soil mesofauna, the population of plant parasitic nematodes may be more related with presence of certain component species associated with organic farming. The competence to detect shifts on individual microbial taxon in an agroecosystem indicated the potential direction that sustainable farming system should follow to promote soil health. Corresponding changes observed at multi-trophic levels explained by convergent effects of organic and conventional farming systems indicated that these parameters must be determined for addressing community relationships and changes in ecosystem processes in agroecosystems.

Material and methods

The agricultural Soil Health Experiment (SHE) model system

The long-term Soil Health Experiment (SHE) located at Wageningen University Research (WUR) station in Vredepeel, in the southeast of the Netherlands (51º 32’ 27.10” N and 5º 51’14.86” E) was used to test our hypotheses. The site has been in agricultural cultivation

since 1955. The SHE field (~ 6 ha) was established in 2006, and contains 160 plots (6 m x 6 m) arranged in a randomized block design and continuously managed according to conventional and organic farming systems. Conventional and organic systems only differ in fertilizer application and plant protection strategies. Both conventional and organic systems received similar amount of N, P, K nutrients per hectare/year according to fertilizer recommendations for the crops. Initially, all plots were fertilized with cattle slurry (38 m^3 ha^{-1}). After that, the conventional system received mineral fertilizer (250 kg ha^{-1} of mineral fertilizer) and common chemical plant protection was carried out. The organic system exclusively received organic fertilizers (25,000 kg ha^{-1} of farm yard manure) and when necessary was mechanical weeded. Each year between 2006 and 2013, a crop was grown on the entire experimental field: 2006: Wheat (Conv) or barley (Org); 2007: potato (Conv, Org); 2008: lily (Conv, Org); 2009: Wheat (Conv) or barley (Org); 2010: potato (Conv, Org), 2011: carrot (Conv, Org), 2012: maize (Conv, Org), 2013: maize (Conv, Org).

The Soil Health Treatments (STH)

The Soil health Treatments (SHTs) used in this study was selected based on literature information regarding the efficiency in plant pathogen control. Nine SHTs were applied twice since 2006 until 2013 (from the end of July 2006 till May 2007 and from July 2009 till December 2009) within conventional and organic farming systems with four replicates per treatment: Compost (CO) - 50 t ha^{-1} of mature compost (65% wood, 10% leaves and 25% grass and inoculated with *Trichoderma harzianum* - Orgapower) was incorporated in the 20 cm soil surface; Chitin (CH) - 20 t ha^{-1} of chitin based on shrimp waste material (Gembr, Ecoline) was incorporated in the 20 cm soil surface; Marigold (MA) - *Tagetes patula* (cv. Ground Control) grown from July 2006 till January 2007 and incorporated in the 20 cm soil

surface; Grass-clover (GC) - a combination of four grass species (4 kg ha^{-1} cv. Tetraflorum, 7 kg ha^{-1} cv. Miracle, 2 kg ha^{-1} cv. Pomposo and 1 kg ha^{-1} cv. Tomaso) and two clover species (1 kg ha^{-1} cv. Riesling and 7 kg ha^{-1} cv. Maro) was grown from 27 July 2006 till 12 March 2007 and incorporated in the 20 cm soil surface; Soil Anaerobic Disinfestation (AD) - 50 t ha^{-1} of fresh organic matter (a mixture of different rye-grass species) was incorporated in the 20 cm soil surface on August 2006, irrigated with 20 mm and covered with a virtually impermeable film (VIF - 0.035 mm thick HyTibarrier delivered by Hyplast) till November 2006; Physical Control (PH) - the soil was treated with hot air (Cultivit; 720-780 ºC) into rotavating humid soil; Biofumigation (BF) - *Brassica juncea* (cv. Energy) was grown from 27 July till 20 September 2006, replenished with 117 t ha^{-1} Broccoli (cv. Montop) and then incorporated in the 20 cm soil surface; Combination (CB) - three soil health treatments (MA, CO, and CH) were subsequently combined; and Caliente (CL), only applied in organic system - a byproduct of mustard production (70 L ha^{-1}) was applied in the 20 cm soil surface. Two control treatments were also applied: Chemical control (CC), only in conventional system - 300 L ha^{-1} Metam sodium (Monam 510 g a.i. L^{-1}) was applied with a rotary spading injector on September 2006; and Control treatment (CT) - the soil was given no extra treatment and left fallow after wheat harvest till next growing season. For a complete description of the experimental field, fertilization scheme and details about soil health treatments see[39].

Soil sampling for microbiome analysis and DNA isolation

In order to obtain a picture of eukaryotic microbiome, in May 2013, 60 soil samples (top-layer 0 to 10 cm), and representing 3 replicates per soil health treatment within conventional and organic farming systems were collected. Soil samples were stored in -20^{0}C until molecular analyses. The DNA was extracted from 2 g of soil using the MoBio PowerSoil

DNA extraction kit (MoBio laboratories, Inc.) following the manufacturer's instructions and the yield and quality weredetermined using NanoDrop 1000 spectrophotometer (Thermo scientific, USA).

18S rRNA gene amplification and sequencing

The eukaryotic microbiome was analyzed based on amplicon sequencing of 18S ribosomal marker gene using the primers FR1[59] and the modified version of forward primer FF390w (5′-CGWTAACGAACGAGACCT-3′)[60] designed for Ion Torrent™ technology sequencing. PCR was performed using the FastStart™ High Fidelity PCR System (Sigma-Aldrich) in 25 uL reaction containing a final concentration of 0.056 U (5U/ uL) of FastStart High Fidelity Enzyme Blend, 10x FastStart High Fidelity Buffer with 1.8 mM $MgCl_2$ (Roche Diagnostics Ltd., Burgess Hill, UK), 200 uM of each dNTP (Promega UK Ltd. Southampton, UK), 0.1 uM of each primer and 50 ng of DNA. The conditions used were a hot start of 95 ºC for 5 min, followed by 35 cycles of 95 ºC for 30 s, 58 ºC for 30 s and 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. Each sample was amplified in triplicate in a C1000 Touch thermal cycler (Bio-Rad, Hemel Hempstead, UK), visualized on a 1% (w/v) TBE agarose gel and subsequently pooled for purification using QIAquick gel extraction kit (Qiagen). Total PCR products were quantified using Quant-iT Broad-Range DNA Assay Kit (Invitrogen) in conjunction with BioTek Synergy HT microplate reader, and then combined in equimolar ratios. Template preparation was performed using Ion OneTouch 2 System and Ion PGM Template OT2 400 Kit, and the amplicon library was sequenced using one Ion 318™ Chip and Ion PGM Sequencing 400 kit on Ion PGM™ sequencer. The sequences are available at the European Nucleotide Archive (ENA) (http://www.ebi.ac.uk/ena/data/view/PRJEB10908), study accession no. PRJEB10908 (ERP012207). The complete list of samples and accession

numbers are available on supporting information File S1, Table S1.

