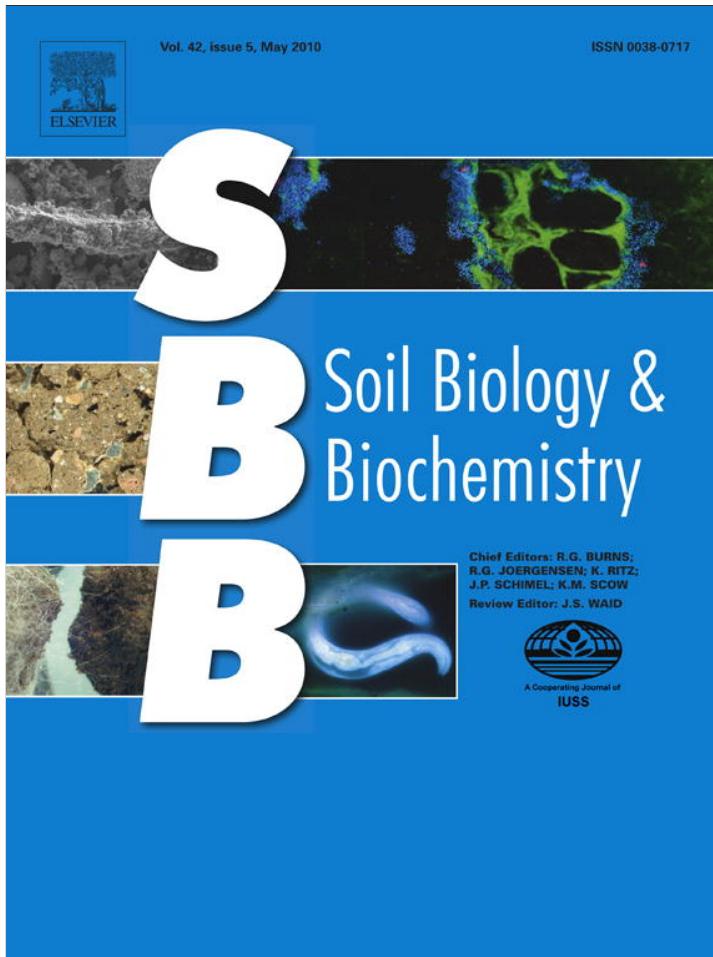


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## Factors affecting soil microbial community structure in tomato cropping systems

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### ABSTRACT

Soil and rhizosphere microbial communities in agroecosystems may be affected by soil, climate, plant species, and management. The management and environmental factors controlling microbial biomass and community structure were identified in a three-year field experiment. The experiment consisted of a tomato production agroecosystem with the following nine treatments: bare soil, black polyethylene mulch, white polyethylene mulch, vetch cover crop, vetch roots only, vetch shoots only, rye cover crop, rye roots only, and rye shoots only. The following hypotheses were tested: (1) Temperature and moisture differences between polyethylene-covered and cover-cropped treatments are partly responsible for treatment effects on soil microbial community composition, and (2) Different species of cover crops have unique root and shoot effects on soil microbial community composition. Microbial biomass and community composition were measured by phospholipid fatty acid analysis. Microbial biomass was increased by all cover crop treatments, including root only and shoot only. Cover cropping increased the absolute amount of all microbial groups, but Gram-positive bacteria decreased in proportion under cover crops. We attribute this decrease to increased readily available carbon under cover-cropped treatments, which favored other groups over Gram-positive bacteria. Higher soil temperatures under certain treatments also increased the proportion of Gram-negative bacteria. Vetch shoots increased the amount and proportion of Gram-negative bacteria, fungi, and arbuscular mycorrhizal fungi in the rhizosphere of tomato plants. The imposed treatments were much more significant than soil temperature, moisture, pH, and texture in controlling microbial biomass and community structure.

