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Cover cropping and no-till increase diversity and symbiotroph:saprotroph ratios of soil fungal communities

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ABSTRACT

Fungi are important members of soil microbial communities in row-crop and grassland soils, provide essential ecosystem services such as nutrient cycling, organic matter decomposition, and soil structure, but fungi are also more sensitive to physical disturbance than other microorganisms. Adoption of conservation management practices such as no-till and cover cropping shape the structure and function of soil fungal communities. No-till eliminates or greatly reduces the physical disturbance that re-distributes organisms and nutrients in the soil profile and disrupts fungal hyphal networks, while cover crops provide additional types and greater abundance of organic carbon sources. In a long-term, row crop field experiment in California's Central Valley we hypothesized that a more diverse and plant symbiont-enriched fungal soil community would develop in soil managed with reduced tillage practices and/or cover crops compared to standard tillage and no cover crops. We measured the interacting effects of tillage and cover cropping on fungal communities based on fungal ITS sequence assigned to ecological guilds. Functional groups within fungal communities were most sensitive to long-term tillage practices, with 45% of guild-assigned taxa responding to tillage, and a higher proportion of symbiotroph taxa under no-till. In contrast, diversity measures reflected greater sensitivity to cover crops, with higher phylogenetic diversity observed in soils managed with cover crops, though only 10% of guild-assigned taxa responded to cover crops. The relative abundance of pathotrophs did not vary across the management treatments. Cover cropping increased species diversity, while no-till shifted the symbiotroph:saprotroph ratio to favor symbiotrophs. These management-induced shifts in fungal community composition could lead to greater ecosystem resilience and provide greater access of crops to limiting resources.

1. Introduction

In the complex soil ecosystem, fungi make up functionally and phylogenetically diverse communities comprised of many distinct ecological guilds (Nguyen et al., 2016). The net effects of their activities influence soil health, carbon sequestration and crop yields. Saprotrophic fungi are involved in organic matter decomposition, carbon cycling, nutrient mobilization, and creating soil structure (Kramer et al., 2012; van der Wal et al., 2013). Symbiotrophic fungi vastly expand the surface area of plant roots, giving plants greater access to nutrients and water in exchange for carbon (Kramer et al., 2012). Pathotrophic fungi attack crop plants but also control populations of nematodes, insects and other animal, plant or fungal pests (Vega et al., 2009; Raza et al., 2017; Wang and Wang, 2017). Fungi are major agents in creation and maintenance of soil structure through secretion of extracellular compounds and physical binding of soil via hyphae

(Bergmann et al., 2016; Horn et al., 2017). Higher fungal biomass (Helgason et al., 2009), species richness and diversity have been observed in the top 5–10 cm than deeper in the soil profile (Rahman et al., 2008; Degruene et al., 2016; Schlatter et al., 2018). Fungi can adapt to intensive agricultural practices, such as excessive tillage and reduction of carbon inputs. However, long fallow periods and high rates of fertilizer application have been shown to reduce fungal biomass, diversity and arbuscular mycorrhizal fungi (AMF) root colonization rates (Tsiafouli et al., 2015), potentially diminishing the fungal contribution to soil ecosystem services (Bender et al., 2016).

Studies of tillage disturbance effects on the soil fungal community have shown mixed results. Some studies, based on phospholipid fatty acid analyses, found increased ratios of fungal to bacterial biomass in no-till systems (Schutter et al., 2001; Acosta-Martinez et al., 2010), no changes (Feng et al., 2003; Helgason et al., 2009; Mathew et al., 2012) or lower fungi to bacteria (F:B) ratios under no-till (Mbuthia et al.,

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2015). DNA sequencing studies have reported both increases in fungal diversity (Wang et al., 2010) and no changes to diversity (Dong et al., 2017) in systems that adopted no-till. These variations reflect the complexity of the soil environment, and can be due to different soil conditions, intensities and duration of land use, climatic conditions, plant cover or other factors. Effects on specific functional groups of microorganisms, however, seem to be more consistent in their findings. For example, AMF consistently experience reduction in numbers, root colonization rates and diversity with tillage (Helgason et al., 2010; Schnoor et al., 2011; Wetzel et al., 2014; Sale et al., 2015; Jesus et al., 2016). Far less is known about other groups of fungi such as the saprotrophs that make up 50–80% of the overall fungal community (Balser et al., 2005; Nguyen et al., 2016), although saprotrophs that colonize fresh, mature plant residues turned into the soil by tillage have been shown to increase under standard tillage (Sharma-Poudyal et al., 2017).

The diversity and duration of seasonal coverage by plants also play a role in shaping soil microbial communities due to the quantity and variety of organic substrates they provide via exudates and residues to the soil community (Cline et al., 2018; Sievers and Cook, 2018; Zhahnina et al., 2018). A meta-analysis of 122 studies concluded that microbial biomass increased by 20.7% when one or more crops were added to a monoculture, and the biomass increases correlated with increases in soil C (3.6%) and N pools (5.3%) (McDaniel et al., 2014). Where cover crops specifically were included in the rotation, much greater increases in C and N were noted (8.5 and 12.8% respectively), likely due to the longer period under plant cover resulting in greater root inputs (McDaniel et al., 2014). In turn, the greater microbial diversity and biomass observed in some soils with higher SOM has been shown to play a role in suppression of some root rot and *Rhizoctonia* disease (Page et al., 2013; Larkin, 2015). More specific effects are linked to crop and cover crop diversity. Increases in numbers of plant symbiotrophs, such as AMF, under more plant-diverse crop rotation and cover crop systems, have been linked to increases in niche diversity (Jesus et al., 2016).