Processing of sequencing data and taxonomic affiliation

Sequence data was analyzed using vsearch 1.4.0[61] combined with a Snakemake pipeline[62]. The multiplexed reads were filtered for perfectly matching primer, quality score >25 and length of 150 bp on flexbar[63]. After trimming, the reads were de-replicated, sorted by abundance, clustered into OTUs at 97% sequence similarity cut-off using USEARCH[64] and chimera filtered using the *uchime*[65] on UPARSE package[66]. The representative OTU sequences were taxonomically assigned against The Protist Ribosomal Reference database (PR2), http://ssu-rrna.org/)[32] through BLAST search using QIIME (max E value 0.001, min percentage identity 90.0)[67, 68]. The representative sequences were aligned using the Silva rRNA database (release SSU_Ref_119)[69] and the phylogenetic tree was constructed using a distance matrix with MUSCLE algorithm[70] available in QIIME. The biom file was created using the biom-format package version 2.1.5[71]. The pipeline and commands used for sequence processing are available online (https://gitlab.bioinf.nioo.knaw.nl/amplicon-metagenomics/iontorrent-vsearch/commits/vredepeel/).

Soil sampling for nematode counting and maize yield

To analyze how farming systems and soil health treatments affect the population of plant-parasitic and non-parasitic nematodes, in April 2013, 60 soil samples (25 cm depth, 3 replicates per soil health treatment within conventional and organic systems) were collected and nematode extraction were performed according[39]. In summary, nematodes were extracted from 100 mL soil using a modified Oostenbrink elutriator[72, 73]. Nematode numbers were determined based on counting of two 10 mL aliquots from the suspension after Oostenbrink elutriator, identified at Family level and expressed as total numbers per 100 mL. In the same

month, maize plants were harvested mechanically from the centre of each plot using an experimental field-corn combine harvester (De Kemper bek, type Champion) and a sample of 700 g was dried for 48 hours at 70°C to determine dry weight of maize productivity.

Data analysis

All statistical analyses were performed using the R package, version 3.2.3[74]. The statistical tests performed were considered significant at $P<0.05$ unless indicated otherwise when smaller significance was obtained. To analyze the microbiome, the biom file was imported to R using "phyloseq" package[75]. The Good's coverage[76] was calculated to evaluate the sequencing depth per sample at OTU level (97% similarity cutoff) for entire eukaryotic community and for fungi, protist and others eukaryotic taxa separately.

The alpha-diversity was evaluated based on observed number of OTUs, estimated compositional OTU diversity (Shannon index diversity, H'[77]) and phylogenetic diversity (Faith's phylogenetic diversity index - Faith's PD[78]) and evenness (Pielou's evenness[79]) using two different data sets: (1) the diversity of total microbiome was calculated with the complete data set rarefied to 9,405 sequences; (2) the diversity of the fungal and protist microbiome was constructed separately and calculated based on the data set rarefied to 1,610 sequences. The observed number of OTUs and Shannon index were calculated using *estimate_richness* function in "phyloseq" package, the Faith's index was calculated using *pd* function in "picante" package[80] and Pielou's evenness was calculated using *diversity* function on "vegan" package[81].

The effects of farming systems and soil health treatments on fungal and protist microbiomes diversity, on parasitic and free-living nematodes and on plant productivity were tested using two-way analysis of variance (ANOVA) after checked for homogeneity of

variance following the Fligner-Killeen Test using the *fligner.test* function on “stats” package[74]. When the effects were significant, they were further analyzed using a post-hoc *pairwise.t.test* (for pairwise comparison between farming systems) in the “stats” package or *HSD.test* (for pairwise comparison between treatments) in the “agricolae” package[82].

Overall farming systems and soil health treatments effect on fungal and protist communities was examined using PCoA combined with multivariate PERMANOVA of Bray-Curtis distances based on relative abundance data. The association among structure and diversity of the fungal and protist communities (at OTU level) and soil mesofauna were analyzed using coinertia analysis (CIA)[83] computed with "ade4" package in R[84]. To further test the statistical significance of the associations, a randomization test based on Monte-Carlo method with 999 permutations using the function *randtest* on ade4 package was calculated.

For the in-depth ecological analysis, we identify the component microbial taxa associated with farming systems and soil health treatments responsible for the patterns observed on structure of microbial communities by performing the indicator species analysis[85]. Based on the evidence that an microbial species (at OTU-level) can prevail (in abundance and frequency) in a certain niche provided for agricultural management considering all possible combinations, is important to obtain that information for elucidate their ecological rules in agroecosystems[86]. The OTUs associated with farming systems and soil health treatments were identified using the *multipatt* function available in “indicspecies” package using 999 permutations[85]. The representative sequences were used to construct a maximum likelihood dendrogram what was displayed using iTOL[87] tool.

ACKNOWLEDGEMENTS

We thank Adriano Lucheta for helpful discussions of fungal classification, Leonardo Pitombo

for the soil sampling, Agata Pijl and Victor Carrion for laboratory assistance, Johnny Visser for field assistance and Rodrigo J.S. Jacques for general discussion. This study was supported by BE-BASIC Foundation (F08.002.05). The scholarship to the first author was granted by FAPERGS/CAPES (Research Agency of the State of Rio Grande do Sul/Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil) under the Graduate Program of Soil Science (PPGCS) at Federal University of Santa Maria.

