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Effects of Annual and Perennial Alleyway Cover Crops on Physical, Chemical, and Biological Properties of Soil Quality in Pacific Northwest Red Raspberry

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Abstract. **Cover crops can lessen soil erosion and compaction, improve water infiltration, enhance nutrient availability, suppress weeds, and assist with pest management. However, cover crops are not commonly used in alleyways of established red raspberry (*Rubus idaeus*) fields in the Pacific Northwest of the United States. Rather, the space between red raspberry beds is repeatedly cultivated and the soil is kept bare, which has detrimental effects on soil quality. Adoption of alleyway cover crops is limited because red raspberry growers are concerned about resource competition between a cover crop and red raspberry crop. A 2-year study was conducted in an established 'Meeker' red raspberry field in northwest Washington to evaluate the effects of eight annually seeded alleyway cover crops (cultivars of wheat, cereal rye, triticale, oat, and ryegrass), one perennial ryegrass alleyway cover crop, mowed weed vegetation, and the industry standard of cultivated bare soil (Till) on the physical, chemical, and biological properties of soil quality in alleyways and raised beds. This included evaluating soil bulk density (D_b), compaction, organic matter, pH, cation exchange capacity (CEC), macro- and micronutrients, and bacterial and fungal community structure; red raspberry yield and fruit quality were also evaluated. Although there were statistically significant differences among treatments across sampling dates for CEC, there were no consistent trends. Alleyways planted with the perennial ryegrass mix had the lowest mean D_b 6 and 24 months after seeding. Tilled alleyways had the lowest D_b 12 and 18 months into the study. Red raspberry grown adjacent to Till did not result in a significantly higher estimated yield or fruit total soluble solids than raspberry grown adjacent to cover crops in either year of the experiment. Differences in microbial community structure were observed among seasons rather than treatments. These results do not demonstrate significant effects of alleyway cover crops on red raspberry productivity when applied to established fields. The potential benefits of alleyway cover cropping on soil quality may outweigh any concerns regarding resource competition. Changes in soil quality are often difficult to quantify and require long-term study.**

In 2017, approximately 35,600 t of raspberry (*Rubus idaeus* L.) were produced in Washington and Oregon and valued at over $57 million (U.S. Department of Agriculture, 2019). Washington leads the United States in the production of red raspberry for processing, and it is an important crop to the Pacific Northwest (PNW; U.S. Department of Agriculture, 2019). Red raspberry is also a unique perennial fruit crop as it has an expansive perennial root system and biennial canes that fruit on 1-year-old floricanes. The production of red raspberry in the PNW is on a 5 to 8-year cycle. Because suitable land is limited, red raspberry is often replanted in the same location following field renovation and a possible short-term winter cover crop (Rudolph et al., 2017). Replanted fields are usually broadcast fumigated in the fall before replanting (Walters et al., 2011). The following spring, 0.5 m-wide raised beds are formed and then planted with raspberry either as tissue culture plugs, roots, or bare-rooted plants.

In the PNW, and in western Washington specifically, alleyways of established red raspberry plantings are commonly kept bare and maintained by repeated and frequent rototilling to suppress weeds and unwanted canes (Barney et al., 2007; Walters et al., 2011). Alleyway cover cropping, annual or perennial, is not common practice. Over 3800 ha of raspberry were harvested in Washington alone in 2018 (U.S. Department of Agriculture, 2019). Only 20% of those hectares are occupied by red raspberry plants, which means that ≈3075 ha of soil is being disturbed by repeated rototilling and passage of heavy machinery and equipment for harvesting and other field operations.

Over time, repeated rototilling can have negative impacts on soil quality. Soil quality has been defined as the "continued capacity of the soil to function" as a living ecosystem that sustains plants, animals, and humans (Karlen et al., 1997; U.S. Department of Agriculture, 2001). Soil quality involves physical, chemical, and biological properties. Tillage can directly or indirectly affect any of these properties. Repeated tillage can increase soil erosion, which leads to the loss of soil quality and productivity (Pierce and Lal, 1994). Tillage can also cause soil compaction, contribute to the loss of soil physical structure, reduce the nutrient and water holding capacity of the soil, and increase dust during the dry season—which may increase spider mite (*Tetranychus* spp., *Eotetranychus* spp., and *Panonychus* spp.) activity and reduce photosynthetic capacity of plants (Barney et al., 2007; Golchin et al., 1995; Jackson et al., 2003; Magdoff and van Es, 2009; Tanigoshi et al., 2003). The highest percentage of soil organic matter is present on or near the soil surface, which makes it especially susceptible to wind and water erosion. Losses of soil organic matter from erosion have been shown to affect soil productivity (Bauer and Black, 1994). Cover crops may counteract the effects of repeated tillage. Cover crops are annual, biennial, or perennial living groundcovers that are grown with, before, or after a cash crop to the benefit of the cash crop and the surrounding soil. Cover crops can produce more biomass than resident vegetation, leading to enhanced rainfall infiltration and decreasing the potential for soil erosion and runoff (Dabney, 1998). Additionally, cover crops can improve soil structure; suppress weeds, pests, and pathogens; promote beneficial insect and soil microorganisms; and improve nutrient cycling (Forge et al., 2000; Freyman, 1989; Magdoff and Van Es, 2009; Mazzola and Gu, 2002; Sarrantonio, 2007; Zebarth et al., 1993).

Although challenging to demonstrate, the physical and chemical benefits to soil from the long-term use of cover crops and groundcovers have largely been accepted (Dabney et al., 2001; Hartwig and Ammon, 2002; Magdoff and Van Es, 2009; Sarrantonio, 2007). However, the biological ramifications are even more complex to establish. Soil microorganisms play an important role in soil quality and nutrient cycling by

Table 1. Cultivar, crop type, and species name for alleyway cover crops included in the western Washington floricane red raspberry (*Rubus idaeus*) field study, 2014–16.

