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Biosolids and conservation tillage: Impacts on soil fungal communities in dryland wheat-fallow cropping systems

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ABSTRACT

Organic amendments and conservation tillage are important management tools for reducing soil erosion and improving soil health in agricultural systems, yet the impacts of these practices on soil microbial communities is poorly understood. We evaluated the effects of biosolid amendments and conservation tillage on soil fungal communities in a dryland wheat (*Triticum aestivum* L.) –summer fallow cropping system in the inland Pacific Northwest, USA (PNW). Biosolids or synthetic fertilizer was used in combination with conventional (disk) or conservation (undercutter) tillage. Fungal communities were characterized from soil and biosolid aggregates after the second application of biosolids in 2015 and before and after the second application of biosolids in 2016 using high-throughput amplicon sequencing. Biosolid amendments substantially altered fungal community composition, but not diversity, relative to synthetic fertilizer. In contrast, although many more fungal taxa were influenced by conservation tillage when synthetic fertilizer was applied, conservation tillage had relatively little effect on soil fungal communities receiving biosolids, suggesting that the form of N supplied (mineral or organic) may mediate the effects of increasing surface crop residue on fungal communities. Biosolid-mediated shifts in fungal communities were correlated with differences in soil characteristics, especially C, N, and P, and were persistent for at least three years after the initial biosolid application. A small number of taxa, including *Fusarium, Ulocladium, Gymnoascus, Mortierella,* and *Neurospora*, were highly enriched by biosolids in soil and dominated fungal communities of biosolid aggregates. Results show biosolids can have strong and lasting impacts on soil fungal communities, likely due to their effects on soil nutrients, and select for a small number of fungi capable of utilizing biosolids as a food source.

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1. Introduction

Soil loss is a global problem for the long-term sustainability of agricultural systems as the rate of soil loss is 10–40 times greater than that of soil formation (Uri and Lewis, 1999). Billions of tons of topsoil are lost to erosion in the US every year (Montgomery, 2007; Uri, 2001) with a social cost in the billions of dollars (Crosson, 1995; Pimentel et al., 1995). Wind erosion is an especially serious problem for tillage-intensive dryland wheat production in the low-precipitation (<350 mm annual) region of the Pacific Northwest (PNW) (Singh et al., 2012). The major cropping system is a 2-year winter wheat-summer fallow rotation where only one crop is produced every other year on a given parcel of land. Soils are primarily composed of silt or fine sand, have low organic matter (<1%), and are poorly aggregated. Moreover, because of often meager residue cover on the soil surface, tillage during fallow, drought, and high winds makes these soils especially prone to wind erosion and PM_{10} small particulate emissions (Sharratt and Schillinger, 2016; Singh et al., 2012). Adoption of management practices to minimize or eliminate tillage and increase residue cover are key for mitigating soil loss from windblown dust and enhance the sustainability and security of agriculture. Although there has been substantial improvement in management practices to control wind erosion and their acceptance by growers (Wade et al., 2015),

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information is lacking on how these practices impact biotic components of soil health, such as soil fungal communities.

Tillage is a historic hallmark of agricultural systems and has a large impact on the rate of soil loss (Lal et al., 2007; Montgomery, 2007). In the PNW, tillage during spring of the fallow year is practiced by most growers to help maintain seed-zone moisture to ensure successful late-summer planting of winter wheat. A rod-weeder implement is used once or twice during the late spring and summer to control broadleaf weeds. The dry soil mulch created by tillage helps restrict evaporation by cutting off capillary flow of water from the seed zone and by thermally insulating the moist subsoil (Wuest and Schillinger, 2011). Tillage also disrupts soil aggregates and fungal mycelial networks, promotes microbial activity and decomposition of residue, reduces soil organic matter, and accelerates soil erosion (Hobbs et al., 2008; Ritz and Young, 2004; Young and Ritz, 2000). Because of these drawbacks, no-till or direct seeding, is increasingly practiced around the world (Wade et al., 2015). No-till summer fallow is practiced by an increasing number of growers in the winter wheat-summer region of the PNW, but the majority of growers continue to use tillage during spring of the fallow cycle for the aforementioned seed-zone moisture retention benefits.

The undercutter method of conservation tillage has been promoted as a best management practice for summer fallow during the past 15 years and the USDA Natural Resources Conservation Service has provided cost-sharing to growers to purchase undercutter implements. The undercutter implement has overlapping V-shaped blades that slice beneath the soil at the desired depth to sever capillary pores to preserve soil moisture during the warm, dry summer. With the undercutter, there is minimal soil inversion and most residue remains on the soil surface. In contrast, a tandem disk implement is a conventional primary spring tillage implement that mixes and stirs the surface soil, buries considerable residue, and can pulverize surface soil aggregates (Papendick, 2004; Sharratt and Feng, 2009; Sharratt et al., 2012).

Amending soils with organic material is a management practice used to stabilize soils, reduce erosion, and improve soil heath (Diacono and Montemurro, 2010; Medina et al., 2015; Reardon and Wuest, 2016). Re-purposing of processed sewage sludge, or biosolids, for application to agricultural land can be an economical means to use human waste and enhance soil quality and plant productivity. Biosolids are produced from wastewater where the solid fraction is separated, digested to stabilize organic matter and reduce pathogen loads, and concentrated prior to field application (Lu et al., 2012). Biosolids have high (up to 50%) organic matter and are rich in N, and P, as well as several plant micronutrients (Lu et al., 2012; Rigby et al., 2016; Stehouwer et al., 2000). Biosolid application enhances soil organic matter and soil aggregate size and stability, and can completely replace the use of synthetic fertilizers (Brown et al., 2011; Cogger et al., 2013a; Powlson et al., 2012).

Fungi are critical components of all soils and are intimately associated with plant and soil health as saprophytes, pathogens, symbionts, and decomposers. Tillage can substantially restructure soil fungal communities by disrupting soil aggregates and mycelial networks, altering the accessibility of plant residue to degradation, and modifying the soil chemical and physical environment (Acosta-Martínez et al., 2007; Ritz and Young, 2004; Simmons and Coleman, 2008; Young and Ritz, 2000). Although tillage may be detrimental to some beneficial components of the soil community, such as arbuscular mycorrhizal fungi, populations of some major soilborne plant pathogens, such as *Rhizoctonia* or *Fusarium*, may proliferate when tillage is reduced or eliminated (Bailey, 1996; Paulitz et al., 2002; Pumphrey et al., 1987). Biosolid amendments may also stimulate soil microbial activity and alter soil microbial community structure and diversity (Barbarick et al., 2004; Sullivan et al., 2006; Zerzghi et al., 2010). However, with some exceptions (Hazard et al., 2014), many questions remain on how fungal communities respond to biosolid applications. Biosolids can impact soil fungal communities via the introduction of organic substrates, which may benefit those taxa able to use biosolids as a food source. In addition, the presence of antimicrobials or other toxic compounds (eg., heavy metals, pharmaceutical compounds, nanomaterials) sometimes found in biosolids may have detrimental impacts on soil microbes (Anderson et al., 2008; Colman et al., 2013; Judy et al., 2015; Kao et al., 2006; Waller and Kookana, 2009). Together, biosolid amendments and conservation tillage have the potential to significantly improve soil quality by increasing organic matter, reducing wind erosion, and increasing the financial sustainability of dryland wheat production in the PNW. However, how a combination of these practices impacts soil communities, especially plant pathogens, remains unknown. In this work we investigate the impacts of conventional and conservation tillage and biosolid amendments on soil fungal communities in a winter wheat-summer fallow cropping system. We hypothesize that biosolid amendments and conservation tillage will modify the soil fungal community and increase fungal diversity relative to conventional tillage and fertilization.