Figures

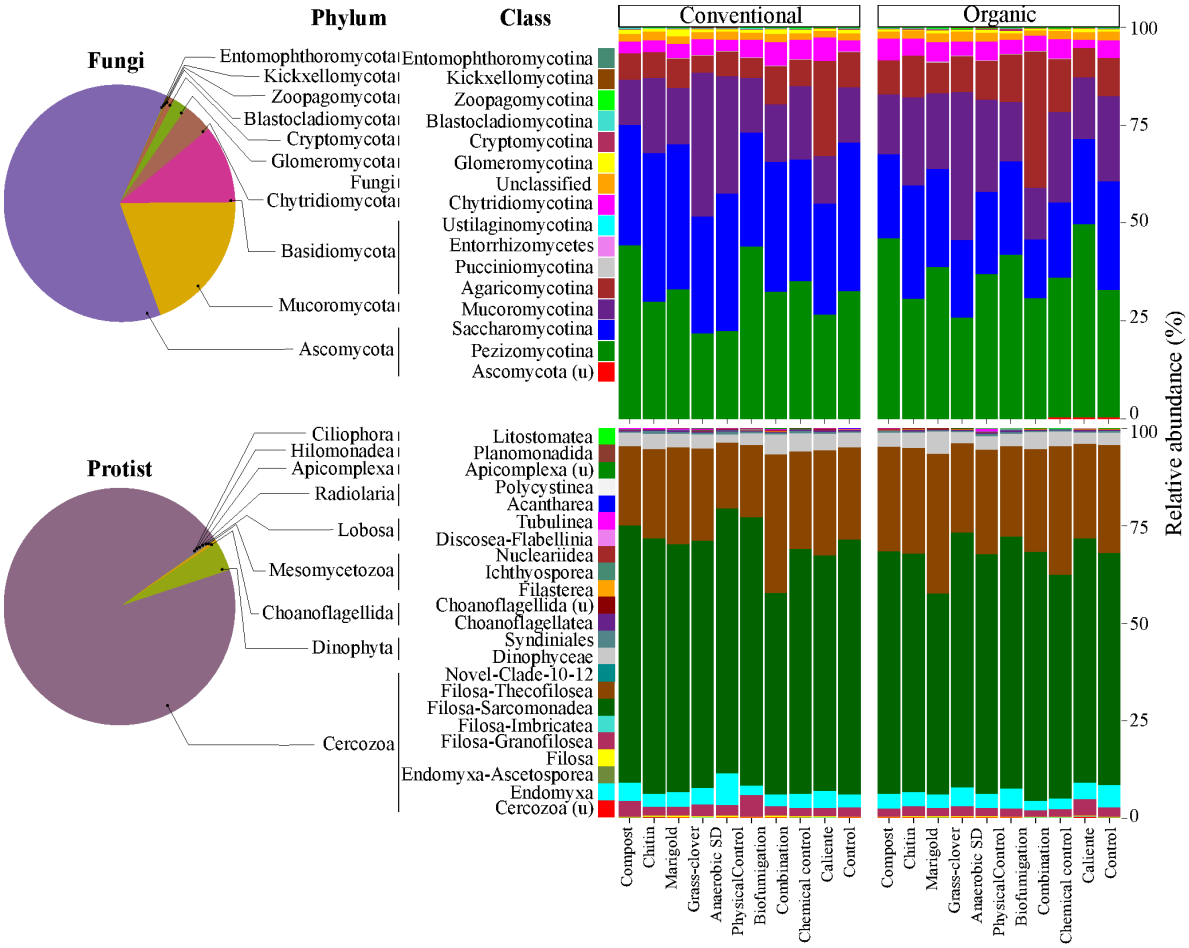

Figure 1. Average composition of fungal and protist communities. The phyla are represented in pie charts and class are represented in bar plots showing the relative abundance variation between soil health treatments and farming systems. The relative abundances are represented on y-axis (left) and soil health treatments are represented on x-axis. The class of fungal and protist are represented by different colors on y-axis (right panel). (u) means unclassified.

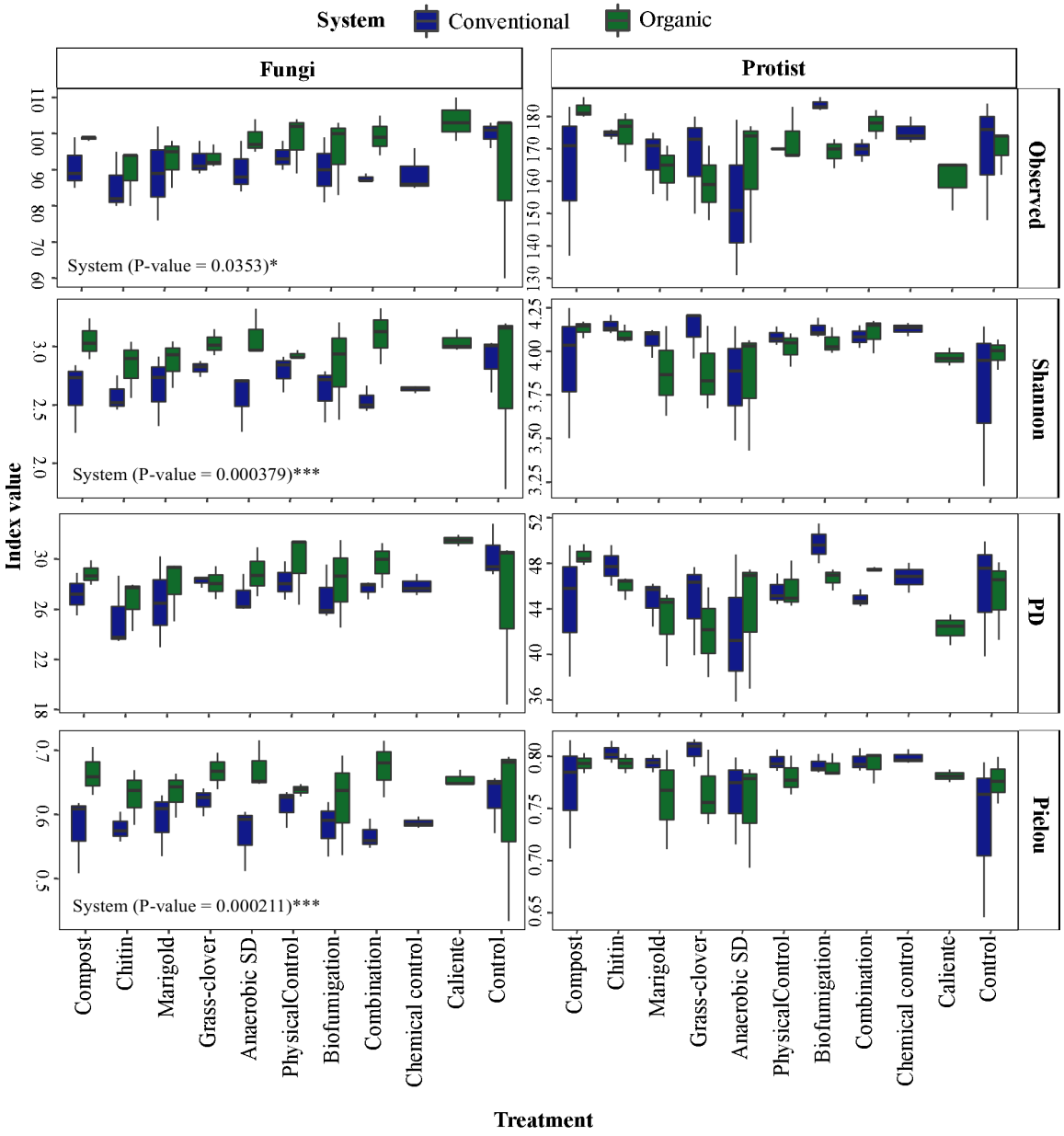

Figure 2. Fungal and protist operational taxonomic units (OTU, 97% cut-off level) richness, diversity and evenness in soil health treatments (SHT) within conventional and organic farming systems. Conventional system is represented in blue and organic in green color. The diversity index values are showed on y-axis and soil health treatments on x-axis. Components of the boxes: bottom and top of box (25th and 75th percentiles), center lines (medians), bottom and top whiskers (1.5 times the interquartile range from the 25th and 75th percentiles). P-value and the significance of farming systems based on two-way ANOVA are represented by * ($P < 0.1$) and *** ($P < 0.001$). *ns* = non-significative. Anaerobic SD = Anaerobic soil disinfestation. PD = Phylogenetic distance.