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## 1. Introduction

The microbial community composition of agricultural soils is influenced by a wide variety of factors. Physical, chemical, and biological factors that are believed to affect microbial community composition include soil type and texture (Buyer et al., 1999, 2002; Cavigelli et al., 2005; Gelsomino et al., 1999; Girvan et al., 2003; Ulrich and Becker, 2006), aggregate size (Schutter and Dick, 2002), moisture (Buckley and Schmidt, 2001b; Griffiths et al., 2003; Williams and Rice, 2007), predation (Griffiths et al., 1999), pH (Fierer and Jackson, 2006), and temperature (Norris et al., 2002). Agricultural management factors include tillage (Buckley and Schmidt, 2001a; Cookson et al., 2008), cover cropping (Carrera et al., 2007; Schutter et al., 2001), fertilizer (Grayston et al., 2004), organic amendments (Saison et al., 2006), and crop

rotation (Olsson and Alström, 2000). Many of these factors interact with each other and have both direct and indirect effects on the soil microbial community. For example, a winter cover crop would add organic carbon to the soil through rhizodeposition, and if the crop residue was mowed and left in place it would both reduce water evaporation and suppress weeds. In this example one management factor has at least three potential mechanisms for affecting the soil ecosystem.

This complexity of interacting factors makes it difficult to parse the dominant drivers of microbial community structure into explicitly measurable variables. In many cases, studies are limited due to an inability to control many of the factors influencing the microbial community and evaluation must rely on alternative metagenomic approaches which can be cost prohibitive (Shi et al., 2009). Agroecosystems, on the other hand, offer environmental sites that generally have well defined histories and often have well controlled factors that have been shown to influence soil community dynamics (Minoshima et al., 2007; Wang et al., 2007a). Additionally, determination of factors that influence microbial community composition in the field will have significant impact on understanding how management practices affect crop quality

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(Barrett et al., 2007), disease ecology (Zhou and Everts, 2007), and biogeochemical cycling (Hawkes et al., 2005; Mills et al., 1999). The agroecosystems described in this paper included several gradients across many of the variables that influence microbial populations and offered an opportunity to test hypotheses regarding the relative importance of these factors.

Tomatoes and other high-value crops are often grown on raised beds covered with black polyethylene (Hochmuth et al., 2008). The black polyethylene raises early season soil temperature, suppresses weeds, and conserves soil moisture. When combined with drip irrigation, synthetic fertilizers, and pesticides, this cropping system can produce high yields of marketable produce. However, use of this system can result in degradation of soil quality, increased runoff of contaminated water, and raised production costs (Rice et al., 2001). Alternative systems that use renewable resources and minimize soil tillage have been developed to improve environmental quality while maintaining profitability (Abdul-Baki and Teasdale, 1997; Rice et al., 2001).

In a tomato production system developed at the Beltsville Agricultural Research Center (Abdul-Baki and Teasdale, 1997), raised beds are formed each fall and seeded with hairy vetch. In the spring the vetch is mowed and the tomato seedlings are planted through the vetch residue without tilling. The vetch provides nitrogen and organic carbon to the soil and suppresses weeds. The surface layer of decomposing vetch shoots also reduces surface runoff and prevents splashing of soil onto the lower tomato leaves and fruit. While tomatoes grown under black plastic produce an earlier crop, tomatoes grown under vetch produce for a longer period of time and are less susceptible to disease (Kumar et al., 2004).

In a previous study on tomato cropping systems (Carrera et al., 2007) we found that cover cropping with hairy vetch had a greater impact on soil microbial communities than amending with compost or manure. We were unable to determine whether the differences in microbial communities between cover-cropped and black polyethylene-covered soils were due to nutrient inputs from the cover crops, soil temperature increases from the black polyethylene, both of these, or other unexplored factors. In this paper we use phospholipid fatty acid (PLFA) analysis to test the following hypotheses:

- (1) Temperature and moisture differences between polyethylene-covered and cover-cropped treatments are partly responsible for treatment effects on soil microbial community composition, and
- (2) Different species of cover crops have unique root and shoot effects on soil microbial community composition.

## 2. Materials and methods

### 2.1. Field experiment

This experiment was conducted at the USDA-ARS Beltsville Agricultural Research Center, Beltsville, Maryland, in the same field in 2005 and 2007 and in an adjacent field in 2006. Soils were mixed Hapludults and Endoaquults in the order Ultisols. Soils were classified according to the USDA texture classification scheme as sandy loam or loamy sand, varying between 63 and 83 percent sand, 7–27 percent silt, and 2–16 percent clay.