2. Materials and methods

2.1. Field experimental design

Field plots were sampled at the Washington State University Dryland Research Station near Lind, Washington (47°00′N, 118°34′W) in 2015 and 2016. This site receives on average 242 mm annual precipitation. The study was conducted on a Ritzville silt loam (coarse-silty, mixed, superactive, mesic Calcidic Haploxerolls) in 2015 and a Shano silt loam (coarse-silty, mixed, superactive, mesic, Xeric Haplocambids) in 2016. Ritzville silt loam is composed of 13% clay, 61% silt, and 26% sand while Shano silt loam is composed of 9% clay, 56% silt, and 35% sand. Organic matter content in the surface 15 cm is 0.7%. The soils had a pH of 6.7 and an EC (1:1) value of 0.24 m. mhos/cm. Water holding capacity of soils at Lind are 2.5 inches of water per foot of soil. Experimental treatments were established in a split-plot design with tillage (conventional vs. conservation) as the main-plot factors and fertilizer (biosolids vs. synthetic) as the secondary factor. Size of individual main plots was 76 × 8 m and subplots 38 × 8 m. Each treatment combination was replicated four times. Glyphosate [N-(phosphonomethyl) glycine] was applied in mid-March at a rate of 0.43 kg acid equivalents/ha to control weeds. Biosolid material (Class B) was obtained from the King County Wastewater Treatment Division, Seattle, WA, and was applied with a manure spreader on 4 May in 2015 and on 19 April 2016 at a rate of 6508 kg/ha (dry weight) to meet the nutrient requirements of two wheat crops. Each plot had a previous history of biosolid use; the 2015 plots first received an application of biosolids in 2011 and the 2016 plots first received an application of biosolids in 2012. No biosolids were applied in the intervening fallow years. Synthetic fertilizers were applied every 2 years to meet the nutrient requirements of each crop. Conventional tillage was with a tandem disk implement and conservation tillage with an undercutter implement. Tillage was conducted immediately after biosolid application during both years. For the synthetic fertilizer treatments, liquid aqua NH_3-N plus thiosol was applied at a rate of 56 kg N plus 11 kg S/ha. With conventional tillage, fertilizer was stream jetted on the soil surface with a sprayer fitted with large-orifice nozzles and immediately incorporated into the soil with the tandem disk. For conservation tillage, fertilizer was injected into the soil with the undercutter implement. Depth of tillage was 13 cm. Plots were subsequently rodweeded at a depth of 10 cm on

15 June in 2015 and on 3 June and 10 July in 2016.

2.2. Soil sampling

2.2.1. Soil sampling in 2015

Soil samples were collected to a depth of 3.0 cm on 18 June, 45 days after fertilizer treatments were applied (soil-post). Samples were taken near the soil surface (0–3 cm) with a flat-bladed shovel by digging a trench and then inserting the shovel into the face of the trench at a 3 cm depth. A composite sample was obtained by taking 0.25 kg samples from six random locations in each plot. Soils were air dried at least 48 h inside a laboratory and then stored at room temperature (25 °C) for six months prior to DNA extraction.

2.2.2. Soil sampling in 2016

Soil samples were collected from plots prior to imposing treatments (soil-pre) (13 April) and on 6 June three days after the first rodweeding in 2016 (soil-post). Samples were collected in 2016 as described above and were transported in a cooler to the laboratory and stored at −20° C until DNA extraction.

2.2.3. Sampling of biosolid aggregates

In 2015, biosolid aggregates were collected from plots on 18 June after biosolids had been applied, by extracting from the soil with a sterile tweezers; biosolid aggregates were shaken free of loose soil, and treated as a distinct sample type (aggregate-post). In 2016, biosolid samples (aggregate-pre) were collected from the biosolid stockpile before application to the field (biosolids were placed in a stockpile adjacent to field plots upon delivery to the Station). Biosolid samples were also collected from plots on 6 June (aggregate.-post) after the biosolids had been applied to the soil, by extracting from the soil with a sterile tweezers; biosolid aggregates were shaken free of loose soil, and treated as a distinct sample type (aggregate-post).

2.2.4. Sample preparation and soil analysis

DNA was extracted from soil samples (~0.25 g) and biosolid aggregates (~0.1 g) using the Qiagen/MoBio Powersoil DNA extraction kit according to the manufacturers' instructions (one extraction per sample). Soil chemistry was evaluated for samples collected in June in each year for both nutrient and metal analysis. Samples were analyzed for heavy metal concentrations regulated by the USEPA, including As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, and Zn. Macronutrient analyses were performed for C, Ca, K, Mg, N, P, and S while micronutrient analyses were performed for Co, Fe, Mn, and V. Chemical properties were measured by the University of Idaho Analytical Sciences Laboratory. Carbon and N content was determined using a LECO CNS 2000 combustion analyzer while nutrient and metal contents were determined using a Leeman 200 inductively coupled argon plasma spectrophotometer and Hewlett Packard 4500 Series ICP-MS Plus. Additional soil analysis data are presented in Supplemental Table 1.

2.3. Fungal community characterization

2.3.1. PCR and illumina 2 × 300 sequencing

The ITS1 region of the fungal rRNA gene was amplified using a dual-indexing approach. Briefly, ITS1-F and ITS2 adapted to include a linker, phase offset, and consensus tag were combined in equimolar ratios and used as gene-specific primers (Supplemental Table 2). The first round of PCR reactions consisted of 1 μl DNA extract, 10 μl of 2x Roche FastStart Master Mix, 0.7 μl of primers (10 μM), and 8.3 μl of PCR-grade H_2O. Reactions were conducted in triplicate using three different annealing temperatures for each reaction to reduce PCR bias and then pooled. PCR conditions consisted of an initial denaturation at 95° C for 4 min, followed by 30 cycles of 95° C for 30 s, annealing at 50, 53, or 55° C for 30 s, 72° C for 45 s, and a final extension at 72° C for 7 min. After pooling, reactions were checked for successful amplification on a 1.5% agarose gel. Despite good quality DNA and numerous PCR attempts using dilutions, fungal ITS amplicons could not be successfully attained from fresh biosolid material, indicating a minimal fungal presence in these sample types and/or high concentrations of PCR inhibitors. For successful amplicons, a second round of PCR was used to add unique barcodes and Illumina tags for each sample. The second PCR included 1 μl of product from the first PCR, 10 μl of 2x Roche FastStart Master, 0.75 μl of barcoding primers (provided by University of Idaho), and 8.25 μl water. PCR conditions consisted of an initial denaturation at 95° C for 4 min, followed by 10 cycles of 95° C for 30 s, 60° C for 30 s, and 72 °C for 1 min, with a final extension at 72° C for 7 min. Barcoded amplicons were checked on a 1.5% gel for a size shift indicating a successful addition of barcodes, quantified using a PicoGreen fluorometry kit (Invitrogen, Carlsbad, CA), combined in equimolar ratios, bead purified, and sequenced using the Illumina MiSeq platform (version 3 2 × 300 chemistry) at the University of Idaho iBEST sequencing core facility.

2.3.2. Data processing

Primer and barcode sequences were removed and reads were paired using PEARv0.9.6; (Zhang et al., 2014). Sequences were clustered into operational taxonomic units (OTUs) using the UPARSE pipeline (Edgar, 2013). Briefly, reads were quality filtered to include only sequences longer than 200 bp, no ambiguous bases, and a maximum expected error rate of 1, singletons were removed, and reads were dereplicated prior to clustering with the cluster_-otus command in USEARCH. Raw reads were mapped to OTUs using vsearch (Rognes et al., 2016) to generate an OTU table. Taxonomy was assigned to OTU representative sequences (centroids) with BLAST + to the UNITE database (31.1.2016 general release). OTUs were filtered to include only those with a match length >75%, a percent identity >80% to UNITE representatives, and a total read count of five or more. OTU tables were rarefied to 12,186 sequences per sample using QIIME (Caporaso et al., 2010) prior to analyses.