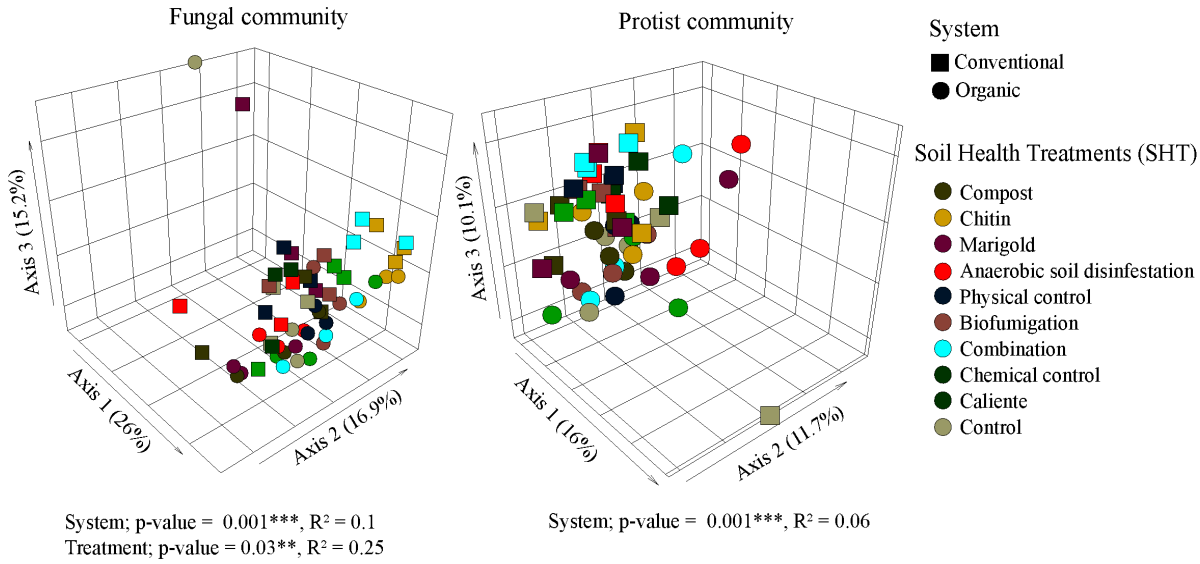

Figure 3. Ordinated community structure based on OTU-level and explained variances of farming system and soil health treatments. Soil health treatments are represented by colors according to the left panel in conventional (represented by square) and in organic (represented by circle) farming systems. The variances were assessed by permutational multivariate analysis of variance (PERMANOVA) based on a distance matrix created using Bray-Curtis method. R^2 indicate the estimation of the variance and significance levels of farming systems and soil health treatments are represented by **P < 0.01 and ***P < 0.001.

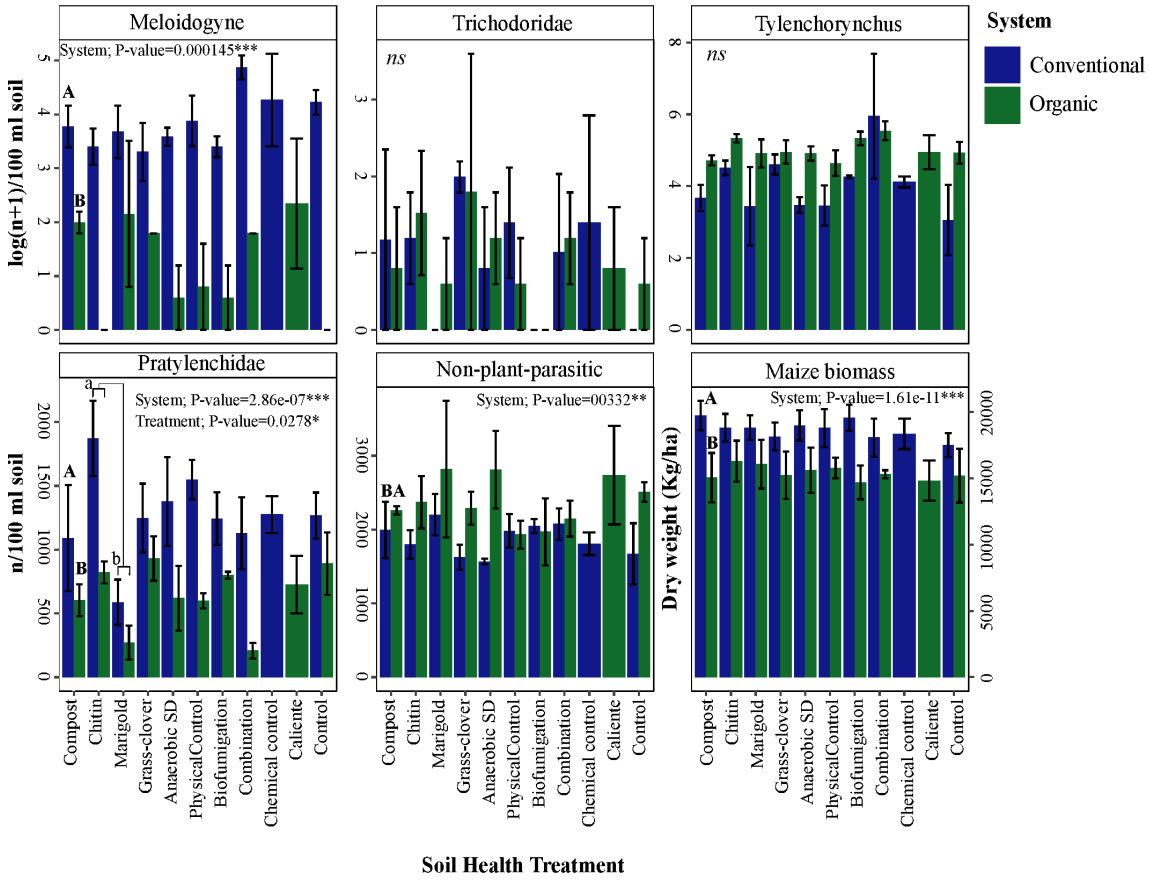

Figure 4. Nematode abundance belonging to parasitic and non-parasitic nematodes and maize biomass (t ha^{-1}) in soil health treatments in conventional and organic farming systems. Organic system is represented in green and conventional in blue color. Average densities (n/100 mL soil or expressed as log(n+1)/100 mL soil) of nematodes are showed on y-axis and soil health treatments on x-axis. Different uppercase letters indicate significant differences between systems and different lowercase letters significant differences between treatments. The significance of farming systems and soil health treatments based on two-way ANOVA are represented by *P < 0.05, **P < 0.01, ***P < 0.001 and *ns* = non-significative. n = number of nematodes.