The long-term effects of no-till and cover cropping have been studied extensively in temperate, rain fed systems, but their impacts on soils in irrigated Mediterranean systems have received far less attention (Mitchell et al., 2015). In semi-arid climates, impacts might be expected to differ from other climates due to extreme summer temperatures, existence of wet-dry rather than freeze-thaw cycles and reduced periods of precipitation. A field scale no-till and cover cropping experiment has been in place at the UC Davis West Side Research and Extension Center at Five Points, CA since 1999 (Wang et al., 2010). No-till and cover cropping have led to significant changes in soil C, N and other soil characteristics, and also distinctly different bacterial communities from those in conventional agricultural systems that do not use these practices (Veenstra et al., 2006; Mitchell et al., 2017).

Farming practices have specific and distinct effects on different phylogenetic groups. We recently reported the analysis of tillage influences on bacterial communities at Five Points using a functional trait assignment to bacterial 16S rDNA sequence data. We linked changes in traits associated with increased specialization and rapid response to substrate availability to homogenization of environmental micro-niches and periodic release of nutrients rather than physical disturbance alone (Schmidt et al., 2018). These results are in line with growing appreciation of the importance of analyzing soil processes at the microbial scale in order to interpret molecular data describing microbial communities (Bach et al., 2018). Moreover, the effects of tillage on the bacterial community were largely eclipsed by cover cropping effects (Schmidt et al., 2018). Similar analyses have not been performed, until this time, on the effects of tillage and cover crops on soil fungal communities.

The purpose of this study was to describe the effects of two conservation agriculture practices – no-till and cover cropping – on the composition and functions of resident fungal communities in a

Mediterranean climate row crop system under a tomato-cotton rotation. Using fungal ITS sequence data, we conducted both a phylogenetic and functional group analysis based on the recently developed open annotation tool FunGuild (Nguyen et al., 2016) to assign fungal taxa into three ecologically relevant trophic modes – saprotrophy, symbiotrophy and pathotrophy. These modes were further subdivided into specific guilds comprised of fungi that share similar lifestyle modes (e.g. animal pathogens, endophytes, mycorrhizae) (Nguyen et al., 2016), and their relative abundances were linked to soil physicochemical data and specific management practices. We hypothesized that:

- fungal communities under different management strategies will diverge both in phylogenetic composition and relative proportions of saprotrophs, symbiotrophs and pathotrophs.
- standard tillage will alter the functional composition of soil fungal communities, selecting for higher relative numbers of saprotrophic taxa.
- amendment with cover crops will select for higher relative numbers and greater diversity of symbiotrophic taxa.

2. Materials and methods

2.1. Site description

The 427 m by 100 m study site is located at the University of California's West Side Research and Extension Center (<http://ucanr.edu/sites/westsiderec>) in Five Points, CA (36°20'29"N, 120°7'14"W). The soil is a Panoche clay loam (fine-loamy, mixed superactive, thermic Typic Haplocambids) (Arroues, 2006). Four treatments of tomato-cotton rotations in place since 1999 included – no-till (NTNO), no-till plus cover crop (NTCC), standard tillage (STNO), and standard tillage plus cover crop (STCC). All systems had buried drip irrigation. Each treatment consisted of four replicate plots arranged in a semi-randomized block system, for a total of 32 914 m² plots (8 blocks, each containing the 4 study treatments in random order). We focused on the southern half of the experimental site, specifically on plots 1–16 that were under tomato crop in 2013. Soil chemical and physical parameters were collected regularly in the fall between 1999 and 2013 at two depths: 0–15 cm and 15–30 cm below ground surface (bgs) (Table S2; Dhainaut Medina, 2015; Mathesius, 2015). Data from 2013 were used in statistical analyses.

Soil samples were collected on 11/22/2013. Twelve one-inch diameter cores were collected from each of three depths (0–5, 5–15 and 15–30 cm bgs) in each plot. The twelve cores from each plot/depth were homogenized, placed on ice in the field, and stored at –20 °C until analysis.

2.2. Crops and cover crops

The two-year crop rotations consist of processing tomatoes (*Solanum lycopersicum* L.) and cotton (*Gossypium hirsutum* L.). The cover crop (CC) mix included Juan triticale (*Triticosecale* Wittm.), Merced rye (*Secale cereale* L.) and common vetch (*Vicia sativa* L.). In 2010 the CC mix was expanded to include pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.), radish (*Raphanus sativus*), and Phacelia (*Phacelia tanacetifoli*) (Mitchell et al., 2015). Treatments received pre-plant fertilizer (11–52–0 N–P–K) applied at 89.2 kg ha^{–1} (9.8 kg ha^{–1} N and 46.4 kg ha^{–1} P). Additional N (urea) was side dress applied at 111.5 kg ha^{–1} for a total of 51.3 kg N ha^{–1} about 4 weeks after transplanting (Mitchell et al., 2015).

2.3. DNA extraction

Soil DNA was extracted in triplicate from 0.25 g (total humid weight) of soil using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's

instructions. DNA extraction was performed in triplicate for each soil sample. The quality and relative quantity of the extracted DNA was determined using a Qubit Fluorometer (Invitrogen, NJ, USA).