2.4. Data analysis

Due to differences in experimental plot location (two locations on the same farm) and sample storage methods in 2015 and 2016, samples from each year were analyzed separately unless otherwise noted. Differences in relative abundances of fungal phyla were evaluated in each year using ANOVA on arcsine-square root transformed proportions at each sampling date (June 2015; April and June 2016) with the 'aov' function in the R stats package using a nested design (v3.3.1; (R Core Team, 2016)). Similarly, differences in genera comprising >0.5% of sequences were evaluated within each sampling point and between April and June samples in 2016 using a two-way ANOVA on Log10(x+1) transformed sequence counts followed by a Benjamini-Hochberg correction for false discovery rate (FDR). Richness and diversity indices (Shannon H′ and Simpson's 1/D) were calculated using the vegan package in R (Oksanen et al., 2016) and evaluated using two-way ANOVA. Similarity in fungal community composition was evaluated among all samples from both years with non-metric multidimensional scaling (NMDS) on Bray-Curtis distance matrices using the metaMDS function in vegan. Differences in community composition among fertilizer and tillage treatments were evaluated for 2015 samples and among treatments and sampling month for 2016 samples with PERMANOVA using the 'adonis' function in the vegan package with 1000 permutations to determine significance. Soil chemical data were standardized to a mean = 0 and standard deviation = 1 and vectors

were fitted to the NMDS ordination for June samples using the 'envfit' function of the vegan package with 1000 permutations to determine significance. To identify OTUs that differed in abundance among treatments, DESeq2 (Love et al., 2014) was used with two separate sets of samples. Soil samples from the June sampling date were used to compare the impacts of fertilizer treatment on the abundances of fungal taxa in each tillage treatment using the model:~ Year + Tillage*Fertilizer. Soil samples from April and June in 2016 were used to compare differences in fungal communities with different fertilizer treatments three years after a single application (April), and relative shifts in taxa abundances from April to June after an additional biosolid or synthetic fertilizer treatment using the model: ~Tillage + Month*Fertilizer. Prior to running the deseq model all OTUs with normalized counts <5 or found in <3 samples were excluded and those with a maximum Cook's distance >15 and a baseMean<5 were excluded to reduce false positives. Unrarefied OTU tables (after excluding low-abundance OTUs) were used as input for DESeq2 analyses.

3. Results

3.1. Impact of tillage and biosolids on fungal community composition

Soil and biosolid samples from all treatments were dominated by members of the phylum Ascomycota (mean = 86.4 ± 9.2% of sequences), followed by Basidiomycota (mean = 7.9 ± 9.2% of sequences) and Zygomycota (mean = 4.4 ± 3.4% of sequences) (Fig. 1). Proportions of Zygomycota, but no other phyla, varied significantly with biosolid applications in 2015 ($F = 29.48$, $p = 0.002$), tending to be of greater relative abundance in biosolid-treated versus synthetically fertilized soils. Similarly, in 2016 proportions of Zygomycota varied significantly with biosolid amendment in June ($F = 18.71$, $p = 0.005$) but not in the April sample before biosolid application ($F = 0.002$, $p = 0.96$), tending to be higher in the June sampling date in biosolid-amended plots. Tillage also significantly impacted proportions of Zygomycota in the 2016 June samples after biosolids were applied, where their proportions were greater under conventional versus conservation tillage ($F = 47.73$, $p = 0.006$).

Tillage practice (conventional versus conservation) had no significant impact on soil fungal community composition in either year, although in 2015 communities tended to cluster by tillage treatment (Fig. 2, Table 1). Similarly, there was no significant impact of tillage practices on the relative abundance of fungal genera in 2015 or in 2016 (Supplemental Tables 3 and 4). In contrast to the weak effects of tillage on overall fungal community composition, communities from biosolid-amended plots were clearly distinguished from those receiving synthetic fertilizer in 2015, and in both pre- and post-treatment in 2016 (Fig. 2; Table 1), indicating that biosolid amendments select for a substantially different suite of soil fungi compared to synthetic fertilizers. Vectors of soil C ($r^2 = 0.33$, $p = 0.002$), N ($r^2 = 0.44$, $p = 0.001$), P ($r^2 = 0.42$, $p = 0.001$), S ($r^2 = 0.29$; $p = 0.009$), and Cu ($r^2 = 0.21$, $p = 0.027$), along with other trace metals (Zn, Ag; data not shown) were significantly correlated with NMDS ordinations of fungal communities and tended to be higher in biosolid-amended soils, especially

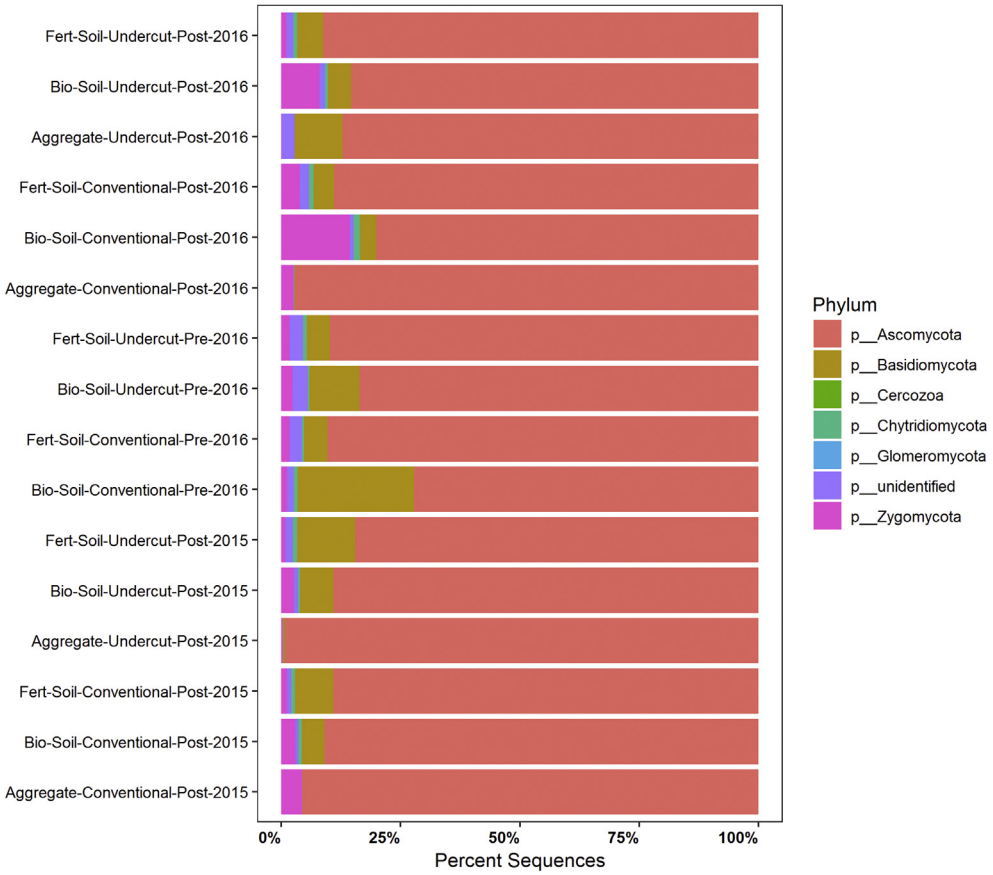

Fig. 1. Phyla-level composition of fungal communities from different treatments (Bio = Biosolid treated; Fert = Synthetic fertilized; Conventional = conventional tillage; Undercut = conservation tillage (undercutter)), sample types (Soil = soil samples; Aggregate = biosolid aggregates); date of sampling (Pre = Pre-treatment [in April], Post = Post-treatment [in June]), and years (2015 and 2016).