Treatment designation	Treatment description
Mow	Resident weeds mowed and tilled annually
Till	Cultivation two times during growing season
‘Norwest’ wheat	Hard, red winter wheat (*Triticum aestivum*)[z] mowed, tilled, and reseeded annually
‘Rosalyn’ wheat	Soft, white winter wheat (*T. aestivum*)[z] mowed, tilled, and reseeded annually
‘Nora’ oat	Winter-hardy oat (*Avena sativa*)[y] mowed, tilled, and reseeded annually
‘TAM 606’ oat	Winter-hardy oat (*A. sativa*)[y] mowed, tilled, and reseeded annually
Perennial Grass	Intermediate and tetraploid perennial ryegrass mix (*Lolium hybridum*, *Lolium perenne*)[x] mowed, but not tilled or reseeded
Annual Grass	Perennial ryegrass mix (*L. perenne*)[x] mowed, tilled, and reseeded annually
‘Trical 103BB’ triticale	Triticale (*Triticosecale* sp.)[w] mowed, tilled, and reseeded annually
‘TriMark 099’ triticale	Triticale (*Triticosecale* sp.)[w] mowed, tilled, and reseeded annually
Cereal rye	Cereal rye (*Secale cereale*)[x] mowed, tilled, and reseeded annually

[z] WSU Northwest Research and Extension Center Plant Breeding Program, Mount Vernon, WA.

[y] Justin Seed Co., Justin, TX.

[x] Bailey Seed Co., Salem, OR.

[w] ProGene Plant Research, Othello, OR.

decomposing soil organic matter and storing nutrients (Turco et al., 1994). Soil disturbance through tillage can alter the soil environment and the associated microbial community structure, which has been shown to change dramatically following tillage (Calderón et al., 2000; Jackson et al., 2003; Peixoto et al., 2006). However, total microbial biomass in tilled soils has been shown to be both reduced (Angers et al., 1993; Beare et al., 1997) and not (Jackson et al., 2003; Reicosky et al., 1997) when compared with reduced tillage or no-till practices. Biological activity, specifically fungal, can also be reduced in compacted soil (Whalley et al., 1995).

Alleyway groundcovers are common in other perennial systems such as grape (*Vitis* spp.) and blueberry (*Vaccinium* spp.) (Hartwig and Ammon, 2002; Julian et al., 2011). Raspberry growers in the PNW cite concern for potential competition for water and nutrients between the cover crop and the raspberry crop, a situation that could decrease fruit yield and fruit quality. Previous results from cover crop trials in raspberry were mixed and only evaluated a small number of cover crop species. The Fraser Valley of British Columbia is geographically like the northwestern Washington region where much of the floricane red raspberry is grown. Zebarth et al. (1993) conducted a field study in the Fraser Valley and observed reduced primocane diameter of ‘Willamette’ raspberry when grown adjacent to perennial grasses (*Lolium* spp.). However, raspberry yield was not significantly affected. Bowen and Freyman (1995) observed a significant reduction in raspberry yield when alleyways were planted with perennial ryegrass (*Lolium perenne* L.) compared with bare soil in the same region. In the same study, when alleyways were planted with white clover (*Trifolium repens* L.), there was no difference in yield compared with bare soil. Freyman (1989) also observed reduced fruit yield and primocane diameter when alleyways were planted to perennial ryegrass in the Fraser Valley, but this was not consistent across all 3 years of the study. On Prince Edward Island, ‘Festival’ raspberry grown adjacent to oat (*Avena sativa* L.) that was annually seeded in alleyways produced similar fruit yields to raspberry grown adjacent to bare soil alleyways during a 4-year study (Sanderson and Cutcliffe, 1988).

The objectives of this study were to evaluate effects on the physical, chemical, and biological aspects of soil quality of one perennial and eight annual cover crops grown in the alleyways between raised beds of raspberry relative to the industry standard of cultivated, bare soil alleyways. We hypothesized that cover crops would improve physical and chemical properties of alleyway soil compared with bare, repeatedly cultivated soil.

Materials and Methods

Experimental design. A field experiment was conducted in an established, 3-year-old commercial ‘Meeker’ red raspberry field in Lynden, WA beginning in Fall 2014 and concluding in Fall 2016. The experiment had 17 rows of raised beds that were $\approx$150 m long with 3 m of spacing between bed centers. The space between the raised beds is referred to as “alleyways.” The raised beds of every other row were the location of the treatment plots where data were collected. The adjacent alleyways on either side of the raised beds were where the cover crops were seeded. The raised beds were $\approx$25 cm tall, and 45 cm wide. Before this experiment, alleyways had been routinely cultivated in the late spring and summer and no groundcover had been planted in the alleyways during the 3 years since establishment. To establish a baseline for soil chemistry before treatment application, a composite of 10 soil cores (2.5 cm in diameter and 20 cm deep) were uniformly collected from the bed of each row in Sept. 2014 and submitted to Brookside Laboratories, Inc. (New Bremen, OH). The soil in this field is a Lynden sandy loam, with a 6.4 pH, 3.94% organic matter (OM), 10 $\text{meq}\cdot 100\ \text{g}^{-1}$ cation exchange capacity (CEC), 100 $\text{kg}\cdot\text{ha}^{-1}$ nitrogen (N; measured as estimated nitrogen release), 201 $\text{mg}\cdot\text{kg}^{-1}$ phosphorus (P), and 227 $\text{mg}\cdot\text{kg}^{-1}$ potassium (K). The commercial grower managed the raspberry crop for the duration of the experiment according to recommended conventional practices for the region (Barney et al., 2007). This included drip irrigation and fertilization through the irrigation lines.

Experimental treatments were arranged in a completely randomized design and replicated four times. Each treatment plot was $\approx$28 m^2, consisting of 9.1 m of raised bed 0.7 m in width with 1.2 m of alleyway on either side. A buffer area 9.1 m long was established between each treatment plot in the same row, with a buffer 18.2 m long at the beginning and end of each row. To avoid undesired interaction between treatments, untreated beds and alleyways were adjacent to each treatment plot.

One perennial and eight annual cover crops (Table 1) were planted in the alleyways during Fall 2014 and 2015 to compare against a cultivated, bare soil control (Till) and a weedy control (Mow) that consisted of resident weeds and was maintained similarly to the cover crops. All cover crop treatments were applied twice over 2 years and were overlaid in the same plots each year except for Perennial Grass, a mixture of 51.25% *Lolium hybridum* ‘Tetralite’ and 48.24% *L. perenne* ‘Kentaur’, which was seeded only

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once at the beginning of the experiment. In 2014, immediately before seeding, all alleyways were rototilled to a depth of 15 cm. Cover crop treatments were seeded on 1 Oct. 2014 using a compact drill (3P500V; Land Pride, Salina, KS). The Perennial and Annual Grass treatments were seeded at 28 kg·ha^{-1} per the seed company's instructions. All other cover crop treatments were seeded at 112 kg·ha^{-1}. In both years, the cover crops were seeded in the alleyway at 0.9 m in width, leaving a 15-cm-wide band unseeded between the treatment and the raised bed on both sides. This area is where the tires of machinery and equipment pass throughout the season. The alleyways were not irrigated or fertilized.