2.4. Sequencing

Amplification of the ITS1 region of the ribosomal RNA gene was carried out using primer pair ITS1F (CTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) that had a six-nucleotide barcode and a partial Illumina adapter (Mueller et al., 2014). All primer sequences and a detailed PCR protocol are provided in Supplemental Table 1. Libraries were sequenced using an Illumina MiSeq system, generating 250bp paired-end amplicon reads. The amplicon data was multiplexed using dual barcode combinations for each sample. Amplicons were mixed at roughly equivalent ratios based on electrophoretic band intensity and purified using Agencourt Ampure XP magnetic bead purification kit (Beckman Coulter, CA, USA). Pooled samples were submitted to the University of California Davis Genome Center - 250-bp paired-end sequencing on the MiSeq platform. Raw Illumina fastq files were demultiplexed, quality filtered (Q30), and analyzed using QIIME 1.8.8 pipeline and the GreenGenes 13.5 reference database. QIIME was used to assign Operational Taxonomic Units (OTUs) using UCLUST, with a threshold of 97% pairwise identity. Unweighted Unifrac distances were used to estimate Beta diversity. Raw sequence data was submitted to the NCBI Sequence Read Archive (SRA) (<https://www.ncbi.nlm.nih.gov/sra>) – project accession number PRJNA501845.

2.5. Functional group analysis

To identify different functional groups within fungal communities and link their relative abundance to particular management practices, we used a recently developed open annotation tool (FunGuild (Nguyen et al., 2016);) to assign fungal taxa into three ecologically relevant trophic modes – saprotrophy, symbiotrophy and pathotrophy. These modes were further subdivided into specific guilds comprised of fungi that share similar lifestyle modes (e.g. animal pathogens, endophytes, mycorrhizae) (Nguyen et al., 2016).

2.6. Statistical analysis

Data were analyzed using R statistical software package in RStudio version 0.99.446 (RStudio, Inc. 2015) as a mixed model with depth, tillage and cover cropping as fixed effect variables. The significance level for the variables and their interactions was set at 0.05. Canonical Correspondence Analysis (CCA) (R package Vegan (Oksanen et al., 2015)) was used to model community composition constrained by soil physicochemical parameters. Shannon, Simpson, inverse Simpson, richness and evenness (R package Vegan (Oksanen et al., 2015)) were used as diversity indicators. Spearman rank correlation analysis was carried out using R package Hmisc (Harrell and Dupont, 2008). As physicochemical data were available for two depths only (0–15 and 15–30 cm), the sequence data from 0 to 5 and 5–15 depths were combined as weighted sum averages for all comparisons to physicochemical data. Data for fungal numbers were log transformed for analysis to meet the assumption of homogeneity of variance. FunGuild software (Nguyen et al., 2016) was used to assign fungal OTUs to trophic groups and guilds with subsequent manual BLAST searches employed to identify and assign additional OTUs not formally assigned by FunGuild.

3. Results

3.1. Phylogenetic markers and diversity

Fungal communities were dominated by the phylum Ascomycota, with Zygomycota and Basidiomycota accounting for less than 10% of assigned sequences, and other phyla accounting for less than 1%

(Table 1). At the phylum level, Basidiomycota showed significantly higher relative numbers under no-till, Ascomycota relative numbers were higher in the absence of cover crops, Zygomycota and Chytridiomycota were higher with cover crops and Glomeromycota relative numbers were lowest in the 0–5 cm bgs soil depth (Table 1). The top 42 genera (each representing at least 0.1% of total) made up 90% of total fungi. More than half of these (23 genera; 42% of total fungi) were sensitive to tillage, with an essentially equal split between genera significantly higher under no-till - *Peziza*, *Chaetomium*, *Pseudogymnoascus*, *Gibberella*, *Neosetophoma*, *Cryptococcus*, *Microascales*, *Geomyces*, *Cordyceps*, *Phoma* and *Haematonectria* (21% of total fungi; Table S3), and standard till - *Pseudeurotium*, *Heydenia*, *Preussia*, *Gymnostellatospora*, *Podospora*, *Pithoascus*, *Schizothecium*, *Chaetomidium*, *Ascobolus*, *Doratomyces*, *Gelasinospora* and *Cladorrhinum* (21% of total fungi; Table S3). In contrast, less than one quarter of the top genera were sensitive to cover cropping. These included *Chaetomium*, *Mortierella*, *Cryptococcus*, *Microascales*, *Ascobolus*, *Plectosphaerella*, *Pleospora*, *Cladorrhinum* and *Phialophora* (11% of total fungi; Table S3), all showing significantly higher relative numbers with cover crops. Only three of the top genera showed significant differences with soil depth; *Gymnostellatospora*, *Pithoascus* and *Doratomyces* (3.4% of total fungi; Table S3), all had significantly lower numbers in the surface 0–5 cm of soil.

Fungal diversity at the genus level, measured by Shannon diversity index, was significantly higher in cover crop than non-cover crop systems ($P = 0.024$) (Table 2). Overall, the same pattern is observed for all other indices ($P < 0.05$) except Richness, which did not show significant differences between treatments (Table 2). No differences were observed, however, between the different tillage treatments or between different soil depths. Using canonical correspondence analysis (CCA), soil communities at the genus level (constrained by soil physicochemical properties) could be distinguished based on tillage practice; however, the two standard tillage treatments were not distinguishable regardless of the presence of cover crop (Fig. 1). As evidenced by the greater spread of points, the NTNO community showed greater variability than the other three treatments (Fig. 1). Increases in OM, K and total C and N corresponded with no-till, while NO_3^- correlated with standard tillage (Fig. 1).