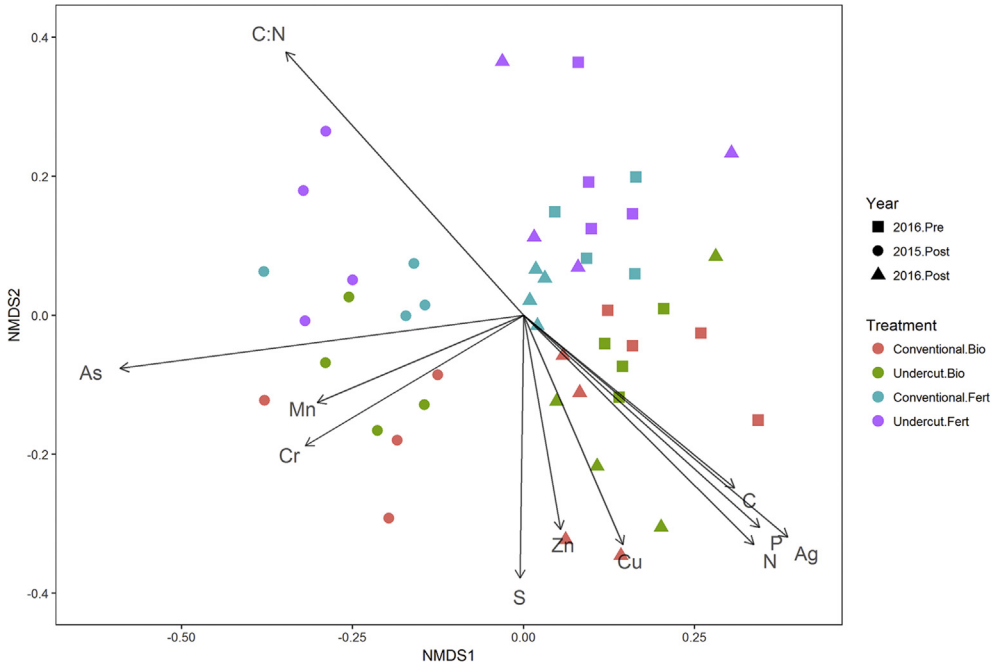

Fig. 2. NMDS ordinations of soil fungal communities in June in 2015 and 2016 and in 2016 prior to (Pre) and following the second treatment (Post). Vectors represent significant correlations ($p < 0.05$) with soil chemical characteristics in post-treatment samples, where the vector length is scaled by the correlation coefficient.

Table 1
Effects of biosolids (vs. synthetic fertilizer) and conservation tillage (vs. conventional) on fungal community composition in 2015 and 2016 assessed with PERMANOVA.

Year	Factor	F-val	r^2	p-val
2015	Tillage	1.43	0.075	0.157
	Biosolid	**4.95**	**0.259**	**0.002**
	Tillage X Biosolid	0.72	0.038	0.736
2016	**Month**	**3.06**	**0.073**	**0.003**
	Tillage	**1.69**	0.041	0.064
	Biosolid	**7.73**	**0.185**	**0.001**
	Month X Tillage	1.09	0.026	0.352
	Month X Biosolid	1.76	0.042	0.051
	Biosolid X Tillage	1.24	0.03	0.217
	Month X Tillage X Biosolid	1.67	0.028	0.252

in 2016 (Fig. 2). In contrast, the C:N ratio was strongly associated with fungal communities in synthetically fertilized plots ($r^2 = 0.54$, $p = 0.0001$). Soil Cr ($r^2 = 0.25$, $p = 0.017$), Mn ($r^2 = 0.18$, $p = 0.046$), and As ($r^2 = 0.72$, $p = 0.001$) were also significantly correlated with NMDS ordinations and tended to be greatest in biosolid-amended soils in 2015. Differences in fungal communities between years may be due to sampling of distinct locations on the farm, different environmental conditions between years, or differential sample storage.

Biosolid-amended soils had greater relative abundances of many fungal genera after fertilization and tillage treatments in both years, including *Fusarium*, *Gymnoascus*, *Neurospora*, *Ulocladium*, and *Mortierella* (Fig. 3, Supplemental Tables 3 and 4), suggesting consistent enrichment for members of these genera in biosolid-amended soils despite year-to-year variation in field conditions and sampling year. Moreover, prior to treatments in April 2016, *Fusarium*, *Gymnoascus*, and *Ulocladium,* were enriched in those plots that had received biosolid amendments in 2012, whereas *Capronia* was enriched in those plots that had received synthetic fertilizers (Supplemental Tables 3 and 4). There were no significant differences in relative abundances of *Mortierella* or *Neurospora* prior to treatments in 2016 with any significant interactions between fertilizer and tillage treatments in determining fungal community composition as a whole or the relative abundances of genera in either year (Fig. 1; Table 1; Supplemental Tables 3 and 4).

3.2. Differentially abundant taxa between tillage and fertilizer treatments

Differences in the relative abundances of fungal taxa between conventional and conservation-tillage practices were similar in both fertilizer treatments, though more taxa differed with tillage in synthetically fertilized plots compared to those amended with biosolids (37 vs. 7 OTUs; Fig. 4). Taxa that differed with tillage in both fertilizer treatments included Chaetotmiaceae (OTU6) and *Acremonium* spp. (OTU77), which were more abundant in conventionally-tilled soils. Many taxa differed between tillage treatments only in synthetically fertilized plots. For example, OTUs related to *Pseudaleuria* (OTU15), *Neurospora* (OTU10), and *Trichoderma* (OTU29) species were more abundant with conventional tillage only in plots with synthetic fertilizers, whereas those related to *Podospora* (OTU3), Coniochaetales (OTU23), and Lasiosphaeraceae (OTU38) were relatively more abundant with conservation tillage.

Differential abundance analysis identified 47 OTUs that differed between fertilizer treatments in plots with conservation tillage and 36 that differed in plots under conventional tillage (Fig. 5). With conservation tillage, 26 OTUs significantly decreased in relative abundance while 21 increased in biosolid-amended soil. Similarly, with conventional tillage 13 OTUs decreased and 23 increased in relative abundance with biosolids versus synthetic fertilizer. Of those OTUs that increased in relative abundance with biosolid amendments, 12 were shared between tillage practices. Shared OTUs included many of the most abundant taxa in biosolid-amended soils, such as *Fusarium* spp. (OTU1), *Mortierella* spp. (OTU7), *Ulocladium chartarum* (OTU2), *Gymnoascus reesii* (OTU14), and *Neurospora terricola* (OTU10). Four OTUs decreased with biosolid amendments under both tillage regimes, including an