All treatments, except Till, were mowed for the first time on 27 Apr. 2015. The Till plots were cultivated on 9 June and 17 Aug. 2015. On 22 Sept. 2015, all treatments, except for the Perennial Grass, were ended by sub-soiling and rototilling the alleyways. The floricanes were pruned, flail-mowed, and incorporated into the alleyways by the grower on 5 Oct. 2015. Treatments were seeded on 8 Oct. 2015 at the same rates and in the same locations as 2014, but seeding was done with a custom-made double-disc planter with a cone seeder that seeded seven rows with 15 cm spacing. Cover crops and Mow treatments were mowed once on 13 May 2016. Till plots were cultivated on 20 May and again on 27 July 2016.

Soil physical and chemical measurements. Soil bulk density (D_b) measurements were collected in treatment plot alleyways in Spring and Fall of 2015 and 2016. Two D_b measurements were collected in the alleyways on either side of the raised bed for a total of four samples per treatment plot. Measurements were never collected from the outer 15 cm of the alleyways (the area closest to the beds). Chopped canes, weeds, and other debris were brushed away to place the metal cylinder, 347.3 cm^3 in volume, directly on the soil surface. The D_b collection and measurement procedures were as described by the U.S. Department of Agriculture (2001) with a few modifications. In the field, soil in each cylinder was emptied into a plastic bag, stored in a cooler, and transported to the laboratory. Soil was immediately weighed, then a subsample was weighed before and after being dried in an oven dryer for 5 d at 65 °C to determine D_b.

Separate soil cores, 2.5 cm in diameter and 20 cm deep, were collected from the alleyway and bed for each treatment plot in the spring and fall of each year. Four cores were collected from each side of the bed and from each adjacent alleyway. Composite samples of eight cores for alleyways and beds were split into subsamples. On the same day of collection, one subsample was sent for chemical analysis (Brookside Laboratories, Inc.), including pH, OM, CEC, estimated N release, and concentrations of P, K, S, Ca, Mg, Na, B, Mn, Zn, Cu, Al, and Fe. Another subsample was stored in a freezer at –10 °C for future DNA extraction and molecular analysis (see section Soil microbial analysis).

Soil compaction measurements were collected in the spring and fall of each year using a Field Scout SC900 soil compaction meter (Spectrum® Technologies, Inc., Aurora, IL). Soil compaction was measured in the bare soil control plots immediately after plots were rototilled. Four soil compaction measurements were collected for each treatment plot to a depth of 30 cm, except in Fall 2016 when measurements were collected at a depth of 20 cm. The outer 15 cm of the alleyways, where no cover crops were seeded, were avoided due to extreme compaction.

Raspberry yield and fruit quality. Fruit was mechanically harvested by the grower from late June to mid-July in 2015 and early June to early July in 2016. Because a single floricane raspberry field can be harvested 12 to 20 times over the course of one season, a modified version of the yield estimation methodology developed by Daubeny et al. (1986) was used. Yield estimation was conducted on the raspberry crop on 12 and 15 June 2015 and on 6 June 2016. Three raspberry plants per treatment plot were selected, and the total cane number from each plant was counted and averaged. The number of laterals on two canes from each of the selected plants were counted and averaged. The fruit, including buds, flowers, and green fruit, on two laterals in five different fruiting zones per plant, were counted and averaged. Early, mid, and late season ripe raspberry fruit collections occurred on 26 June 2015, 7 and 16 July 2015, 14 and 21 June 2016, and 5 July 2016. At each sampling date, 30 fruit were randomly selected from each treatment plot, weighed, and frozen for future analysis of total soluble solids (TSS). Averages for each component were multiplied along with the number of plants/ha and average berry weight to estimate yield. The correction factor is an estimation of the yield lost from berry drop during mechanical harvesting. The fruit from each treatment plot and time point were crushed in a mesh bag (Agdia® Inc., Elkhart, IN) and the juice was strained into a test tube for TSS analysis. Three drops of juice were placed on a Palm Abbe digital refractometer (Model #PA201; MISCO, Solon, OH) for each measurement and measured in °Brix with three technical replicates.

Soil microbial analyses. Not all the treatments were included for rDNA amplicon sequencing and microbial community analysis. Treatments were chosen for sequencing based on performance with regard to *P. penetrans* population densities (Rudolph et al., 2017) and D_b. Additionally, only alleyway soil, not bed soil, was included in amplicon sequencing. The following treatments were selected: Mow, Till, Perennial Grass, Annual Grass, 'Norwest 553' wheat, 'Rosalyn' wheat, and 'Trical 103BB' triticale.

To prepare for DNA extraction, frozen soil samples were thawed in a refrigerator overnight. DNA was extracted from 5 g of alleyway soil from each treatment plot using the PowerMax Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) per the manufacturer's instructions. For bacterial analysis, DNA from each sample was normalized to a concentration of 5 ng·µl^{-1}. Bacterial 16S rDNA was amplified using the forward primer S-D-Bact-0341-b-s-17 and reverse primer S-D-Bact-0785-a-A-21 (Klindworth et al., 2013). The 16S Metagenomic Sequencing Library Preparation protocol created by Illumina® (San Diego, CA) was followed. For fungal analysis, DNA was normalized to a concentration of 200 ng·µl^{-1} using Amicon Ultra centrifugal filters (0.5 mL; EMD Millipore, Billerica, MA). Fungal DNA was amplified using the forward primer ITS1-F (Gardes and Bruns, 1993) and reverse primer ITS2 (White et al., 1990) modified to contain the partial Illumina adapter as described in the Metagenomic Sequencing Library Preparation protocol. A similar preparation protocol was used for fungal amplification as was used for bacterial amplification. All amplified samples were sent to Oregon State University Center for Genome Research and Biocomputing (Corvallis, OR) for library generation and gene sequencing on an Illumina® MiSeq sequencer.

Statistical analyses. Analysis of treatment by sampling date interactions for soil chemistry and plant performance data was conducted before analysis of treatment effects for individual sampling dates. All data, excluding the microbial data, were analyzed using Statistical Analysis System (SAS) software (Version 9.3; SAS Institute Inc., Cary, NC). Alpha was set at 0.05 for all data. All data were initially subjected to an analysis of variance (ANOVA) using Tukey's adjustment for multiple comparisons as the post-hoc test. When the data had unequal variance, means were transformed using log (x + 10) and reanalyzed. In rare cases when ANOVA assumptions could not be met, the nonparametric Kruskal–Wallis analysis was used to analyze differences. All data are presented with original means, even when transformations or rank analyses were performed. For the microbial dataset, the R statistical environment (R Studio Team, 2017) with the "vegan" package (Oksanen et al., 2017) was used to generate nonmetric multidimensional scaling (NMDS) ordinations and test sample groupings of the soil microbial data by calculating pairwise permutational multivariate ANOVA (PERMANOVA) for sampling date and treatment.