3.2. Fungal trophic group and guild assignment

We assigned fungal OTUs to specific trophic groups and then further subdivided them into specific ecological guilds. We used a manually curated set of designations based on FunGuild, a recently established fungal classification tool with rigorously defined and referenced trophic group assignments (Nguyen et al., 2016). The guilds identified in this study are listed in Fig. 2. Three ecological guilds—undefined saprotrophs, plant pathogens and dung/wood saprotrophs—accounted for approximately 82% of all fungal OTUs detected (Fig. 2). The relative abundance of undefined saprotrophs was generally higher under standard than no-tillage, except in the 0–5 cm depth in the presence of cover crops, where their numbers were dramatically lower than in all other treatments and depths (Fig. 2).

3.3. Fungal guild correlation with physicochemical soil parameters

Different ecological guilds had different relationships with soil properties. The relative abundance of four guilds—soil saprotrophs, undefined saprotrophs, plant pathogens and animal pathogens—was most strongly correlated with soil P (Table 3). Although differences in pH were not great (7.4–7.9) (Dhainaut Medina, 2015), the relative proportion of arbuscular mycorrhizae was negatively correlated with soil pH, and also negatively correlated with NO_3^- . Ectomycorrhizae (EcM) fungi were negatively correlated with EC. There was a positive correlation between animal pathogens or endophytes and soil OM. Dung/litter saprotrophs were positively correlated with NO_3^- concentration whereas animal pathogens were positively correlated with K,

Table 1

Relative abundance of fungal phyla under different tillage and cover cropping treatments: No-till (NT) or standard till (ST) treatments; cover crops (CC) or no cover crops (NO).

Parameter		Ascomycota	Basidiomycota	Blastocladio-mycota	Chytridiomycota	Glomeromycota	Mucoromycota	Zygomycota	unidentified
Depth (cm)	0_5	88.2% ± 5.3%	3.3% ± 2.6%	0.02% ± 0.06%	0.1% ± 0.1%	0.1% ± 0.9%	0.7% ± 0.9%	5.7% ± 3.1%	1.9% ± 1.1%
	5_15	88.7% ± 10.0%	1.8% ± 0.8%	0.00% ± 0.01%	0.1% ± 0.1%	0.5% ± 0.7%	0.4% ± 0.7%	4.4% ± 3.2%	4.0% ± 8.2%
	15_30	90.2% ± 5.0%	2.1% ± 1.6%	0.01% ± 0.02%	0.1% ± 0.1%	0.4% ± 0.4%	0.3% ± 0.4%	4.9% ± 2.8%	1.9% ± 1.7%
	P-value	0.633	0.314	0.511	0.424	0.00024	0.511	0.192	0.762
Till	NT	87.6% ± 8.2%	2.9% ± 2.1%	0.01% ± 0.01%	0.07% ± 0.06%	0.38% ± 0.69%	0.43% ± 0.69%	5.2% ± 3.2%	3.4% ± 6.7%
	ST	90.5% ± 5.4%	1.9% ± 1.6%	0.02% ± 0.05%	0.11% ± 0.14%	0.29% ± 0.66%	0.54% ± 0.66%	4.8% ± 2.8%	1.8% ± 1.7%
	P-value	0.104	0.028	0.128	0.227	0.380	0.128	0.606	0.531
Cover crops	CC	85.2% ± 8.1%	2.9% ± 2.3%	0.02% ± 0.05%	0.13% ± 0.13%	0.46% ± 0.82%	0.81% ± 0.82%	6.5% ± 3.6%	4.0% ± 6.6%
	NO	92.9% ± 2.2%	1.9% ± 1.3%	0.01% ± 0.01%	0.05% ± 0.06%	0.21% ± 0.18%	0.16% ± 0.18%	3.6% ± 1.1%	1.2% ± 0.6%
	P-value	0.00015	0.159	0.695	0.0019	0.103	0.695	0.0011	0.0047

N and C concentrations (Table 3).

3.4. Tillage

At the trophic level, no-till practices selected for significantly higher relative symbiotroph numbers ($P = 0.0137$) and lower saprotroph numbers ($P = 0.0033$) than what was detected under standard tillage practices (Fig. 3). There were no significant differences in pathotroph numbers among tillage treatments ($P = 0.131$).

With regard to specific guilds, fungi generally followed trends similar to what was observed for trophic levels. Dung/litter saprotrophs ($P = 0.0002$) and undefined saprotrophs ($P = 0.0006$) had significantly higher numbers under standard than no-till management while ectomycorrhizae ($P = 0.023$), animal pathogens ($P = 0.0002$) and entomopathogenic fungi ($P = 0.0015$) were significantly higher under no-till than standard tillage treatments (Fig. 3).

3.5. Cover crops

There were no significant differences among the three trophic groups between the cover crop treatments; however, there were differences in some of the ecological guilds. The relative abundance of dung/litter saprotrophs ($P = 0.033$), animal pathogens ($P = 0.03$) and endophytes ($P = 0.0057$) was significantly higher in the cover cropped treatments when tillage was not taken into consideration (Fig. 4). The numbers of unidentified fungi—i.e. fungi not possible to assign to specific guilds—were also significantly higher in the cover crop treatments ($P = 0.011$) (Fig. 4).