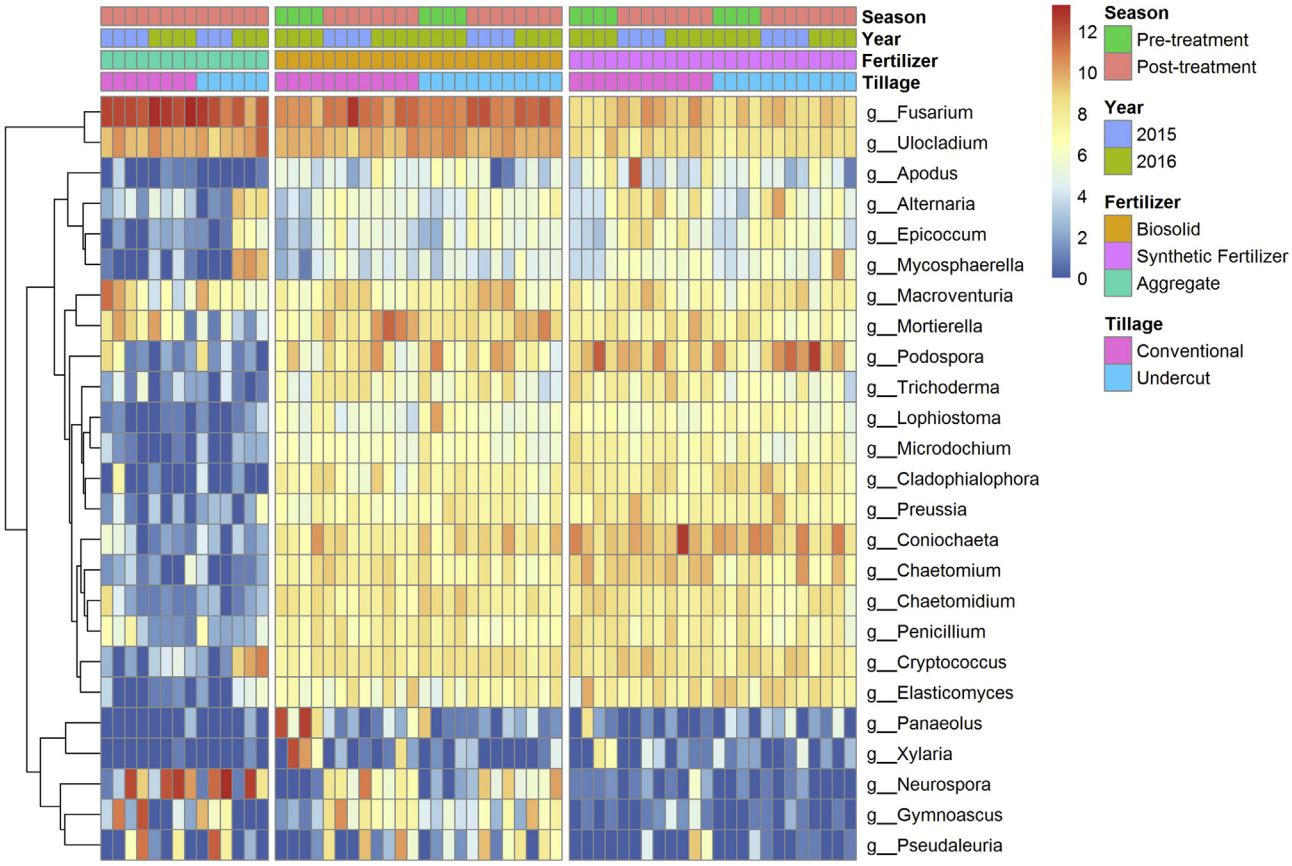

Fig. 3. Heatmap of relative abundances of major fungal genera among years (2015 vs. 2016), tillage treatments (Conventional vs. undercutter), and fertilizer treatments (Biosolid = biosolid-treated soil; Synthetic Fertilizer = Synthetically fertilized soil; Aggregate = Biosolid aggregates).

Alternaria spp. (OTU12), a Helotiales spp. (OTU35), an Ascomycota spp. (OTU45), and a *Coprinellus* spp. (OTU112). Other OTUs that differed with fertilizer treatment were specific to soils under different tillage practices, though these tended to be less abundant. For example, in conventionally-tilled plots, Chytridomycetes spp. (OTU111) and *Panaeolus* spp. (OTU36) tended to be of higher relative abundance than those with conservation tillage, whereas an *Epicoccum* (OTU16) and *Coprinellus* (OTU51) species were less abundant when biosolids were applied. With conservation tillage, there were relative increases in taxa such as *Cryptococcus* spp. (OTU48) with biosolids and relative decreases in others such as in *Podospora* spp. (OTU3), Coniochaetales spp. (OTU23), and *Mycosphaerella* (OTU11).

3.3. Biosolid and tillage impacts on fungal diversity

In general, fungal community richness and diversity were not significantly impacted by either fertilizer treatment or tillage practice (Table 2). Although communities from biosolid-amended plots tended to be less rich and diverse than those from synthetically fertilized plots in 2015, there was a significant effect of fertilizer treatment only on Simpson's diversity (ANOVA $F = 5.99$, $p = 0.05$). However, this pattern was not observed in 2016 (Table 2), where communities from biosolid-amended plots had similar richness and diversity as those from synthetically fertilized plots and soils sampled in April.

3.4. Fungal colonists of biosolid aggregates

Biosolid aggregates extracted from soil harbored numerous fungal taxa (Table 3), although this suite of colonists was less diverse than soil communities, suggesting that biosolid aggregates are colonized by a relatively small number of soil fungi. Biosolid aggregates from soils under conservation tillage in 2016 had significantly more diverse fungal communities than those from conventionally-tilled soil (Table 3). Moreover, fungal communities inhabiting aggregates differed between tillage practices in 2016 (Fig. 6). However, there were no significant patterns in fungal diversity or community composition in aggregates from tillage treatments in 2015.

Despite differences in fungal communities between years and tillage treatments, a few fungal taxa dominated all aggregates (Fig. 7), together comprising more than 50% of sequences in these samples. These aggregate colonists included the largest groups of soil taxa that differed consistently between fertilizer treatments, including *Fusarium* spp. (OTU1), *Ulocladium chartarum* (OTU2), and *Neurospora terricola* (OTU10). This suggests that these taxa are the primary organisms involved in the breakdown of biosolids in soil, and that these amendments consistently select for greater abundances of these taxa in field soil.

3.5. Impacts of biosolids on temporal patterns in fungal taxa

There were significant differences in the relative abundances of many fungal taxa between fertilizer treatments from the pre-treatment April sampling in 2016, before biosolids were applied