Results

Physical soil quality. There were treatment (by sampling date) interactions between Fall 2015 and Fall 2016 for Mow (P = 0.012) and Till (P = 0.0001) in which alleyway D_b was significantly higher in the fall of the second year. There were also treatment (by sampling date) interactions between Spring 2015 and Spring 2016 for nearly all treatments, including these: Till, Mow, 'TAM 606' oat, 'Trical 103BB' triticale, 'TriMark

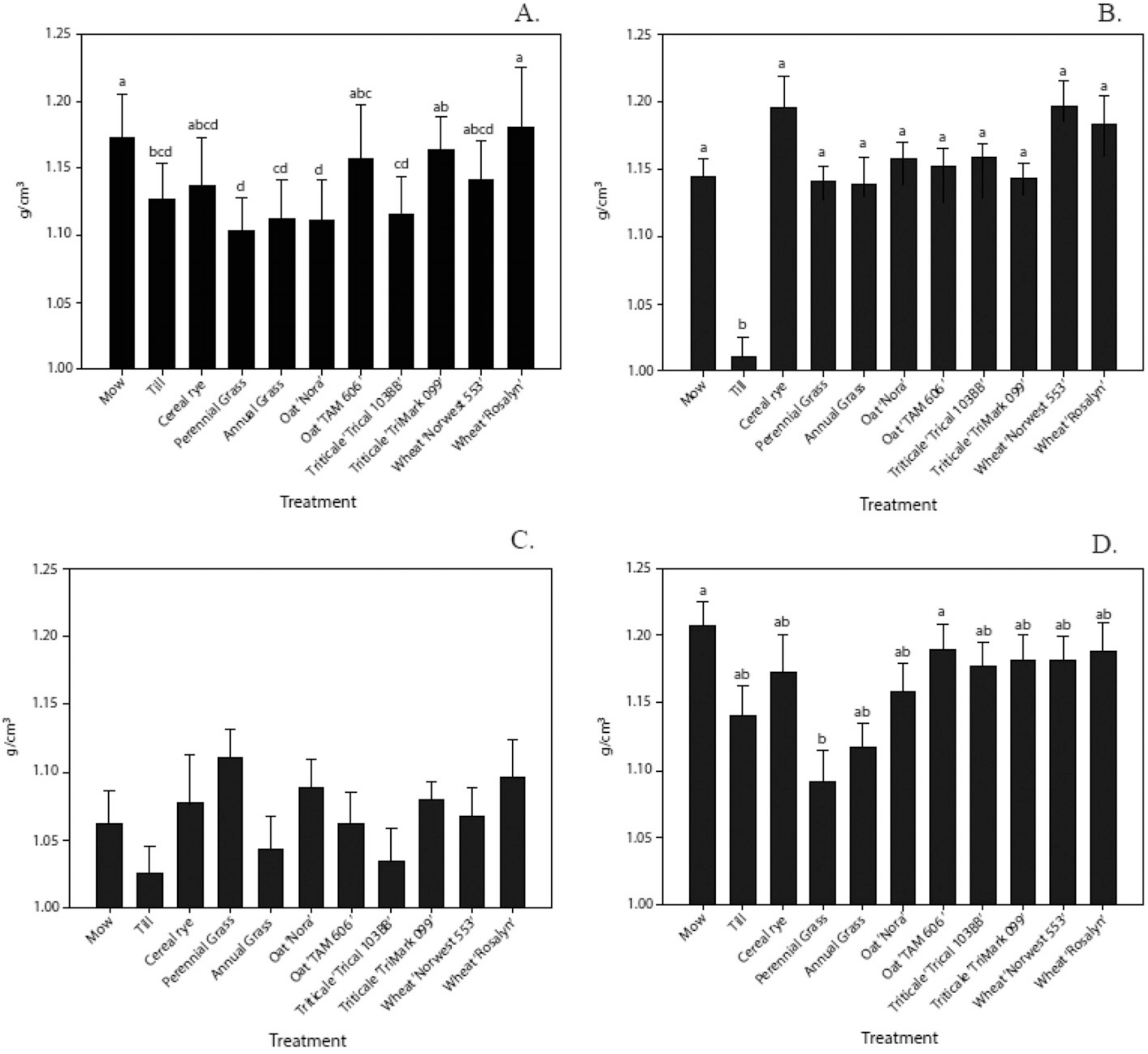

Fig. 1. Bulk density (D_b) of soil seeded with annual and perennial cover crops in Spring 2015 (**A**), Fall 2015 (**B**), Spring 2016 (**C**), and Fall 2016 (**D**) in the alleyways between raised beds of floricane red raspberry (*Rubus idaeus*) in Lynden, WA. Values are the mean of four replications ±SE. Means with the same letter in the same figure are not significantly different at $P \leq 0.05$. No letters indicate no significant differences among treatments.

099' triticale, 'Norwest 553' wheat, 'Rosalyn' wheat, and Annual Grass ($P \leq 0.02$). The D_b of these treatments were significantly lower in Spring 2016 than in Spring 2015. There were no significant differences in D_b between the sampling dates in any other treatment. There were treatment differences in D_b within sampling dates. In 2015, 6 months after seeding the alleyway cover crops, alleyways planted to the Perennial Grass had the lowest mean D_b overall, which was significantly lower than that measured for 'TAM 606' oat, 'TriMark 099' triticale, 'Rosalyn' wheat, or Mow ($P = 0.003$; Fig. 1A), but not Till. In Fall 2015, 1 full year after seeding, Till had the lowest mean D_b and 'Rosalyn' wheat had the highest ($P < 0.0001$; Fig. 1B). In Spring 2016, 6 months after the second seeding, all treatments had lower D_b and there were no significant differences among treatments ($P = 0.30$; Fig. 1C). In Fall 2016, 1 year after the second seeding, Perennial Grass, which had not been reseeded, had the lowest D_b of all treatments, but this was only significantly lower than D_b in Mow and 'TAM 606' oat ($P = 0.004$; Fig. 1D). When compaction was measured, nearly all treatments were observed to be compacted at soil depths >5 cm. There were very few significant differences across treatments, and deep compaction did not improve over time (data not shown).