3.6. Soil depth

There were few differences in relative numbers of any trophic

Fig. 1. Fungal ITS sequence data. Canonical correlation analysis (CCA) of normalized fungal genus richness data constrained by soil physicochemical characteristics.

groups or ecological guilds at different soil depths across all treatments. The only exceptions were arbuscular mycorrhizae ($P = 0.0003$) and undefined saprotrophs ($P = 0.0273$), both of which had lower numbers in the 0–5 cm interval than at greater depths, and animal pathogens ($P = 0.00315$) that were more abundant at 0–5 cm than in deeper layers. The relative numbers of these three guilds did not differ significantly between the 5–15 and 15–30 cm depths, and significant

Table 2

Diversity indices at the genus level for each depth and treatment. No-till (NT) or standard till (ST) treatments; cover crops (CC) or no cover crops (NO). P values indicate likelihood of statistically significant differences between treatments.

Parameter	Shannon	Simpson	Inverse Simpson	Richness	Evenness
Depth					
0–5 cm	2.64 ± 0.34	0.87 ± 0.07	9.11 ± 3.27	74.9 ± 13.5	0.61 ± 0.07
5–15 cm	2.63 ± 0.29	0.87 ± 0.05	8.78 ± 2.90	74.8 ± 14.7	0.61 ± 0.06
15–30 cm	2.70 ± 0.28	0.88 ± 0.05	9.29 ± 3.35	82.4 ± 13.3	0.61 ± 0.05
P value	0.772	0.887	0.896	0.239	0.998
Till					
NT	2.67 ± 0.23	0.88 ± 0.04	8.95 ± 2.43	76.0 ± 14.8	0.62 ± 0.04
ST	2.65 ± 0.36	0.87 ± 0.07	9.17 ± 3.73	78.8 ± 13.4	0.61 ± 0.07
P value	0.815	0.426	0.808	0.516	0.518
Cover Crop					
CC	2.55 ± 0.31	0.85 ± 0.06	7.89 ± 3.08	75.9 ± 11.1	0.59 ± 0.06
NO	2.76 ± 0.25	0.89 ± 0.05	10.23 ± 2.74	78.9 ± 16.5	0.64 ± 0.05
P value	0.024	0.028	0.014	0.478	0.012

Fig. 2. Normalized relative abundance of assigned fungal functional guilds with treatment and depth. NT – no-till, ST – standard till, CC – cover crops, NO – no cover crops; depth in cm below ground surface.

differences with depth were only observed in the no-till system (Fig. 5).

3.7. Plant pathogens

The plant pathogen guild as a whole showed no significant differences ($P < 0.05$) among treatments. The most numerous (> 50%) plant pathogenic fungus identified – the leaf blight agent *Ulocladium dauci* – showed no significant differences among treatments (Table S5). A number of the other organisms in the plant pathogen guild, however, showed differences with tillage and/or cover cropping. *Fusarium chlamydosporum* was significantly higher ($P = 0.0194$) under both no-till and standard till in the absence of cover crops (Fig. 6). The leaf blight agent *Pleospora eturmina* (Fernández and Rivera-Vargas, 2008) was significantly higher ($P < 0.032$) under no-till with cover crop than in the other three treatments (Fig. 6). Lupine leaf blight agent *Phoma schneiderae* was significantly higher ($P = 0.0007$) under no-till, no cover crop treatment (Fig. 6). *Neonectria* was higher ($P = 0.0125$) under standard tillage, while *Fusarium*_sp_CBS119875, *Phoma* sp. MA4621 and *Fusarium oxysporum* were higher ($P = 0.00494$, 0.0422, 0.042 respectively) under no-till (Fig. 6). *Fusarium proliferatum* and *Plectosphaerella melonis* were higher ($P = 0.0468$, 0.0007 respectively) in the presence of cover crops irrespective of tillage regime (Fig. 6). No

significant differences with depth were observed for the plant pathogen guild, nor for any individual plant pathogens under either tillage regime (Table S5).

4. Discussion

4.1. Tillage effects

A number of studies have found that tillage reduces total fungal biomass in agricultural soils (Helgason et al., 2009, 2010; Mathew et al., 2012; Cho et al., 2017). A comparison of no-till and standard till dryland wheat found the fungal community was dominated by Ascomycota in both systems, with Ascomycota and Basidiomycota showing significant changes in the tilled system (Sharma-Poudyal et al., 2017). We also observed Ascomycota as the dominant phylum in all treatments, but it was not responsive to tillage. Only the phylum Basidiomycota responded to tillage showing higher relative abundance in the no-till treatment (Table 1). Almost all genera affected by tillage belonged to Ascomycota, with the exception of the Basidiomycete *Cryptococcus*. Genera showing reduction in numbers with tillage belonged to guilds in all three trophic groups and encompassed ectomycorrhizal symbiotrophs; dung/wood and undefined saprotrophs; and animal and

Table 3

Spearman correlation analysis of soil physicochemical parameters and fungal guilds. Depths at 0–15 and 15–30 cm. Significant correlations ($P < 0.05$) shown.