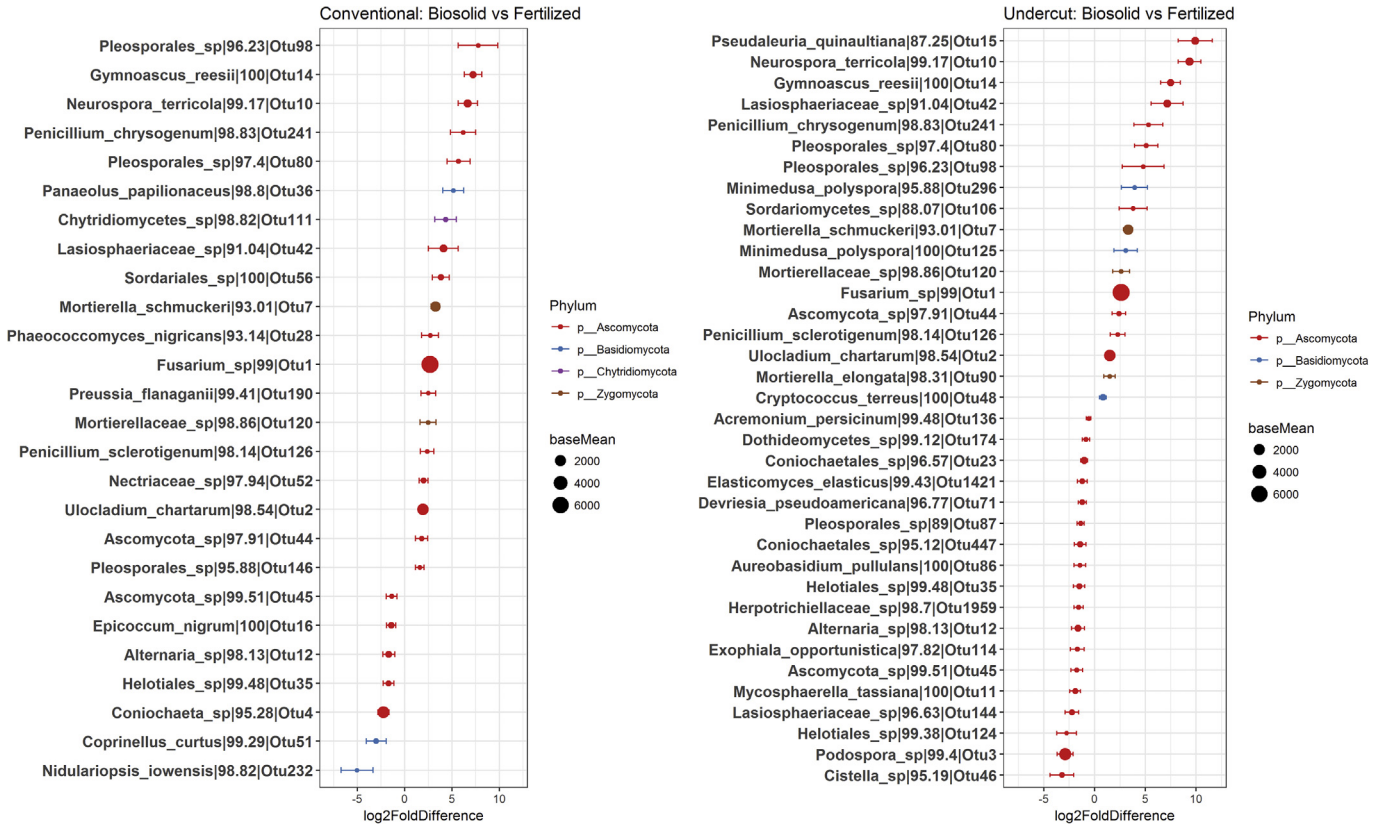

Fig. 4. Differentially abundant OTUs (baseMean>20) between fertilizer treatments in soils under conservation tillage (undercut) or conventional (disk) tillage. OTUs are identified by their best hit to the UNITE database and the blast percent identity. OTUs are colored by the phyla to which they belong and are scaled by their mean abundance among all samples (baseMean).

that year but four years after treatments were first imposed in 2012 (Suppl. Fig. 1). Similar to differences after the second application of biosolids or third of synthetic fertilizer, communities from plots amended with biosolids in 2012 had greater relative abundances of *Fusarium*, *Ulocladium*, and *Gymnoascus* in April 2016. However, there were no significant differences in other taxa that differed between treatments in the post-treatment June sampling, most notably the *Neurospora* and *Mortierella* OTUs that were efficient colonizers of biosolid aggregates, suggesting both sustained and transient impacts of biosolid amendments on fungal communities.

Similarly, fungal taxa that differed significantly in relative abundance between the pre-to post-treatment sampling, after a second round of treatments were applied, depended on fertilizer treatment. In plots amended with biosolids there were relative increases in *Neurospora, Pseudaleuria, Mortierella*, *Gymnoascus, Elasticomyces*, and *Fusarium*, and relative decreases in *Panaeolus* and Ceratobasidiaceae OTUs (Suppl. Fig. 1). In contrast, in plots receiving synthetic fertilizers there were relative increases in *Pseudaleuria*, Coniochaetales and Nectriaceae OTUs, accompanied by relative decreases in *Capronia,* Ascomycota, and *Cladophialophora* OTUs. *Gymnoascus, Ulocladium,* and *Neurospora* OTUs also increased in relative abundance in fertilized plots from April to June, suggesting that although these taxa are highly enriched in biosolids they also increase in relative abundance over the season under synthetic fertilizer regimes.

4. Discussion

There is a pressing need to improve the sustainability of agricultural systems to reduce soil loss and synthetic inputs while enhancing plant productivity. Conservation tillage is one of the primary strategies adopted by farmers to reduce soil loss (Wade et al., 2015). Tillage can induce substantial changes in the composition, activity, and function of soil microbial communities via physical disturbance, altering the distribution of carbon or nutrients in soil, reducing soil aggregation, or by changing soil moisture or temperature (Doran, 1980; Drijber et al., 2000; Fuentes et al., 2009; Govaerts et al., 2007; Griffiths and Philippot, 2013; Jackson et al., 2003). However, the use of an undercutter, a low-disturbance method for primary spring tillage that leaves residue on the surface, had minimal effects on soil fungal community composition or diversity compared to conventional tillage with a tandem disk that mixes and stirs soil and buries residue. Changes in fungal communities as a result of tillage may have been muted due to the cropping system, where differences in tillage for a cycle of wheat-fallow-wheat (four years) may not have had a large impact on the soil physical or chemical characteristics that structure fungal communities. A longer time period in conservation tillage with the undercutter may be necessary for the accumulation of enough residue to significantly increase characteristics such as soil aggregation, C, or N in ways that impact soil fungi. Indeed, Machado (2011) found no differences in soil C after 20 years of using plow, disk, or sweep tillage while Gollany and Elnaggar (2017) found little difference in soil carbon 20 years after switching from conventional to no tillage in a winter wheat-summer fallow field study near Pendleton, Oregon where long-term average annual precipitation is 418 mm. Moreover, microbial communities may need significant amounts of time to recover after many years of disruptive heavy tillage (Griffiths and Philippot, 2013). The response of fungal communities in this system may be further limited due to the short