Chemical soil quality. There were either no significant differences or there were inconsistent differences across treatments and seasons in the raised beds and alleyways for OM, pH, N, P, or K (data not shown). One full year after seeding, in Fall 2015, the CEC in raspberry beds adjacent to 'Norwest 553' wheat was significantly higher than that of raspberry beds adjacent to 'TAM 606' oat (11.9 and 9.8 meq·100 g^{-1}, respectively), but was not significantly greater than the other treatments ($P = 0.04$; data not shown). No other significant differences among other chemical soil properties were observed in the alleyways or beds that season. In Spring 2016, 6 months after the second seeding, there were significant differences among treatments in the raspberry beds' CEC. Beds adjacent to 'Rosalyn' wheat and 'Trical 103BB' triticale had significantly higher CEC than beds adjacent to 'Nora' oat (11.8, 12.1, and 9.5 meq·100 g^{-1}, respectively), but were not significantly different from the other treatments ($P = 0.01$; data not shown).

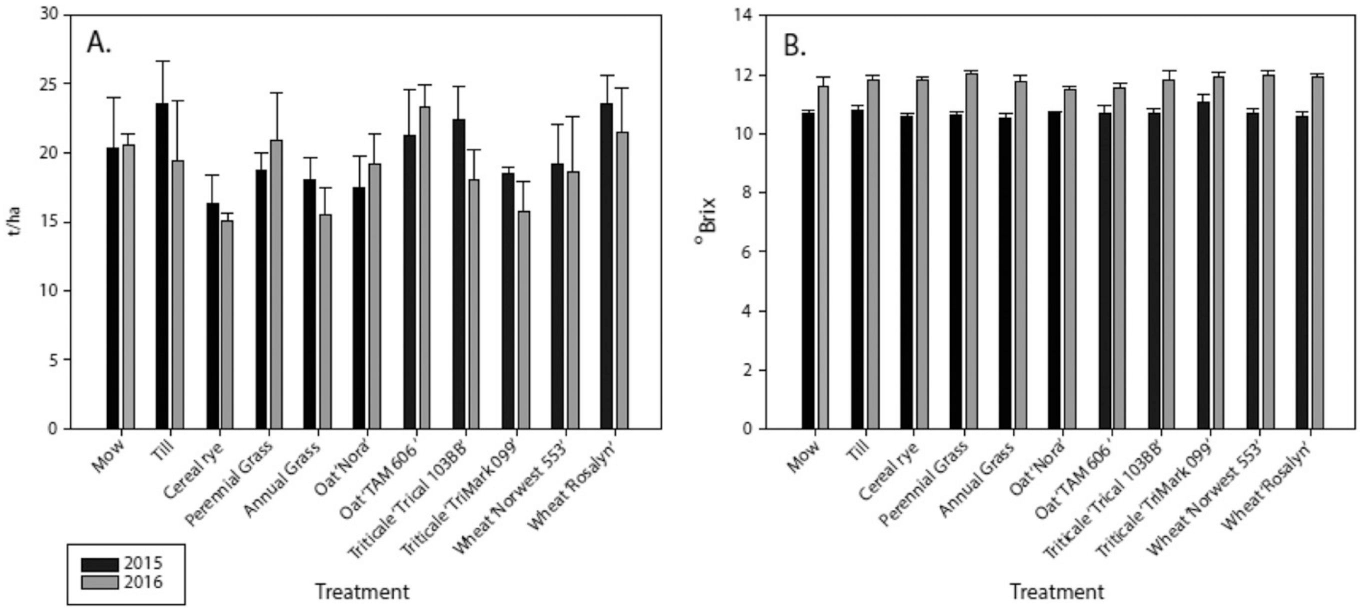

Fig. 2. Estimated yield (**A**) and total soluble solids (**B**) of established 'Meeker' red raspberry (*Rubus idaeus*) growing adjacent to alleyway cover crops, weedy vegetation (Mow), or cultivated bare soil (Till) in Lynden, WA over a 2-year period. Values are the mean of four replications ±standard error.

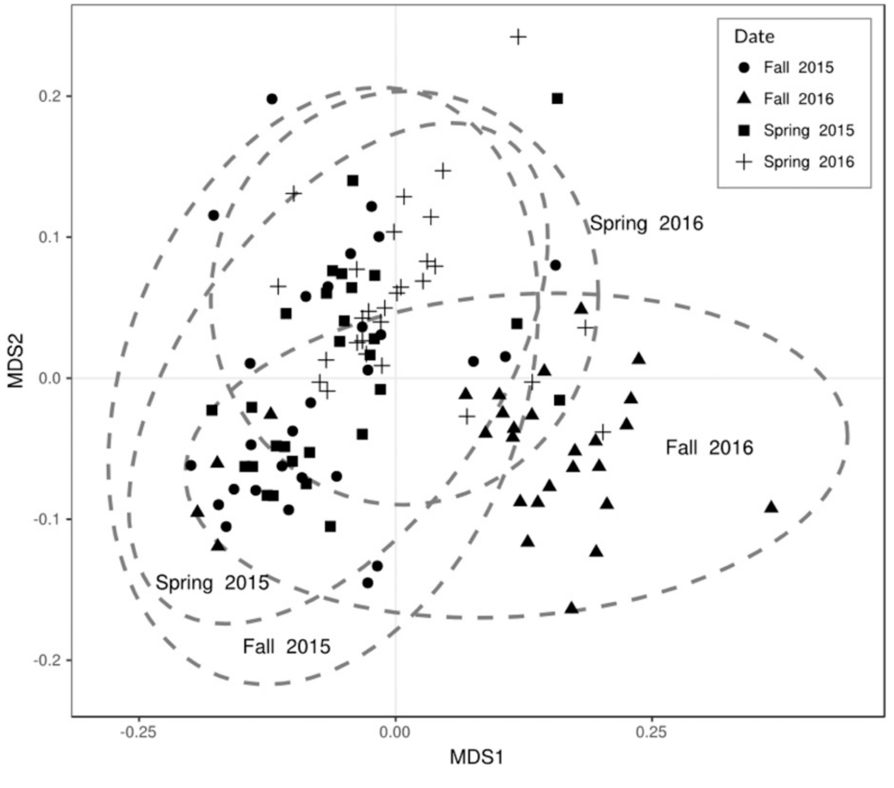

Fig. 3. Bacterial community structure over time from soil collected from floricane red raspberry (*Rubus idaeus*) alleyways under different cover cropping management in Lynden, WA. Treatments included in the analysis were the following: Mow, Till, Perennial Grass, Annual Grass, 'Norwest 553' wheat, 'Rosalyn' wheat, and 'Trical 103BB' triticale. In this NMDS ordination, each dot represents one soil sample from a treatment replication and is based on genus-level phylotype abundance. Permutational multivariate analysis of variance was used to determine significance of sample groupings across sampling dates (P = 0.001).