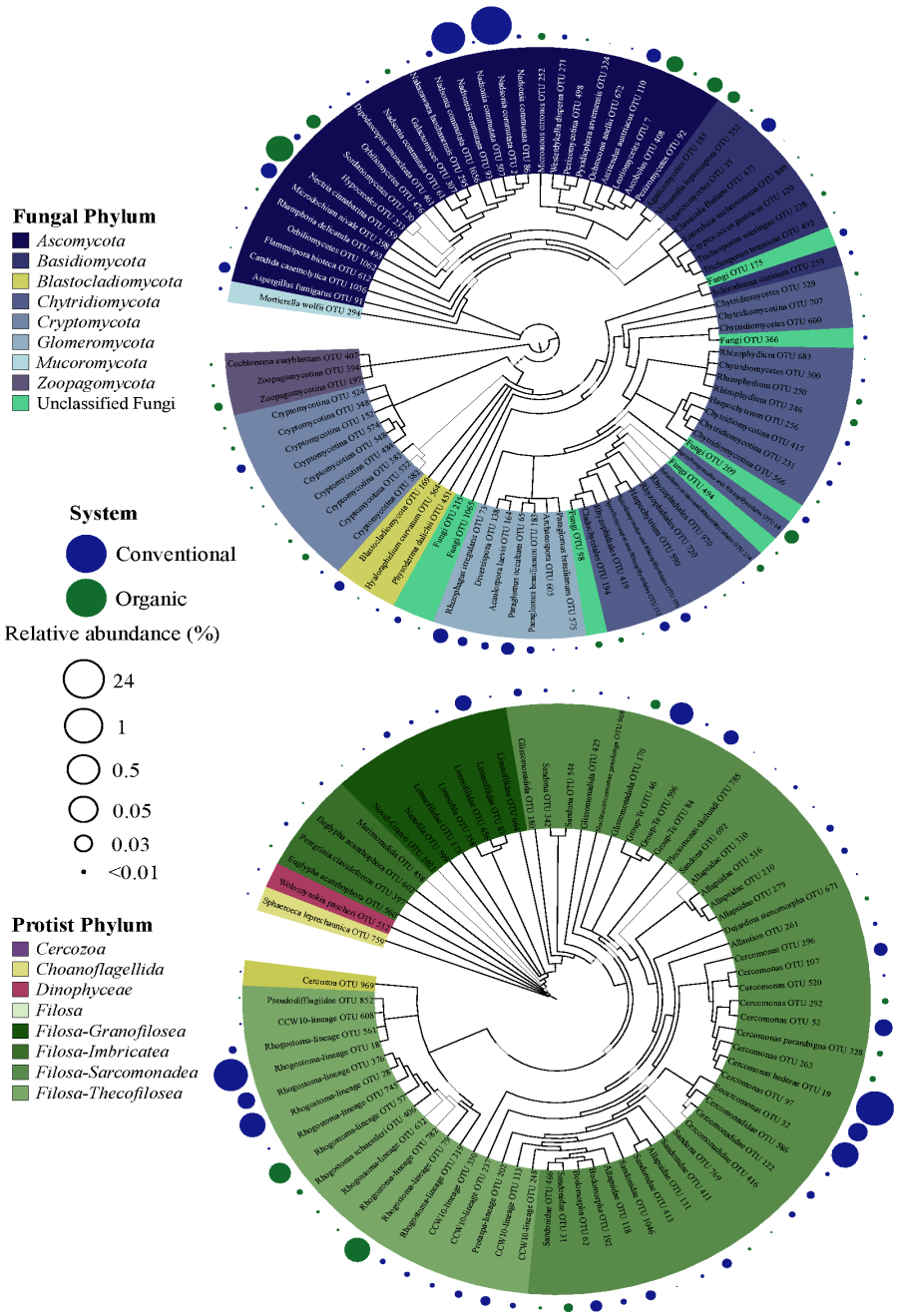

Figure 5. Dendrogram showing the taxonomic information of fungal and protist taxa (OTU level, 97% cutoff) associated with farming systems. The color ranges within the tree identify phyla. The circles outside the tree indicate the association with conventional (in blue) and organic (in green) systems. The diameter of the circles represents the relative abundance (square-root transformed) of the species.

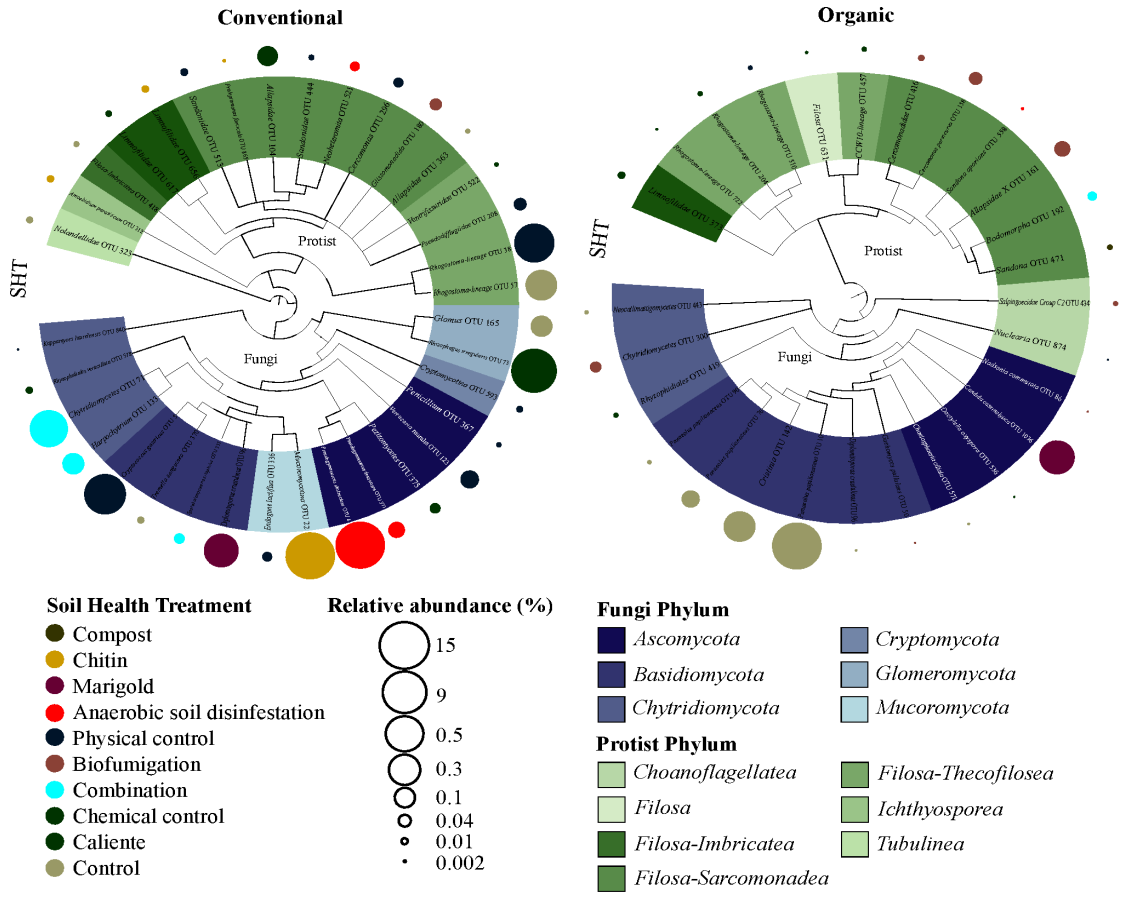

Figure 6. Dendrogram showing the taxonomic information of fungal and protist species (OTU level, 97% cutoff) associated with soil health treatments in farming systems. The color ranges within the tree identify phyla. The fungal phyla are colored by tons of blue and protist phyla by tons of green. The circles outside the tree indicate the association with Soil Health Treatments (their corresponding colors are showed on the left legend). The diameter of the circles represents the relative abundance (square-root transformed) of the species.

Table
Supporting Information File S1

Table S1 Samples names, soil health treatments (SHTs) within farming systems, number of 18S rRNA sequences and sampling coverage of the soil microbiome.

Sample names*	SHT	System	Block	N° of sequences[1](Total)	Good's coverage[2](Total)	N° of sequences[1](Fungi)	Good's coverage[2] (Fungi)	N° of sequences[1](Protist)	Good's coverage[2](Protist)	N° of sequences[1](Other	Good's coverage[2] (Fungi)	Sample unique name**	Sample accession
E1	Control	Organic	1	69550	0.99	46548	0.99	22812	0.99	190	0.97	E1	ERS850211
E2	Chitin	Organic	1	30362	0.99	25196	0.99	5095	0.98	71	0.95	E2	ERS850212
E3	Biofumigation	Organic	1	79832	0.99	62859	0.99	16647	0.99	326	0.97	E3	ERS850213
E4	PhysicalControl	Organic	1	89313	0.99	67696	0.99	21444	0.99	173	0.97	E4	ERS850214
E5	Grass-clover	Organic	1	47512	0.99	37433	0.99	9989	0.99	90	0.94	E5	ERS850215
E6	Marigold	Organic	1	70792	0.99	63285	0.99	7432	0.99	75	0.97	E6	ERS850216
E7	Anaerobic-soil-disinfestation	Organic	1	15539	0.99	11376	0.99	4047	0.98	116	0.95	E7	ERS850217
E8	Combination	Organic	1	48625	0.99	35400	0.99	13005	0.99	220	0.97	E60	ERS850269
E9	Caliente	Organic	1	56043	0.99	41184	0.99	14636	0.99	223	0.98	E9	ERS850218
E10	Compost	Organic	1	16146	0.99	12594	0.99	3517	0.98	35	0.91	E10	ERS850219
E11	Chitin	Conventional	1	12991	0.99	11038	0.99	1911	0.99	42	0.88	E11	ERS850220
E12	Grass-clover	Conventional	1	9677	0.98	8040	0.99	1610	0.99	27	0.77	E12	ERS850221
E13	Chemical-control	Conventional	1	46428	0.99	38581	0.99	7735	0.99	112	0.96	E13	ERS850222
E14	Biofumigation	Conventional	1	82536	0.99	69895	0.99	12495	0.99	146	0.97	E14	ERS850223
E15	Anaerobic-soil-disinfestation	Conventional	1	49634	0.99	39836	0.99	9661	0.99	137	0.96	E15	ERS850224
E16	Combination	Conventional	1	82731	0.99	72662	0.99	9896	0.99	173	0.95	E16	ERS850225
E17	Compost	Conventional	1	21843	0.99	17833	0.99	3875	0.98	135	0.97	E17	ERS850226
E18	Marigold	Conventional	1	55208	0.99	40395	0.99	14540	0.99	273	0.97	E18	ERS850227