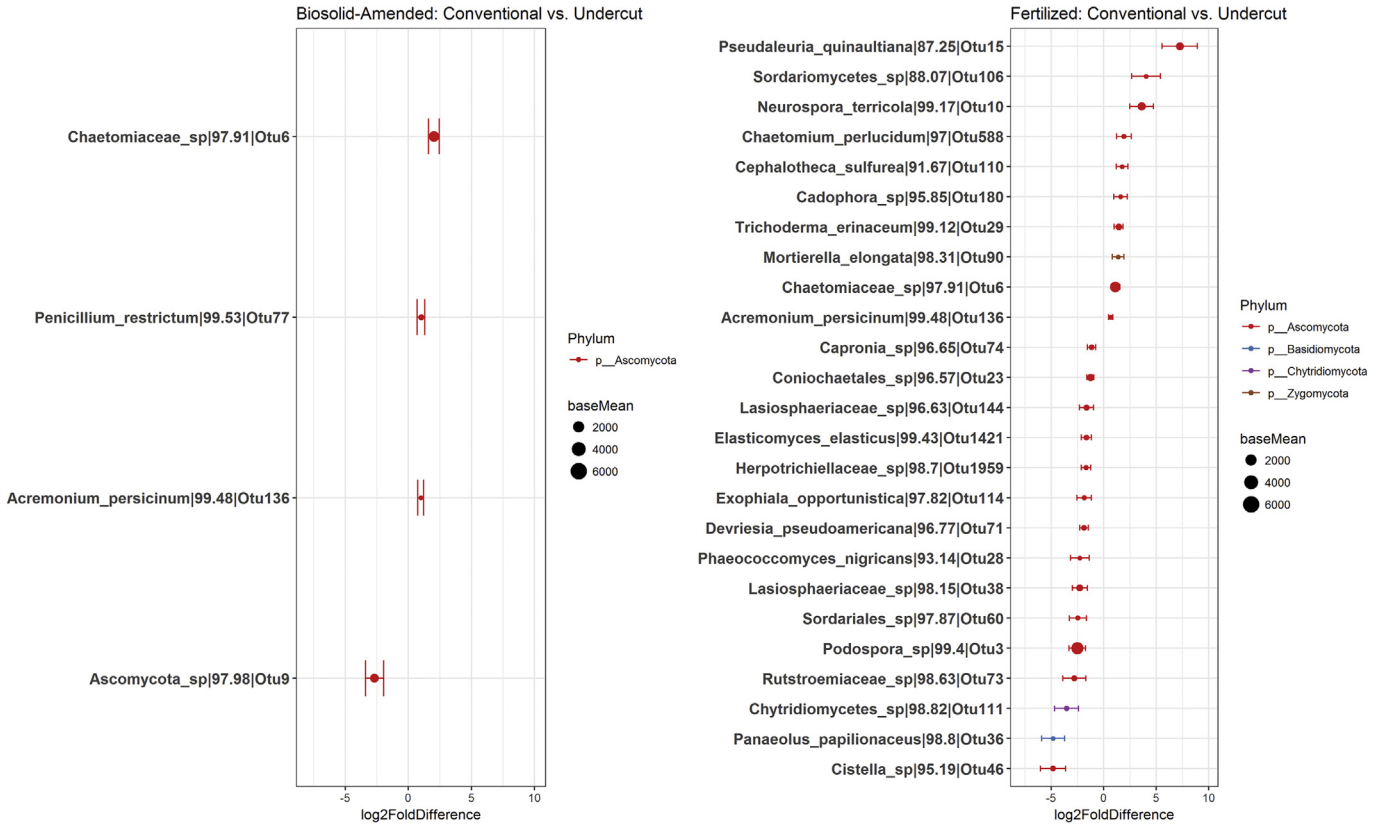

Fig. 5. Differentially abundant OTUs (baseMean>20) between biosolid-amended and synthetically fertilized soils under conventional and conservation tillage. OTUs are identified by their best hit to the UNITE database and the blast percent identity. OTUs are colored by the phyla to which they belong and are scaled by their mean abundance among all samples (baseMean).

Table 2
Fungal community richness and diversity (+/− standard deviation).

Year	Tillage	Treatment	Month	N	Richness	Shannon H′	Simpson 1/D
2015	Conventional	Bio	June	4	234.75 ± 43.1a	3.19 ± 0.62a	**11.23 ± 6.32a**
		Fert	June	4	246.5 ± 31.0a	3.77 ± 0.36a	**22.51 ± 10.08b**
	Undercutter	Bio	June	4	231.25 ± 16.1a	3.39 ± 0.2a	**11.54 ± 4.16a**
		Fert	June	4	258.75 ± 33.3a	3.67 ± 0.46a	**19.63 ± 9.53b**
2016	Conventional	Bio	April	4	223.25 ± 38.9a	3.06 ± 0.73a	10.88 ± 11.19a
		Fert	April	4	262 ± 29.68a	3.77 ± 0.40a	21.03 ± 9.15a
	Undercutter	Bio	April	4	243.75 ± 31.75a	3.62 ± 0.24a	17.43 ± 4.24a
		Fert	April	4	261.7 ± 20.90a	3.56 ± 0.53a	16.94 ± 9.82a
	Conventional	Bio	June	4	246 ± 22.21a	3.42 ± 0.33a	12.35 ± 3.6a
		Fert	June	4	276.25 ± 28.6a	3.69 ± 0.79a	22.25 ± 13.28a
	Undercutter	Bio	June	4	248.5 ± 18.8a	3.3 ± 0.36a	11.35 ± 4.35a
		Fert	June	4	240.5 ± 40.1a	3.2 ± 0.75a	11.84 ± 9.46a

Table 3
Mean fungal community richness and diversity (+/− standard deviation) in biosolid aggregates. T-test p-values (n = 3–4 replicates/treatment) are presented.

Year	Diversity	Tillage		p-value
		Conventional	Undercutter	
2015	Richness	58 ± 16.51	56 ± 30.32	0.92
	Shannon H′	1.77 ± 0.22	1.43 ± 0.38	0.26
	Simpson 1/D	3.75 ± 0.5	2.82 ± 0.91	0.21
2016	Richness	66 ± 15.3	116.67 ± 5.86	**0.004**
	Shannon H′	1.1 ± 0.23	2.23 ± 0.19	**<0.001**
	Simpson 1/D	2.18 ± 0.52	5.15 ± 1.44	**0.059**

window of temperature and moisture conditions conducive to fungal growth in east-central Washington, where the majority of the precipitation occurs over winter when temperatures are low. Alternatively, the minor impact of tillage treatments in this study may be due to the fact that both treatments were subjected to minimum soil inversion rodweeding which may have physically disrupted soil fungi in the upper soil layers in both treatments.

Although conservation tillage had little impact on soil fungal communities as a whole, there were some taxa that increased or decreased in relative abundance with conservation versus conventional tillage. Most notably, OTUs closely related to *Chaetomium* were enriched in the conservation-tillage treatment with both fertilizer treatments. *Chaetomium* is a common soil fungus well-adapted to degrading wheat straw (Abdullah et al., 1985; Katapodis et al., 2007; Sun et al., 2016), and has been found to

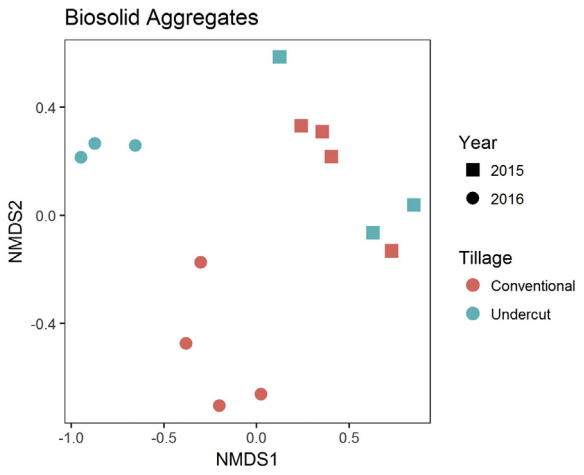

Fig. 6. NMDS plot of fungal communities from biosolid aggregates extracted from soil.

The application of sewage sludge or biosolids has been used in agriculture as a source of N and to increase organic matter (Cogger et al., 2013b; Rigby et al., 2016), which may be especially beneficial for the highly erodible soils of the Columbia Plateau with low levels of organic matter. Numerous studies have found that biosolid applications can modify soil microbial communities by increasing soil C, N, P, or through the introduction of heavy metals or other contaminants. However, in most cases the use of low-resolution methods such as FAME (Cogger et al., 2013b), TRFLP (Reardon and Wuest, 2016), DGGE (Anderson et al., 2008), Biolog (Pascual et al., 2008), or traditional isolation and morphological identification (Awad and Kraume, 2011; Fujii et al., 2013; Kacprzak et al., 2005) lack the ability to identify key fungal taxa.

Biosolid applications resulted in significant shifts in fungal community composition, but not diversity, versus synthetic fertilizers. These changes were most strongly correlated with C, N, and P, which tended to be associated with biosolid amendments. Some metals (Mn, Cu, and Cr) were also correlated with changes in fungal

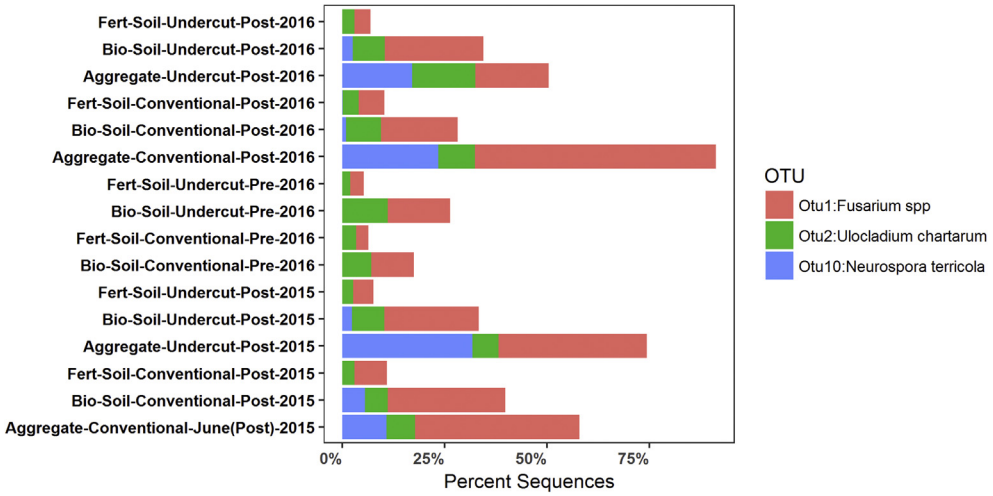

Fig. 7. Proportions of the most abundant fungal colonists of biosolid aggregates in each sample type.