Alleyway CEC for 'Trical 103BB' triticale was significantly higher than for 'Nora' oat (10.2 and 8.4 meq·100 g^{-1}, respectively), but 'Trical 103BB' alleyways were not significantly different from other treatments (P = 0.02; data not shown).

Raspberry yield and fruit quality. There were no significant differences in estimated fruit yield or TSS among treatments in either year of the study. In both years, yield among treatments varied by no more than 35% (Fig. 2A). There was no treatment by year interaction for fruit yield (P = 0.61). However, there was a treatment by year interaction for fruit quality ($P \leq 0.03$): TSS was significantly higher in all treatments in 2016 compared with 2015. There was very little variation in TSS among the treatments within each year (Fig. 2B).

Biological soil quality. Sampling date or season had a stronger influence on the soil bacterial community (P = 0.001, Fig. 3) than treatment that included Mow, Till, Perennial Grass, Annual Grass, 'Norwest 553' wheat, 'Rosalyn' wheat, and 'Trical 103BB' triticale (P = 0.095). The soil bacterial community in Spring 2015 was not significantly different compared with Fall 2015, but every other season was different from one another ($P \leq$ 0.01). There were very few differences in microbial community composition when comparing each cover crop treatment separately to Till. Of genera that were present in 25 sequences or more, unclassified Rhizobiales, *Gemmatimonas*, *Steroidobacter*, unclassified Chlamydiales, unclassified Deltaproteobacteria, and unclassified Rhodospirialles were all significantly more abundant in Till compared with Perennial Grass (Fig. 4). These groups represent a small proportion of the overall bacterial diversity; less than 4% of the total relative abundance for any sample. The ecological effect of the small proportional changes observed in these taxa are not known, however the trend of these taxonomic changes may indicate a bacterial community shift on a longer time scale.

Sampling date and treatment were both significant (P = 0.001 and 0.043, respectively) for the soil fungal community throughout the 2-year study (Fig. 5). However, when making pairwise comparisons of the relative abundance of taxonomic groups between treatments, there were very few significant differences. Till and Perennial

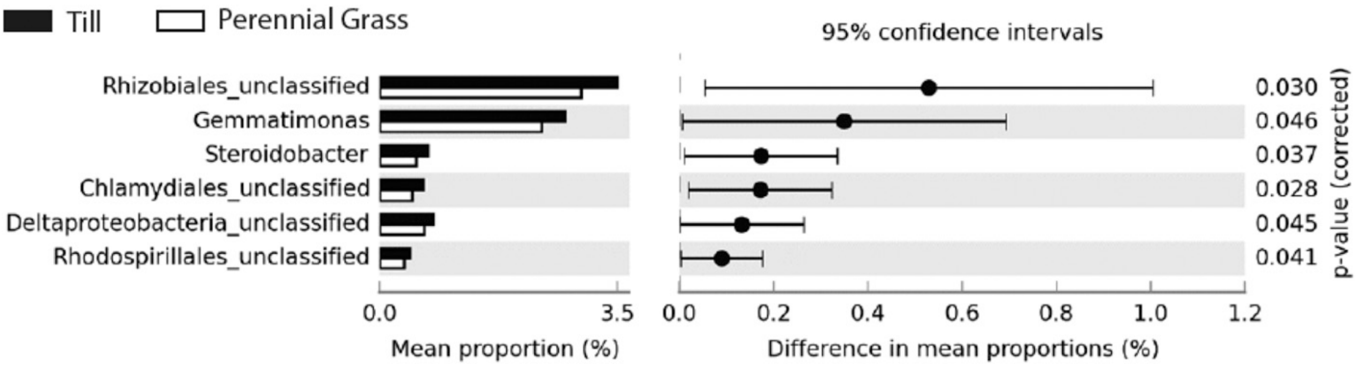

Fig. 4. Bacterial taxa that were significantly different when comparing cultivated, bare soil alleyway soil (Till) to alleyway soil planted to a perennial grass mix (Perennial Grass) for 2 years in an established floricane red raspberry (*Rubus idaeus*) field in Lynden, WA (minimum of 25 sequences). Significance was determined using a two-sided Welch's *t* test.

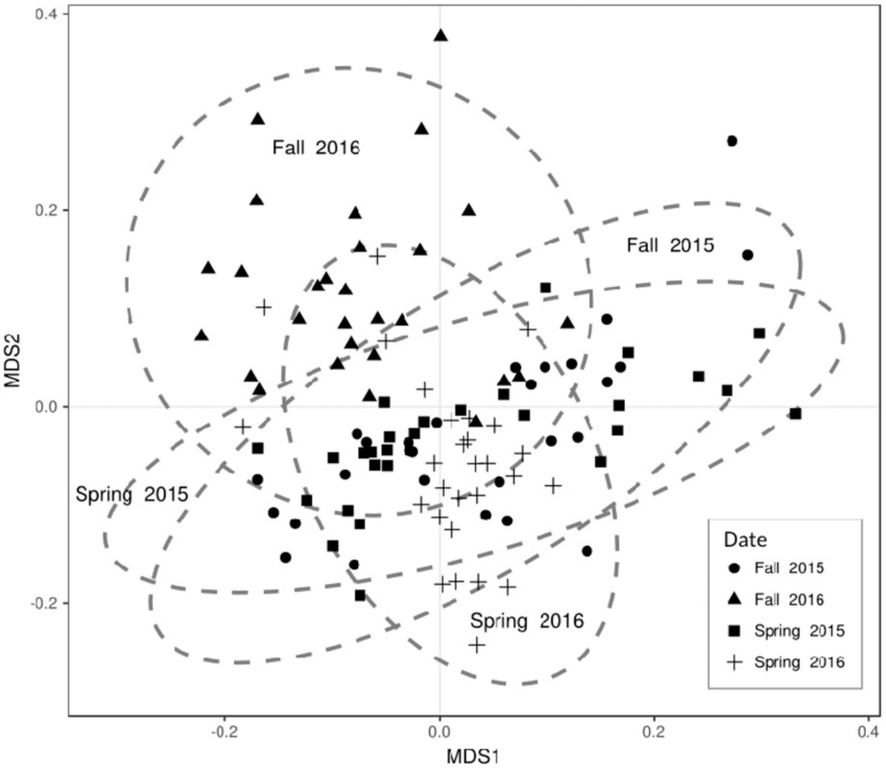

Fig. 5. NMDS ordination of soil fungal community structure based on genus-level phylotype abundance, over time from soil collected from floricane red raspberry (*Rubus idaeus*) alleyways in Lynden, WA. Treatments included in the analysis were the following: Mow, Till, Perennial Grass, Annual Grass, 'Norwest 553' wheat, 'Rosalyn' wheat, and 'Trical 103BB' triticale. Each dot represents one soil sample from a treatment replication. Permutational multivariate analysis of variance was used to determine significance across sampling dates ($P = 0.001$).