Sample names*	SHT	System	Block	N° of sequences[1] (Total)	Good's coverage[2] (Total)	N° of sequences[1] (Fungi)	Good's coverage[2] (Fungi)	N° of sequences[1] (Protist)	Good's coverage[2] (Protist)	N° of sequences[1] (Other	Good's coverage[2] (Fungi)	Sample unique name**	Sample accession
E19	Control	Conventional	1	55853	0.99	46653	0.99	9036	0.99	164	0.96	E19	ERS850228
E20	PhysicalControl	Conventional	1	67445	0.99	53089	0.99	14214	0.99	142	0.95	E20	ERS850229
E61	Grass-clover	Organic	2	22208	0.99	15463	0.99	6721	0.99	24	0.83	E21	ERS850230
E62	Control	Organic	2	33779	0.99	31141	0.99	2617	0.97	21	0.66	E22	ERS850231
E63	Marigold	Organic	2	9404	0.98	7142	0.99	2244	0.97	18	0.77	E23	ERS850232
E64	Chitin	Organic	2	11865	0.99	9976	0.99	1870	0.99	19	0.78	E24	ERS850233
E65	Anaerobic-soil-disinfestation	Organic	2	60778	0.99	42507	0.99	18049	0.99	222	0.98	E25	ERS850234
E66	Compost	Organic	2	54000	0.99	41203	0.99	12588	0.99	209	0.97	E26	ERS850235
E67	Combination	Organic	2	35668	0.99	30614	0.99	4991	0.98	63	0.90	E27	ERS850236
E68	Caliente	Organic	2	64354	0.99	45300	0.99	18807	0.99	247	0.99	E28	ERS850237
E69	PhysicalControl	Organic	2	54828	0.99	41777	0.99	12872	0.99	179	0.98	E29	ERS850238
E70	Biofumigation	Organic	2	80400	0.99	64899	0.99	15265	0.99	236	1	E30	ERS850239
E71	Control	Conventional	2	59326	0.99	32306	0.99	26596	0.99	424	0.99	E31	ERS850240
E72	Marigold	Conventional	2	63980	0.99	52392	0.99	11411	0.99	177	0.99	E32	ERS850241
E73	Chemical-control	Conventional	2	72958	0.99	53261	0.99	19457	0.99	240	0.99	E33	ERS850242
E74	Anaerobic-soil-disinfestation	Conventional	2	47403	0.99	35212	0.99	12046	0.99	145	0.97	E34	ERS850243
E75	Compost	Conventional	2	69085	0.99	53849	0.99	15019	0.99	217	0.98	E35	ERS850244
E76	Grass-clover	Conventional	2	33749	0.99	26542	0.99	7103	0.99	104	0.95	E36	ERS850245
E77	PhysicalControl	Conventional	2	47712	0.99	37357	0.99	10222	0.99	133	0.93	E37	ERS850246
E78	Biofumigation	Conventional	2	67843	0.99	40487	0.99	27116	0.99	240	0.98	E38	ERS850247
E79	Combination	Conventional	2	56231	0.99	46695	0.99	9389	0.99	147	0.95	E39	ERS850248
E80	Chitin	Conventional	2	69323	0.99	53822	0.99	15080	0.99	421	0.98	E40	ERS850249
E101	Chitin	Conventional	3	74463	0.99	59535	0.99	14701	0.99	227	0.97	E41	ERS850250
E102	Grass-clover	Conventional	3	63092	0.99	47328	0.99	15593	0.99	171	0.98	E42	ERS850251

Sample names*	SHT	System	Block	N° of sequences[1] (Total)	Good's coverage[2] (Total)	N° of sequences[1] (Fungi)	Good's coverage[2] (Fungi)	N° of sequences[1] (Protist)	Good's coverage[2] (Protist)	N° of sequences[1] (Other	Good's coverage[2] (Fungi)	Sample unique name**	Sample accession
E103	Compost	Conventional	3	36436	0.99	29997	0.99	6385	0.99	54	0.90	E43	ERS850252
E104	Anaerobic-soil-disinfestation	Conventional	3	45748	0.99	35893	0.99	9769	0.99	86	0.94	E44	ERS850253
E105	Biofumigation	Conventional	3	44283	0.99	34426	0.99	9677	0.99	180	0.98	E45	ERS850254
E106	Marigold	Conventional	3	64107	0.99	53800	0.99	10229	0.99	78	0.89	E46	ERS850255
E107	PhysicalControl	Conventional	3	72523	0.99	56852	0.99	15449	0.99	222	0.98	E47	ERS850256
E108	Combination	Conventional	3	28499	0.99	23557	0.99	4887	0.98	55	0.90	E48	ERS850257
E109	Chemical-control	Conventional	3	83657	0.99	64258	0.99	19234	0.99	165	0.95	E49	ERS850258
E110	Control	Conventional	3	82550	0.99	58290	0.99	23952	0.99	308	0.99	E50	ERS850259
E111	Caliente	Organic	3	38768	0.99	26429	0.99	12209	0.99	130	0.94	E51	ERS850260
E112	Marigold	Organic	3	17788	0.99	15973	0.99	1796	0.99	19	0.78	E52	ERS850261
E113	Anaerobic-soil-disinfestation	Organic	3	50103	0.99	34662	0.99	15293	0.99	148	0.97	E53	ERS850262
E114	Control	Organic	3	65566	0.99	47389	0.99	17722	0.99	455	0.99	E54	ERS850263
E115	PhysicalControl	Organic	3	21712	0.99	14886	0.99	6773	0.99	53	0.88	E55	ERS850264
E116	Compost	Organic	3	33083	0.99	25060	0.99	7978	0.99	45	0.91	E56	ERS850265
E117	Biofumigation	Organic	3	48733	0.99	37084	0.99	11545	0.99	104	0.96	E57	ERS850266
E118	Grass-clover	Organic	3	67696	0.99	54044	0.99	13479	0.99	173	0.98	E58	ERS850267
E119	Chitin	Organic	3	21979	0.99	16214	0.99	5693	0.98	72	0.91	E59	ERS850268
E120	Combination	Organic	3	15	0.66	11	0.72	4	0.5	0	na	E0	ERS850210

1 Number of sequences for total eukaryotic microbiome, fungi, protist and other eukaryotic taxa after filtering and removal of sequences with no blast hit (12 sequences).