In the September before the tomato cropping season, lime and nutrients other than nitrogen were applied to fields according to Maryland soil test-based recommendations and beds were formed. The field was divided into four blocks with three four-bed sets within each block (Fig. 1). Every four-bed set was separated by an

unused bed. Each four-bed set within each block was seeded with either no cover crop, hairy vetch (*Vicia villosa*) at 40 kg ha<sup>-1</sup>, or rye (*Secale cereale*) at 90 kg ha<sup>-1</sup>. The beds within each four-bed set were 42 m long but were split into three 14 m long segments. These 14 m long four-bed areas will be called “plots” and represent the experimental unit for this experiment. Vegetation over the entire length of the beds receiving no cover crop and over one randomly chosen plot of hairy vetch and rye within each block was eliminated with an application of paraquat at 0.6 kg ai ha<sup>-1</sup> in late October and again in early spring if needed.

In spring all beds with no cover crop were rototilled and reformed. In each block these beds were covered with black polyethylene mulch on one plot, white polyethylene mulch on another plot, and the third plot had no mulch (designated bare hereafter). In late May, the hairy vetch and rye cover crops were mowed with a flail mower that dropped the shredded vegetation in place. The next day, cover crop shoots on one plot were moved to the surface of the plot within the same block with no vegetation (eliminated by the paraquat treatment the previous fall). This resulted in three hairy vetch and three rye treatments, intact cover crop, cover crop roots only, and cover crop shoots only. The treatments were randomized annually.

The following day 35-day old tomato (*Solanum lycopersicum* var. ‘Sunbeam’) plants were transplanted at a 51 cm within-row spacing using a punch-style transplanter that could place plants into the polyethylene mulch beds as well as the no-till cover-cropped beds with minimal disturbance to the mulch. Beds were irrigated at least weekly using drip irrigation. In addition, overhead irrigation was used after lengthy dry periods to aid the decomposition and release of nutrients from the surface cover crop residue. N was applied through the drip irrigation system. A total of 224 kg ha<sup>-1</sup> of N was applied to all treatments without hairy vetch and a total of 112 kg ha<sup>-1</sup> of N was applied to hairy vetch treatments based on requirements for achieving optimum yields at this site (Abdul-Baki et al., 1997). Weeds were controlled by a directed application of metribuzin herbicide at 0.56 kg ai ha<sup>-1</sup> approximately three weeks after transplanting and by handweeding as needed. Imidacloprid was applied in the transplant medium to control Colorado potato beetle. Insecticides and fungicides were not applied routinely but were only used according to Maryland IPM recommendations when visible symptoms suggested immediate action. Disease and insect pressure were light in all years so very few applications were needed. When used, herbicides, insecticides, and fungicides were applied to the entire field, so all treatments received the same chemicals at the same application rates. The only exception to this was the paraquat used on the bare treatments as described above.

### 2.2. Sampling

Prior to the flail mowing operation, 0.5 m<sup>2</sup> cover crop samples were removed from each block and separated for determination of above-ground and below-ground biomass. Tomato fruit with color from 24 plants of each plot, 12 each from the center two rows, were harvested weekly from approximately late July to late September. Fruit were separated into marketable and unmarketable categories and each category weighed separately. During the growing season soil moisture was measured 1–3 times per week, depending on field conditions, using a FieldScout TDR-300 (Spectrum Technologies, East Plainfield, IL, USA). In 2006 and 2007 soil temperature was measured for three treatments, black polyethylene, white polyethylene, and hairy vetch, using temperature loggers (Stow-Away Tidbit, Onset Computer Corporation, Bourne, MA, USA) buried 5 cm below the soil surface.

Bulk soil samples were taken five times annually: in late September when beds were formed, in May before the cover crop

Block 1			Block 2			Block 3			Block 4		
Vetch Shoots	Bare	Rye	Black Poly	Rye Shoots	Vetch Roots	Rye Shoots	Vetch Roots	White Poly	Vetch	Rye Roots	White Poly
Vetch Roots	White Poly	Rye Shoots	Bare	Rye Roots	Vetch	Rye	Vetch Shoots	Black Poly	Vetch Shoots	Rye	Bare
Vetch	Black Poly	Rye Roots	White Poly	Rye	Vetch Shoots	Rye Roots	Vetch	Bare	Vetch Roots	Rye Shoots	Black Poly

**Fig. 1.** Field layout for 2005. The layout was re-randomized each year.

was mowed, and in July, August, and September during the tomato harvest season. All four beds of each plot were sampled, with four 0–15 cm cores taken from each bed at 9 m intervals. All cores were combined, sieved, and subsamples stored at 4 °C for nematode analysis and –20 °C for microbial community analysis. Tomato rhizosphere samples were taken in July, August, and September. In each plot, two plants (one from each center bed) were removed and adhering soil was shaken off. The soil from the two plants was combined, sieved, and subsamples stored as described above.