Trophic group	Fungal Guild	NT	CC	Depth	PLOT	Block	pH	EC	OM	P	K	NO ₃ ⁻	N	C	CEC	BDv
Pathotroph	Animal Pathogen	0.41							0.61	0.4	0.51		0.51	0.5		
	Entomo-pathogens	0.37														
	Mycoparasites															
	Plant Pathogens									0.44						
Symbiotroph	AMF	0.53					-0.45					-0.47				
	EcM							-0.44								
	Endophytes		0.37						0.44						0.36	
	Ericoid Mycorrhizae															
	Lichenized		-0.4													
Saprotroph	Dung/Litter Saprotrophs	-0.59										0.39				
	Dung/Wood Saprotrophs															
	Soil Saprotrophs				0.39											
	Undefined Saprotrophs	-0.39								0.42		-0.36				
	Wood Saprotrophs				-0.35	-0.41										
NA	Unclassified fungi		0.39		-0.38	-0.44

Fig. 3. The relative fraction of assigned fungal trophic groups and guilds under different tillage treatments. Trophic groups: a) saprotrophs and b) symbiotrophs c) pathotrophs. Fungal guilds that showed significant differences with tillage: d) undefined saprotrophs, e) ectomycorrhizae; f) dung/litter saprotrophs; g) animal pathogens; and h) entomopathogenic fungi. These guilds accounted for 44.8% of the total fungal community. No-till (NT) or standard till (ST) treatments; different letters indicate statistically significant differences between treatments ($P < 0.05$).

plant pathogens (Table S3). In contrast, genera that increased with tillage were exclusively saprotrophs identified as undefined, dung/litter or dung/wood saprotrophs (Table S3). The net effect on total number of Ascomycetes were negligible due to the similar numbers of taxa positively and negatively affected by tillage. In total, approximately 45% of all guild-assigned fungi showed significant responses to tillage. It has been proposed that fungal taxa selected by conventionally tilled systems are primary colonizers of mechanically damaged plant residues and produce large numbers of tillage-resistant conidia (Sharma-Poudyal et al., 2017). Taxa more abundant in no-till are either endophytes or other groups adapted to utilizing intact roots, or taxa with extensive hyphal networks that can be disrupted by tillage (Sharma-Poudyal et al., 2017). It may be that groups that do not fall into these categories are likely to remain unaffected by the tillage regime.

Relationships of fungal diversity to tillage have shown inconsistent results in the literature. Fungal diversity in a no-till, soybean-rice-corn rotation system resembled that in an adjacent native grassland, while under standard tillage there was a 19% decrease in diversity (Lienhard et al., 2014). In a study of soybean/maize-wheat/lupine crop rotations, soils under no-till showed a 4% higher species diversity compared to standard tilled plots (Souza et al., 2015). In contrast, there were no differences in these parameters between tilled and no-till in a wheat

monoculture (Dong et al., 2017). We also found no significant changes in fungal diversity with tillage (Table 2), but no-till and standard till had distinctly different communities. (Fig. 1), with relatively more symbiotrophs and fewer saprotrophs under no-till (Figs. 2 and 3). These results show that diversity indices are not effective in reflecting functional differences between our different management systems. The relative increase in saprotrophs and decrease in symbiotrophs under standard tillage was associated with reduced SOM and nutrient content (Figs. 1 and 3), in line with the classic model of C decomposition and loss primarily regulated by fungal saprotrophs (Dighton, 2003). In addition, while total N was higher under no-till, NO_3^- concentrations were higher in standard tillage plots. N mineralization rates have been observed to be greater in soils where fungal growth is not N limited (Schimel and Bennett, 2004). This result is consistent with long-standing observations of increased organic matter decomposition and nutrient mineralization with standard tillage on one hand, and greater microbial nutrient uptake and immobilization under no-till on the other (Hendrix et al., 1986). Members of the dung/litter saprotroph guild, observed to be positively correlated with NO_3^- concentrations (Table 3), typically colonize nutrient rich substrates such as leaf litter and animal manures, environments likely to prioritize rapid resource utilization over nutrient use efficiency. Increased prevalence of such

Fig. 4. The relative fraction of fungal guilds that show significant differences with cover cropping. a) dung/litter saprotrophs, b) unidentified fungi; c) animal pathogens; and d) endophytes. These guilds accounted for 9.1% of the total fungal community. Cover crop (CC) or no cover crop (NO) treatments; different letters indicate statistically significant differences between treatments ($P < 0.05$).

Fig. 5. The relative fraction of fungal guilds that show significant differences with depth bgs under different tillage management. a) arbuscular mycorrhizae; b) undefined saprotrophs; c) animal pathogens. Different letters indicate statistically significant differences between treatments ($P < 0.05$).

fungi in tilled systems could therefore help explain the observed higher nutrient mineralization rates. In contrast, undefined saprotrophs, the largest guild increasing with tillage, did not correlate with NO_3^- or total-N. Together with the observation of specific genera within this group either increasing or decreasing with tillage, these results suggest that the undefined saprotroph guild could be further refined to improve its usefulness as a functionally defining category.