Grass displayed the most differences when comparing the different taxa present in the soil of each treatment (Fig. 6). *Cercophora, Minimedusa*, *Acremonium*, unclassified Sordariomycetes, and unclassified Amphisphaeriaceae were more abundant in Till alleyway soil compared with Perennial Grass. *Cercophora* represented, on average, 27.7% of the total bacterial abundance in Till and 23.1% in Perennial Grass, making it the most abundant responsive taxonomic group detected. *Capnobotryella*, *Ramophialophora*, unclassified Myriangiales, and unclassified Fungi were more abundant in Perennial Grass soil compared with Till, however the ecological importance of these small proportional differences among treatments remains uncertain.

Discussion

The implementation of alleyway cover cropping into the PNW raspberry production system instead of the industry standard, continuous cultivation, is supported by our findings. First and foremost, there were no negative impacts of any of the alleyway cover crops on raspberry fruit yield or quality, thus negating the idea of resource competition between cover crops and established raspberry. While consistent and measurable benefits of cover crops on soil quality as measured by physical, chemical, and biological attributes were not observed in this 2-year study, the many potential benefits of cover crops still outweigh the industry standard practice of alleyway cultivation in raspberry. Planting and maintaining a cover crop or groundcover, rather than relying on the resident weed population, ensures a consistent stand of plant material and helps manage the weed population by providing competition.

Raspberry yield and fruit quality were unaffected by the growth of adjacent cover crops compared with that of raspberry growing adjacent to cultivated, bare soil (Till). There are conflicting yield results from previous studies conducted in Canada. British Columbia is often considered part of the PNW because of its similar climate, crop production, and soil types. Zebarth et al. (1993), in the Fraser Valley of British Columbia, like us, also did not observe reduced yield of 'Willamette' raspberry when grown adjacent to alleyway cover crops. On Prince Edward Island (off the east coast of Canada), Sanderson and Cutcliffe (1988) did not observe reduced yield of 'Festival' raspberry grown adjacent to annually seeded oat compared with yield of raspberry grown adjacent to cultivated, bare soil. However, raspberry grown adjacent to timothy grass (*Phleum pretense* L.) did have the lowest yield through the 4 years of the study. Fruit quality was unaffected by treatment in this study. Inconsistent yield results across experiments may be due to whether the raspberry plants were grown in a raised bed vs. a flat ground system. None of the studies mentioned above describe a raised bed production system as part of their experimental design. The raised beds in this study, along with the compacted zones on either side of the raised beds, provided a physical separation from the cover crops. Raised beds also allow for improved soil water drainage (Funt and Ross, 2013), which is especially important in the PNW region where the average rainfall from 2008 to 2015

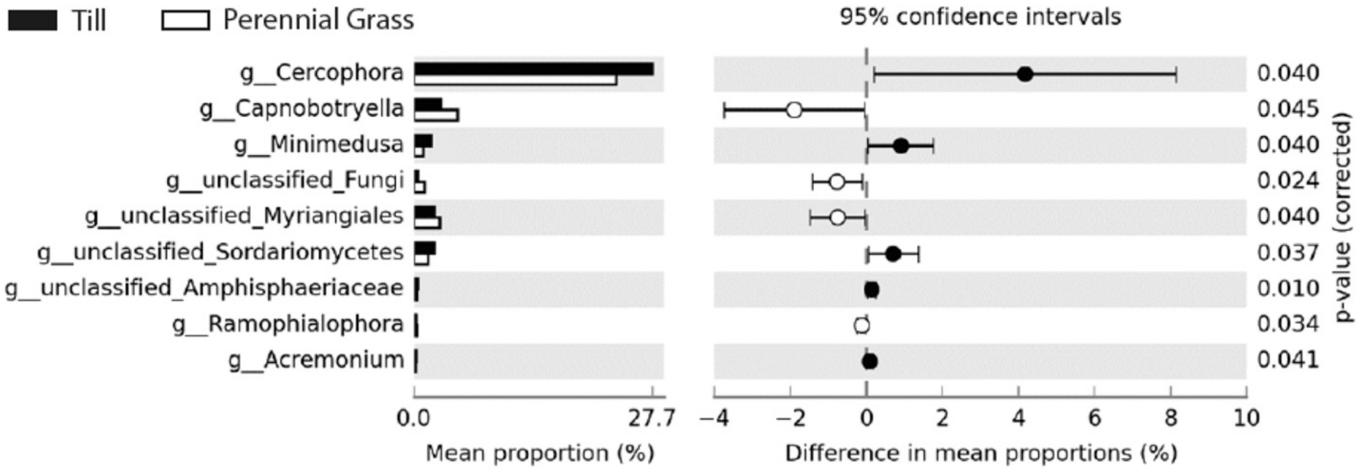

Fig. 6. Nine fungal taxa were significantly different when comparing cultivated, bare soil alleyway soil (Till) to alleyway soil planted to a perennial grass mix (Perennial Grass) in an established raspberry field in Lynden, WA (minimum of 25 sequences). *P* values were determined using a two-sided Welch's *t* test.

was 850 mm (Washington State University AgWeatherNet, 2017). Additionally, raised beds have been shown to reduce incidence of root rot caused by *Phytophthora rubi* compared with flat beds (Maloney et al., 1993).