2 Good's estimator of coverage was calculated using the formula: (1−(singletons/individuals)) × 100 for complete eukaryotic dataset.

* Samples names used on the manuscript according to experimental design and ** corresponding sample unique names used on ENA database.

Table S2 Average densities (n/100 ml soil) of nematodes belonging to parasitic and non-plant-parasitic nematodes and maize yield (t ha^{-1}) in 2013 in soil health treatments withing conventional and organic farming systems.

	System											
	Conventional						Organic					
SHT*	Meloidogyne	Pratylenchidae	Tylenchoryncus	Trichodoridae	Not plant parasitic	Yield maize	Meloidogyne	Pratylenchidae	Tylenchorynchs	Trichodoridae	Not plant parasitic	Yield maize
	n/100ml					Kg/ha	n/100ml					Kg/ha
CO	49.0(28.5)	1091.0(720.6)	44.3(30.9)	11.0(19.1)	1995.0(663.2)	19.7(1.9)	6.7(2.9)	603.3(217.6)	113.3(27.5)	3.3(5.8)	2258.3(101.2)	15.1(3.2)
CH	32.3(17.5)	1871.7(509.0)	93.3(29.3)	3.3(2.9)	1797.7(330.9)	18.8(1.8)	0.0 (0.0)	822.3(150.3)	209.0(39.7)	6.7(7.6)	2369.3(609.3)	16.3(2.7)
MA	47.7(34.1)	588.3(307.0)	71.0(84.1)	0.0(0.0)	2201.7(481.5)	18.8(1.6)	36.7(59.2)	271.7(229.8)	157.7(110.4)	1.7(2.9)	2816.7(1600.9)	16.1(3.2)
GC	35.0(31.2)	1248.3(465.4)	106.7(0.3)	6.7(2.9)	1625.0(297.0)	18.2(1.8)	5.0(0.0)	930.0(299.6)	156.7(94.6)	73.3(127.0)	2290.0(385.4)	15.2(3.1)
AD	36.0(10.1)	1377.3(602.4)	32.7(11.0)	3.3(5.8)	1565.7(65.4)	19.0(2.0)	1.7 (2.9)	620.0(439.7)	140.0(52.0)	3.3(2.9)	2811.7(907.6)	15.6(3.0)
PH	58.3(43.1)	1546.7(269.0)	43.3(44.8)	5.0(5.0)	1981.7(393.5)	18.8(2.5)	3.3 (5.8)	598.3(102.5)	115.0(58.9)	1.7(2.9)	1931.7(324.5)	15.8(1.3)
BF	30.0(10.0)	1243.3(356.5)	70.0(5.0)	0.0(0.0)	2046.7(164.6)	19.6(1.7)	1.7 (2.9)	800.0(45.8)	213.3(75.2)	0.0(0.0)	1968.3(781.3)	14.7(2.2)
CB	136.7(53.5)	1128.3(486.9)	4083.3(6947.0)	6.7(11.5)	2075.0(367.5)	18.1(2.5)	5.0 (0.0)	208.3(104.0)	273.3(136.0)	3.3(2.9)	2148.3(421.4)	15.3(0.5)
CC	146.7(198.1)	1275.0(250.4)	61.7(15.3)	21.7(37.5)	1808.3(267.4)	18.4(2.0)	-	-	-	-	-	-
CL	-	-	-	-	-	-	24.3(26.8)	725.7(388.3)	176.7(151.4)	3.3(5.8)	2736.0(1154.8)	14.8(2.6)
CT	71.0(27.6)	1267.7(310.8)	53.3(79.4)	0.0(0.0)	1671.7(715.5)	17.5(1.6)	0.0 (0.0)	891.0(423.3)	148.7(66.7)	1.7(2.9)	2507.7(231.8)	15.2(3.5)

*The SHTs are represented by CO: compost, CH: chitin, MA: Marigold, GC: grass-clover, AD: anaerobic soil disinfestation, PH: physical control, BF: biofumigation, CB: combination, CC: chemical control, CL: caliente and CT: control treatment.

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DISCUSSÃO

O Soil Health Experiment (SHE) fornece uma visão integrada da influência de sistemas agrícolas e tratamentos alternativos para o controle de patógenos do solo (Soil Health Treatment, SHR) em componentes multitróficos em um agroecossistema. Em estudo anterior, os tratamentos do solo foram avaliados dentro do sistema convencional sobre os efeitos na comunidade do nematóide *P. penetrans* e do fungo *V. dahliae*, ambos parasitas de plantas (Korthals et al., 2014). Alguns tratamentos foram mais eficazes no controle desses patógenos que o controle químico, por exemplo os tratamentos combinação, quitina, desinfestação anaeróbica e *Tagetes*. Em contraste, outros tratamentos como *Grass-clover*, controle físico, biofumigação e composto não foram alternativas eficazes. No entanto, o microbioma e a meiofauna do solo não foram completamente acessados, limitando o entendimento da função da comunidade microbiana neste contexto. Mais de oito anos de contínuo manejo convencional e orgânico alterou a diversidade e a estrutura do microbioma do solo, assim como determinou a presença de determinadas populações da meiofauna e a produtividade de plantas. Táxons microbianos classificados em diferentes grupos taxonômicos foram associados com sistemas agrícolas ou tratamentos alternativos, e muitos considerados importantes para os padrões encontrados entre a microbiota e a meiofauna do solo. Embora estudos sugerem que a comunidade microbiana é temporal e espacialmente estruturada, o acesso do microbioma do solo determinado pelo DNA representa uma comunidade estável e ubíqua de microrganismos, muitos dos quais podem estar inativos, atuando como uma reserva de diversidade e organismos benéficos que podem ser explorados em estudos futuros (Girvan et al., 2004; Williams et al., 2014).

A diversidade e a estrutura das comunidades microbianas foram largamente determinadas pelos sistemas agrícolas, sendo que um maior efeito foi observado na comunidade de bactérias e fungos do solo do que na comunidade de protistas. O sistema orgânico promoveu o aumentou da riqueza, diversidade e equitabilidade das comunidades de bactérias e fungos em comparação com o sistema convencional; a diversidade da comunidade de protistas não apresentou uma resposta aos sistemas de cultivo. A resposta dos grupos microbianos aos sistemas de manejo não é completamente claro (Schneider et al., 2014) e devido à diferenças na fisiologia e ecologia de comunidades de bactérias, fungos e protistas (Pereira e Silva et al., 2012), os mesmos podem responder de diferentes maneiras em condições similares (Reganold e Wachter, 2016). O aumento da diversidade microbiana de

bactérias e fungos esta ligado às práticas de manejo utilizadas nesse sistema, incluindo a ausência de agroquímicos e uso controle biológico (Sun et al., 2004; Chaudhry et al., 2012). Além disso, tem sido observado um aumento da diversidade microbiana através da disponibilidade de recursos e diferenciação de nichos no solo promovidos pelos manejo orgânico (Kamaa et al., 2011). Uma comunidade mais diversa possivelmente contribua para a presença de populações funcionais redundantes e estáveis, a qual interage com outros componentes tróficos do solo, como a presença de certos organismos da meiofauna do solo (Brussaard et al., 2007; Postma et al., 2008; Crowder et al., 2010; Wagg et al., 2014). Além disso, nosso estudo revelou uma comunidade de bactérias mais heterogênea em sistema orgânico, enquanto o sistema convencional promoveu uma maior homogeneidade da comunidade. Não foi observada diferenças na heterogeneidade de fungos e protistas do solo nos diferentes sistemas agrícolas (dados não mostrados). A diminuição da diversidade (bactérias e fungos) e heterogeneidade (bactérias) das comunidades microbianas no sistema convencional é principalmente causada pela redução dos nichos ecológicos provocados pelo manejo intensivo do solo (Olden et al., 2004; Constancias et al., 2013; Guan et al., 2013; Figuerola et al., 2014). A homogeneização das comunidades, conhecido como homogeneização biótica, é um efeito comum dos sistemas convencionais em animais e plantas (Gabriel et al., 2006), e recentemente foi relatado para as comunidades microbianas como uma resposta ao cultivo a longo prazo (Montecchia et al., 2015) e a remoção de florestas nativas para uso agrícola do solo (Rodrigues et al., 2013).