It was surprising to detect relatively high numbers of ectomycorrhizae (EcM) taxa in our row crops systems. Though the relative proportion ($8 \pm 13\%$) was of comparable magnitude to reports for grasslands and shrublands (12%), or regions dominated by AMF trees (8%) (Tedersoo et al., 2014), they were high for a row crop system lacking tree or shrub hosts. Cho et al. (2017) reported similarly high EcM numbers in a mesocosm study with a moist, temperate-zone brown

earth soil, and suggested potential misclassification of species capable of some form of saprotrophy as obligate EcM symbionts (Cho et al., 2017). For example, some EcM, such as *Laccaria bicolor*, have dual lifestyles, alternating between facultative saprotroph and tree root-mutualist phases (Koide et al., 2008; Vincent et al., 2011), making division into strictly saprotrophic or symbiotrophic clades difficult. Recent studies have also shown that EcM fungi can secrete enzymes that digest organic matter and scavenge N (Antunes and Koyama, 2017). Emerging evidence suggests that a wide range of EcM fungi may be able to break down SOM under certain conditions (Shah et al., 2016). The potential reliance on these alternate saprotrophic capabilities could explain the persistence of EcM in row crop systems.

In contrast to EcM, AMF numbers were quite low in all plots (AMF = $0.4 \pm 0.6\%$), even in the no-till system. These numbers are

Fig. 6. Total numbers of fungal plant pathogens that show significant differences with tillage and cover crops. a) tillage and cover crops i) *Fusarium chlamydosporum*, ii) *Pleospora eturmiuna*; ii) *Phoma schneiderae*; b) tillage i) *Pleospora eturmiuna*, ii) *Phoma schneiderae*; iii) *Neonectria*; iv) *Fusarium_sp_CBS119875*; v) *Phoma sp. MA4621*; vi) *Fusarium oxysporum*; c) cover crops i) *Fusarium proliferatum*, ii) *Plectosphaerella melonis*; iii) *Pleospora eturmiuna*; iv) *Phoma schneiderae*. No-till (NT) or standard till (ST), cover crop (CC) or no cover crop (NO) treatments; different letters indicate statistically significant differences between treatments ($P < 0.05$).

similar to AMF numbers previously reported for ITS (Sommermann et al., 2018) and 18S (Jesus et al., 2016) sequence analysis. A recent meta-analysis found standard tillage practices have a significant negative impact on AMF diversity (Bowles et al., 2016). Tillage disruption of hyphal networks has also been strongly linked to decreased AMF abundance (Helgason et al., 2010; Larkin, 2015). However, we did not observe large differences in AMF between standard and no-till treatments. The soil physicochemical conditions at the test site – namely mildly alkaline pH, abundant phosphorus and relatively high organic content (Mitchell et al., 2015, 2017) – do not provide optimal conditions for AMF which appear to be favored by low pH, low available P and low organic matter content (Jansa et al., 2006) and may thus dominate and mask the effects of management practices.

We observed significant increases in relative AMF numbers with depth under no-till (Fig. 5), and an inverse relationship with NO_3^- and pH. AMF spore counts at the end of maize growing season did not change from 0 to 30 cm in a Calcic Regosol, while spore counts decreased in 10 cm depth intervals in Haplic Alisol to the same depth (Oehl et al., 2005). AMF spore counts also decreased from 0 to 40 cm depth in Vertic Cambisols under reduced till, but remained constant with depth under conventional tillage (Säle et al., 2015). In contrast, lower AMF hyphal density in the 0–5 cm than deeper soil depths has been reported in both standard and no-till maize soils (Kabir et al., 1998). These results may indicate that spore counts do not reflect the actual distribution of AMF biomass with depth. Increased NO_3^- availability has been linked to lower AMF infection rates as plants can then directly uptake nitrate and don't have to rely on AMF to provide N (Camenzind et al., 2014; Berruti et al., 2016). In contrast, pH has been shown to have limited effect on fungi (Rousk et al., 2010), and the narrow range of range of pH values in this study (7.4–7.9) is not likely to lead to detectable changes.

4.2. Cover cropping effects

Ecosystem services provided by cover crops are many, including improved soil quality, nutrient cycling, pest regulation, and crop productivity (Tonitto et al., 2006; Lundgren and Fergen, 2011; Ryan et al., 2011; Schipanski and Drinkwater, 2011). Including cover crops in crop rotations appears to significantly increase soil C and N pools in comparison to crop-rotations that do not contain cover crops (McDaniel et al., 2014). Rotations with annual cover crops increased fungal and AMF biomass (Maul et al., 2014), as well as the relative proportion of fungi making up the soil microbial biomass (Lehman et al., 2014), but decreased plant pathogen numbers (Benitez et al., 2016). In grasslands, a higher plant biomass was associated with increases in both fungal phylogenetic and functional group diversity (Cline et al., 2018). In our study, genera that increased with cover crops were dominated by Ascomycetes, but also included members of Zygomycota and Basidiomycota. They included endophyte symbiotrophs; dung/litter, dung/wood and undefined saprotrophs; and plant and animal pathogens (Table S3). Cover crops increased overall phylogenetic diversity but did not change the relative abundance of saprotrophs, symbiotrophs or pathotrophs. Therefore cover cropping did not appear to contribute to functional changes in the fungal community.

Specific guilds with significantly higher relative numbers in the presence of cover crops were either those that utilize fauna excrement and plant litter (dung/litter saprotrophs), or were pathogens of animals or symbiotic partners (endophytes). However, the taxa associated with these functional groups together accounted for only 9% of guild-assigned fungal taxa. Excrement cycling is an important component of the soil nutrient cycle (Doughty et al., 2016). At the field scale, nitrogen-rich mesofauna excretions may contribute up to 30% of mineralized N (De Ruiter et al., 1993; Griffiths, 1994). Our observations of increases in fauna-dependent fungi with cover crops is consistent with observations of greater soil meso- and macrofauna in other cover cropped systems (DuPont et al., 2009; Leslie et al., 2017). Higher endophyte numbers

likely reflect the greater extent and variety of plant root carbon availability with cover crops. By providing a longer period of time during the year when the soil is vegetated, there is less disruption to habitat for endophytes, with potential greater benefit from those associated with pest and disease suppression (Backman and Sikora, 2008).