increase with no-till in other studies (Penton et al., 2014; Stubbs et al., 2004). Intriguingly, more taxa were responsive to differences in tillage system in synthetically fertilized soils versus biosolid-amended soils, suggesting that increases in surface residue under conservation tillage may be less important for fungal communities when exogenous organic substrates are also supplied. Since biosolids are high in both organic C and N (~7:1), they may provide a high-quality substrate that is preferred by microbes over wheat straw residue, which has a much higher C:N ratio (~80:1). Indeed the C:N ratio in soil was among the strongest correlates of fungal community composition and was related to community differentiation between fertilizer treatments. Thus, the impacts of different fertilizers on nutrient stoichiometry, either with the combined addition of C and N in organic amendments or solely N in mineral fertilizers, may be a substantial driver of the observed shifts in soil communities (Delgado-Baquerizo et al., 2017). Further, because the organic forms of N in biosolids are not immediately available to microbes and are mineralized over time, fungal communities may respond more rapidly to the presence of wheat residues when readily-available mineral N is also supplied. In either case, organic N supplied via biosolids may slow the fungal community response to increased surface residue and reduce the rate of residue breakdown in conservation tillage systems.

communities and tended to be greater in biosolid-amended soils in 2015. However, Anderson et al. (2008) and Gomes et al. (2010) showed that Zn, Cd and Cu had minimal effects on fungal communities. Thus, it is likely that changes in fungal communities are largely driven by increasing C, N, and P in biosolid-amended soils rather than heavy metal contamination.

We identified a relatively small number of taxa that were greatly enriched by biosolids, including *Fusarium*, *Ulocladium*, *Neurospora*, *Gymnoascus*, and *Mortierella* in both years. These taxa were also commonly found in non-amended soil, and probably proliferate on biosolids as a source of C, N, or P, which is supported by the finding that these taxa dominated biosolid aggregates recovered from soil. *Neurospora terricola* was originally described as a soil fungus (Howe and Page, 1963), but does not produce conidia. *Ulocladium* is a common soil fungus that can degrade cellulose, plant residue and produce large numbers of dark colored spores that can survive in the soil. *U. chartarum* is also a common component of the aerial mycoflora. *Morierella,* a zygomycete, is a common soil fungus, capable of rapid growth on organic substrates. Zygomycetes and *Mortierella* have been noted in other long-term fertilization studies, and are hypothesized to be enriched in fertilized soils (Francioli et al., 2016), though the prevalence of these groups after biosolid applications versus synthetic fertilizer suggests that are likely

rapidly proliferating on the introduced organic material. *Gymnoascus* has been isolated from treatments with sewage sludge (Awad and Kraume, 2011) and is also considered a coprophilous fungus (Bills et al., 2013). *Gymnoascus* is keratinolytic and may be utilizing hair in the biosolids that survives the sewage processing and some are considered to be dermatophytic human pathogens causing skin diseases. In 2016, we were not successful in obtaining fungal ITS amplicons from fresh biosolid material, suggesting that these were probably not present in the fresh biosolid material. Previous work has shown that fresh biosolids are colonized by yeasts, but are bacteria dominated. The human gut microbiome is also dominated by bacteria, with very few fungal groups, primarily *Saccharomyces*, *Geotrichum* and *Candida* (Huffnagle and Noverr, 2013; Sam et al., 2017). However, there was little evidence for enrichment of these groups in soils that had received biosolids, likely due to strong competition from soil microbes and the lack of survival structures for fungi adapted to the human gut.

Fusarium spp. are among the dominant components of most soils. In fact, one of the OTUs (OTU 1) was the most abundant taxon in our study. However, it is not possible to distinguish down to the species level for *Fusarium* with ITS sequencing, and this OTU cluster may represent multiple *Fusarium* species. Most *Fusarium* are saprophytic soil inhabitants, such as *F. oxysporum*, and are important in the decomposition of straw, and a number of species have been isolated from wheat crowns (Gebremariam et al., 2017). Some *Fusarium* are important plant pathogens, such as *Fusarium pseudograminearum* and *F. culmorum,* causing Fusarium crown rot. However, preliminary results with a specific qPCR assay targeting these species suggested that biosolids were not selecting for these plant pathogens (Schlatter and Paulitz, unpublished), suggesting that the enriched *Fusarium* are saprotrophic. Although the risk of crown rot is not increased by enhancing populations of the pathogen, interactions with N levels may still be possible. Excessive N application can enhance Fusarium crown rot (Cook, 2001) through indirect drought stress by allowing plants to more rapidly deplete soil moisture, thus increasing disease. In cases where biosolids have enhanced corn (*Zea mays*) stalk rot caused by Fusarium (Ghini et al., 2016), this may have been due to enhanced levels of N rather than pathogen enrichment.

Surprisingly, enrichment for some fungal taxa was long term and persistent, since an application four years prior to sampling still showed consistent enrichment of *Fusarium*, *Ulocladium* and *Gymnoascus.* For other taxa, such as *Neurospora* and *Mortierella*, there was no apparent increase in relative abundance after four years. Reardon and Wuest (2016) examined soils in wheat cultivation seven years after biosolids were applied but found no significant shifts in fungal communities or increases in fungal populations. Cogger et al. (2013a) applied biosolids every four years from 1994 to 2010 to dryland wheat, which resulted in an increase in the bacterial/fungal ratio as determined by FAME, but no increase in fungal biomarkers. However, Barbarick et al. (2004) found significant impacts of biosolid on microbial respiration and AMF six years after a one-time application. Together, these studies suggest that biosolids may have lasting effects on soil communities, but these may depend on the land-use history or initial soil characteristics.

To our knowledge, this is the first study to use high-throughput sequencing to dissect in detail the response of fungal communities to biosolid applications in a monoculture wheat cropping system.

In conclusion, although the use of an undercutter tillage implement for soil conservation had minor impacts on fungal community composition over four years, biosolid application caused long-term shifts in fungal communities versus synthetic fertilizer in a dryland winter wheat-summer fallow cropping system. Still, more taxa responded to conservation tillage when synthetic fertilizer was used as an N source rather than biosolids, suggesting that differences in mineral versus organic N may impact residue degradation. Changes in fungal communities due to biosolids were characterized by relative increases in a small number of soil fungi that selectively colonize and likely decompose the biosolids, with little significant impact on community richness or diversity. However, the consequence of this shift in fungal communities for other microbial functions, such as disease suppression, is unknown. Native bacteria in these dryland wheat production areas have been shown to reduce take-all and Rhizoctonia root rot, two important root diseases (Kwak and Weller, 2013; Yin et al., 2013). Organic amendments, manures, and composts can reduce plant diseases and pests (Litterick et al., 2004). Although Ghini et al. (2016) found that sewage sludge increased suppressiveness to *Sclerotium* and *Rhizoctonia,* decreased suppressivness to *Pythium*, *Sclerotinia,* and had no effect on *F. oxysporum,* the potential for biosolids to suppress plant diseases merits study. Further work is needed to characterize the functions of the specific fungal taxa enriched by biosolids and their role in plant and soil health.

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Appendix A. Supplementary data

Supplementary data related to this chapter can be found at https://doi.org/10.1016/j.soilbio.2017.09.021.

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