A meiofauna do solo e a produtividade de planta foram principalmente determinadas pelos sistemas convencionais e orgânicos, mas não foram detectadas significantes efeitos dos tratamentos alternativos. Em sistemas orgânicos, a dimunição do número de nematóides parasitas e um aumento de nematóides de vida livre sugere que o manejo orgânico pode suprimir a comunidade de patógenos do solo e promover condições benéficas aos organismos de vida livre, importantes componentes de procesos do solo, responsáveis por regular a população de organismos de outros níveis tróficos e decomposição da matéria orgânica do solo (Oka, 2010; Reilly et al., 2013). Ao contrário dos efeitos dos tratamentos alternativos observados na comunidade de nematóides do solo em prévio estudo (Korthals et al., 2014), os resultados mostrados nessa tese mostram que a população da meiofauna solo não foi fortemente influenciada. Após 2 anos de última aplicação de tratamentos no solo, o restabelecimento da população de nematóides indica que essa comunidade é resiliente à

distúrbios do solo causados pelos tratamentos alternativos, os quais podem ter um efeito de curto prazo no controle de patógenos (Carrascosa et al., 2014). Em nosso estudo, também observamos que a agricultura orgânica pode produzir rendimentos mais baixos em comparação com a agricultura convencional. Embora esperado que as práticas menos sustentáveis aplicados em sistemas convencionais e a maior população de organismos patogênicos possa reduzir a produtividade das plantas em longo prazo (Alaru et al., 2014), a produtividade das plantas pode depender de vários factores interligados, em vez de um único aspecto da gestão. Por exemplo, tem sido demonstrado que sistemas convencionais promovem uma maior produtividade de plantas comparado ao sistema orgânico devido principalmente à rápida disponibilidade de nutrientes dos fertilizantes químicos (Khalil et al., 2016).

Os sistemas agrícolas determinaram o padrão de múltiplos componentes tróficos, os quais podem estar ecologicamente relacionados dentro de um contexto de interações promovidas pelos sistemas convencional e orgânico. Essas relações entre organismos que compõe as "food-webs" do solo são refletido ao longo das cadeias tróficas, determinando diversos padrões em agroecossistemas agrícolas, como biomassa de planta e supressão de patógenos do solo (Scherber et al., 2010; Eisenhauer et al., 2013). A diversidade e a composição da comunidade de fungos e bactérias pode ter sido um dos principais fatores explicando a composição da meiofauna do solo. Estudos recentes mostram que a diversidade da comunidade microbiana é positivamente correlacionada com a diminuição de doenças em plantas, e responsável por múltiplos processos no solo (Zavaleta et al., 2010; Bonilla et al., 2012). Além da diversidade, a estrutura da comunidade representada por um conjunto de espécies microbianas pode determinar alguns dos principais processos envolvidos na supressão de fungos e nematóides patógenos de plantas (Harrier e Watson, 2004). Várias grupos microbianos pertencentes à bactérias, fungos e protistas foram associados aos sistemas agrícolas e tratamentos alternativos do solo, e podem estar relacionados à população da meiofauna do solo, principalmente ao controle de patógenos.

Por exemplo, táxons microbianos pertencentes a *Bacillus* e *Clostridiales* (*Firmicutes*) foram associados com tratamentos eficazes no controle de *P. penetrans* e *V. dahliae*, especialmente no tratamento desinfestação do solo anaeróbica. Espécies pertencentes à família *Bacillales* familiares são econômica e agronomicamente importantes por serem responsáveis pela supressão de fungos do solo causadores de doenças e nematóides parasitas de plantas através da produção de compostos antimicrobianos, toxinas formadoras de poros

(proteínas de cristal) e indução de resistência de plantas (van Loon et al., 1998; Wei et al., 2003; Mowlick et al., 2012). Membros microbianos do gênero *Lysobacter* foram associados ao tratamento quitina e podem ajudar a elucidar os mecanismos que inibem a população de nematóides nesse tratamento. Bactérias do gênero *Lysobacter* possuem propriedades quitinolíticas que podem degradar a parede celular dos fungos e os ovos de nematóides (Nour et al., 2003; Postma et al., 2008; Oka, 2010). Táxons pertencentes a *Flavobacteriales* e *Chitinophagaceae* foram observados no tratamento com *Tagetes*, sugerindo que além de produzir compostos nematicidas, essa planta também pode recrutar microrganismos antagonistas à certas espécies de nematóides (Tian et al., 2007; Hooks et al., 2010; Kharade e McBride, 2013). Bactérias do gênero *Chitinophaga* e a maioria das espécies de fungos parasitas (*Rhizophydium*, *Hyaloraphidium*, *Zoopagomycotina* e *Cryptomycotina*) foram associados com o sistema orgânico e portanto reduzidos em sistema convencional. Apesar da pouca informação sobre o processo envolvido na supressão de patógenos, esses táxons tem sido descritos na literatura como possíveis candidatos responsáveis pela redução de nematóides do solo (Stirling, 1991; Kharade e McBride, 2013; Benny et al., 2014).

CONCLUSÃO GERAL

No geral, os resultados desse estudo indicam que os sistemas de cultivo convencional e orgânico exibem efeitos convergentes em múltiplos componentes bióticos em um agroecossistema. Além disso, os sistemas agrícolas exercem uma grande influência sobre a diversidade e composição de comunidades microbianas, meiofauna do solo e produtividade de plantas, enquanto os efeitos dos tratamentos alternativos para o controle de patógenos solo são de menor magnitude. O sistema orgânico promove efeitos benéficos sobre a diversidade e a heterogeneidade da microbiota, principalmente na comunidade de bactérias e fungos do solo; não foi observado efeito na diversidade de protozoários do solo. No sistema orgânico também foi encontrado uma maior população de nematóides de vida livre, enquanto nematóides parasitas e a produtividade de plantas foram menores no sistema convencional. Embora a diversidade de bactérias e fungos parecem estar relacionados com a população da meiofauna do solo, a população de nematóides parasitas pode estar mais relacionado com a presença de certas espécies microbianas associados com os sistema orgânico e determinados tratamentos alternativos. Ainda não está claro como as mudanças na diversidade e composição do microbioma está relacionado ao funcionamento dos ecossistemas e quais as consequências a longo prazo. Porém, os resultados apresentados nesse estudo fornecem novos conhecimentos ecológicos em relação ao microbioma do solo, interações multitróficas e controle de patógenos em agroecossistemas, indicando alternativas originais para melhorar a sustentabilidade dos ecossistemas agrícolas por meio de microrganismos benéficos.

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