4.3. Depth effects

We did not observe differences in diversity indices or species richness with depth. Similar lack of change in fungal diversity and community composition with depth has been described in fallow fields dominated by Ascomycetes (Ko et al., 2017), although several other studies have observed decreasing richness and diversity with increasing soil depth (Rahman et al., 2008; Degruene et al., 2016; Schlatter et al., 2018). The observed increase in relative abundance of AMF and undefined saprotrophs below 5 cm in the no-till treatments may have been due to specific taxa within these groups showing greater sensitivity to daily temperature swings, wet-dry cycles or other environmental effects that are more pronounced near soil surface. The observed lack of change in community composition with standard tillage is likely due to the homogenizing effect of tillage (Sipilä et al., 2012).

4.4. Plant pathogens

One concern for some farmers in adopting conservation practices is a potential increase in crop plant diseases. Studies in wheat have shown that reduced tillage favors some fungal plant pathogens by protecting them from high temperatures, limited water availability and disturbance, or by providing a refuge in the plant litter that accumulates on the soil surface (Bockus and Shroyer, 1998; Schroeder and Paulitz, 2006). On the other hand, reduced tillage can obstruct the movement of spores of plant pathogens, reduce plant stress through improving soil conditions (Bockus and Shroyer, 1998), and maintain a more diverse and abundant microbial community that suppresses invasion and establishment of soil borne plant pathogens (Larkin, 2015; van Bruggen and Finckh, 2016). The use of crop rotations and cover crops that increase SOM (McDaniel et al., 2014) and break plant pathogen infection cycles is considered important for effective reduced till management (Bockus and Shroyer, 1998; Larkin, 2015; van Bruggen and Finckh, 2016). Interestingly, no significant differences were observed in the relative abundances of the phytotroph group as a whole among treatments in our study. While diagnosis of plant pathogens was not part of this study, the relatively similar crop yields consistently reported for all treatments (Mitchell et al., 2017) suggest no major differences in plant pathogen pressure across the systems, consistent with our observation of statistically similar phytotroph numbers among treatments.

Several taxa known to be plant pathogens varied with treatment. *Fusarium chlamydosporum* had higher numbers under no-till than tilled treatments in the absence (but not presence) of cover crops (Fig. 6). Strains of *F. chlamydosporum* have been shown to be pathogenic to cotton (Khalil et al., 2003), but not tomato (Charoenporn et al., 2010). We sampled after tomato harvest, so a full year had passed since cotton harvest. Over this one-year period, cover crops appeared as effective as tillage in reducing the relative *F. chlamydosporum* numbers. In addition, five other plant pathogens – *P. eturmiuna*, *P. schneiderae*, *Fusarium* sp. CBS119875, *P. sp.* MA4621 and *F. oxysporum* (Fig. 6) – showed higher numbers in no-till than tilled soils irrespective of cover crops. Only one pathogen, an undefined species of *Neovectria*, had higher relative numbers under standard than no-till treatments. These results suggest the importance of incorporating management practices that reduce pathogens, such as crop rotation, residue management or compost amendment, in reduced till systems (Bailey and Lazarovits, 2003; van Bruggen et al., 2016).

4.5. Bacteria vs fungi

In a study conducted on the same field site, we explored the effect of no-till and cover crops on bacterial communities (Schmidt et al., 2018). The bacterial community shifted towards more efficient resource utilization and greater metabolic and phylogenetic diversity under no-till with cover crops (Schmidt et al., 2018). Management with no-till but not with cover crops, led to increased bacterial biodiversity (Schmidt et al., 2018). In contrast, management with cover crops but without no-till, led to an increase in fungal biodiversity.

5. Conclusions

We observed significant differences in the responses of the fungal community to tillage and to cover crop treatments in clay loam soils under a Mediterranean climate. No-till led to increased soil total C and total N in the top soil layers and changed the functional composition of the fungal community. It did not, however, affect fungal species phylogenetic diversity. Correlations with total N and NO_3^- , suggest that tillage may select for groups that grow more rapidly and therefore lead to higher rates of nutrient mineralization. There were no significant changes in fungal community composition with depth under standard tillage, and only three groups showed significant changes with depth under no-till. In contrast to tillage, addition of cover crops promoted greater fungal diversity, but did not change the functional composition of the soil fungal community. All systems were dominated by Ascomycetes; although the inclusion of cover crops resulted in a significant, but relatively small increase in phylogenetic diversity. Benefits linked to increased diversity, such as increased system resilience, would also be expected to be comparatively minor. The fungal community was more affected by tillage than presence of cover crops – on average, 45% of guild-assigned fungi responded to tillage, while only 10% responded to cover crops. The responses of the fungal community to tillage and cover crops contrast with previously reported bacterial community responses in the same system where cover crops played a more prominent role than tillage in functional shifts in the bacterial community. Ultimately, the long-term combination of reduced disturbance and increased plant cover led to a more diverse, symbiotroph-enriched fungal community.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2018.11.010>.

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