Throughout the study, all of the treatments were below what is considered high D_b (1.60 g·cm^{-3}) in both seasons (Arshad et al., 1996). During two sampling dates, Spring 2015 and 2016, soil planted to Perennial Grass had the lowest D_b. Although not significantly lower than Till, this suggests that physical soil quality can be improved over time. Improvement of soil structure and lessening of D_b by cover cropping is a long-term solution and thus requires more time to demonstrate positive results. It is not unusual for D_b to be lower in cultivated soil compared with noncultivated soil, especially a few weeks after soil has been cultivated (Ismail et al., 1994). This occurred in Fall 2015 when Till was significantly lower than all other treatments. In a 10-year study with maize (*Zea mays*), no significant differences were observed in the D_b of cultivated soil compared with uncultivated soil (Blevins et al., 1983). Tillage of soil instantly relieves compaction and aerates the soil, but the effects are temporary. If repeated, however, cultivation can give the continuous impression of low D_b at shallow soil depths, but soil structure may be lost or deteriorated and OM will decompose more rapidly and decrease.

There were few significant differences among treatments in the soil chemical variables considered in this study. This was expected in the raised beds because of the physical separation from the alleyways and because of the uniform fertilization and irrigation performed by the grower. The absence of soil chemical differences in the alleyways may be attributed to the annual cultivation and deep ripping conducted in the fall before the second seeding of annual cover crops. This was done to all treatment plots, except those planted to the Perennial Grass. Soil OM accumulates slowly over time with continual inputs. The alleyways in this field had relatively high soil OM (2.9% to 4.6%) and received a yearly addition of plant biomass when raspberry canes were pruned, chopped, and incorporated into the soil. The total CEC of a soil is calculated based on OM and clay content. Given that the soil in this study was a sandy loam, soil OM likely accounted for a large portion of the total CEC (Magdoff and van Es, 2009). This may have influenced soil OM and CEC differences, or lack thereof. Additionally, increases in soil OM or available N may be depleted after one tillage event (Sarrantonio, 2007). Because nearly all treatments were under similar management, they would all be depleted equally. It is possible that differences were not observed in Perennial Grass soil chemical properties compared with all other treatments because significant effects on the soil take longer than 2 years to manifest.

Although soil bacterial community differences were not significant across treatments, significant differences were observed in the relative abundance of some taxonomic groups when performing pairwise comparisons of treatments. The greatest number of taxon differences occurred when comparing Till to Perennial Grass. Members of Rhizobiales, *Gemmatimonas*, *Steroidobacter*, Chlamydiales, Deltaproteobacteria, and Rhodospirillales were all significantly more abundant in alleyway Till soil compared with soil planted to Perennial Grass. Rhizobiales is an order of gram-negative bacteria that contains several genera capable of fixing N. Symbiotic *Rhizobium* spp. are in this order, as are many common plant-pathogens that can cause galling in plant roots, such as *Rhizobium radiobacter*. *Steroidobacter* is a gram-negative bacteria found in both grape roots and soil and is thought to be important to plant physiology and development through the production of brassinosteroids (Zarraonaindia et al., 2015). Deltaproteobacteria is a class of bacteria within the phylum Proteobacteria. This class includes a largely uncultivated group of bacteria that play an important role in the cycling of carbon, N, and sulfur (Kersters et al., 2006).

There were also soil fungal differences observed between Till and Perennial Grass. Till alleyway soil contained a larger proportion of *Cercophora, Minimedusa*, and *Acremonium*, while Perennial Grass soil had a larger proportion of unclassified Myriangiales. *Cercophora*, detected as a major taxonomic component of both the Till and Perennial Grass treatments, is a genus within the Lasiosphaeriaceae family, one of 31 polyphyletic genera in this family (Kruys et al., 2015). This genus is known for degrading plant material and animal manure. The genus *Minimedusa* has been isolated from soils around the world. Species within the genus have been shown to have antifungal properties and have suppressed the spread of *Fusarium* spp. and *Pythium violae* on various plants (Beale and Pitt, 1992, 1995). *Acremonium* is a genus within the family Hypocreaceae. The organisms within this genus have a broad range of ecological roles. Most species have been isolated from soil or plant material, either free-living or as endophytes (Wicklow et al., 2005). *Acremonium* has also been observed to be antagonistic toward plant-parasitic nematodes and other fungal pathogens (Anisha and Radhakrishnan, 2015; Cook et al., 1991; Goswami et al., 2008; Yao et al., 2015). Fungi from the order Myriangiales are often found on living leaves and twigs, while other members are saprobes or mycoparasites (Alexopoulos et al., 1996).

The exact biological ramifications of the presence of the bacteria and fungi observed in this study are unknown and confounded by the diverse ecological roles present within each taxonomic group. Additionally, the proportional abundance of most of the responsive taxonomic groups was very small, generally representing less than 4%, on average, of the total community in any treatment. The ecological impact of these minority members of the soil community may be difficult to detect simply due to their low abundance. The fungal genus *Cercophora* was commonly detected in all treatments and represented greater than 20% of the bacterial community in Till and Perennial

Grass treatments. The ubiquitous presence of this manure degrading group may be due to historical manure amendment on the site.

The lack of biological differences across the different cover crop treatments and the control, Till, is likely because all alleyways with cover crops, except those planted to Perennial Grass, were cultivated in the fall of each year. Tillage can have lasting effects on the soil microbial community (Calderón et al., 2000; Jackson et al., 2003; Peixoto et al., 2006). It can take many years of practicing no-till or not disturbing the soil for there to be significant differences in the soil microbiota. Soil that shares a land-use history has been shown to have similar microbial communities despite having different plant species growing in different areas of a field (Buckley and Schmidt, 2001; Jangid et al., 2011). This is likely the case with the soil in raspberry fields. In this production system, the soil has been managed the same for many years with repeated plantings of raspberry and repeated cultivation of alleyways. Although there are different areas of the field being treated differently or planted with different species of cover crops, the history of the land remains an overwhelming factor in determining the microbial community structure.

Conclusion

The absence of significant differences in the physical, chemical, and biological parameters considered in this study does not demonstrate how invaluable alleyway cover crops could be for PNW raspberry production. These positives include reduced soil erosion in continuously cultivated alleyways, improved water infiltration during the wet winters in the PNW, and weed suppression with minimal herbicide or mechanical weeding inputs. The lack of differences observed in this study also does not demonstrate that alleyway cover cropping is detrimental to raspberry production. Yield and fruit quality were maintained throughout the study and did not differ by treatment. Many of the benefits of planting cover crops may only be achieved through long-term implementation. The 2-year duration of this research was potentially insufficient to realize the long-term benefits of alleyway cover cropping in this production system. Future research should include long-term studies using more perennial cover crops rather than annual cover crops. It should also include an economic evaluation of maintaining alleyway cover crops compared with repeated cultivation.

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