### 2.3. Phospholipid fatty acid analysis

All solvents were HPLC or GC grade (Sigma–Aldrich, Milwaukee, WI, USA). Chloroform was stabilized with ethanol. Sodium phosphate, potassium hydroxide, and acetic acid were reagent grade. Water was purified by reverse osmosis and deionization to 16–18 MΩ cm. Glassware was washed with detergent, dried, and heated at 500 °C overnight, except for centrifuge tubes which were heated at 400 °C for 2 h. Screw caps were sonicated with detergent, rinsed and dried, and then washed with chloroform.

Five grams of lyophilized soil were placed in a 25 ml centrifuge tube with Teflon-lined screw cap, and 4 ml of 50 mM phosphate buffer (pH 7.4), 10 ml of methanol, and 5 ml of chloroform were added. Tubes were sonicated for 10 min in a sonicating water bath at room temperature and then rotated end over end for 2 h at room temperature. After centrifugation for 10 min at 2500 rpm, the liquid phase was transferred to a 30 ml test tube with Teflon-lined screw cap. Five ml each of chloroform and water were added, shaken vigorously, and allowed to separate overnight. The bottom (organic phase) was evaporated under N<sub>2</sub> and stored at –20 °C.

Lipid classes were separated by solid phase extraction (SPE) chromatography as follows. A 500 mg silica gel column (Part No. 188-0156, Agilent Technologies, Wilmington, DE, USA) was washed with 5 ml chloroform. After loading the extract in chloroform, neutral lipids were eluted with 5 ml of chloroform, polar lipids were eluted with 10 ml of acetone, and phospholipids were eluted with 5 ml of methanol. The phospholipid fraction was evaporated under N<sub>2</sub> and stored at –20 °C.

Fatty acids were transesterified with 1 ml of 1:1 methanol:toluene and 1 ml of 0.2 M methanolic KOH at 37 °C for 15 min. After adding 2 ml of hexane, 0.3 ml of 1 M acetic acid, and 2 ml of H<sub>2</sub>O, the mixture was vortexed and the phases allowed to separate. The top (organic) phase was removed and the extraction repeated with an additional 2 ml of hexane. The combined organic phase was evaporated under N<sub>2</sub> and stored at –20 °C.

The extracts were cleaned up by SPE using an NH<sub>2</sub> SPE column (Part No. 8B-S009-EAK, Phenomenex, Torrance, CA) washed with 1 ml of chloroform. Samples were loaded onto the column in chloroform and eluted with 1 ml of chloroform. After evaporation under N<sub>2</sub>, samples were dissolved in 200 µl of 1:1 hexane:methyl *tert*-butyl ether and transferred to a gas chromatography vial with a limited volume insert. Samples were stored at 4 °C until analysis.

Gas chromatography was performed on an Agilent 6890 GC equipped with autosampler, split-splitless injector, and flame ionization detector. The system was controlled with Agilent Chemstation and MIDI Sherlock software (Microbial ID, Inc., Newark, DE, USA). An Agilent Ultra 2 column, 25 m long × 200 µm I.D. × 0.33 µm film thickness, was used with hydrogen as the carrier gas at 66.9 kPa constant pressure. The MIDI Eukaryote method and fatty acid library was used, with the split ratio changed from 1:100 to 1:50 in order to increase sensitivity. The oven temperature was programmed from 170 °C to 300 °C at 5 °C/min, with a final hold of 12 min at 300 °C. An external standard of 16:0 methyl ester was used for quantification.

Random samples were also run on a Clarus 500 GC–MS (Perkin–Elmer, Waltham, MA, USA) in order to confirm fatty acid identifications. A DB-5MS column (Agilent), 50 m long × 200 µm ID × 0.33 µm film thickness, was used with helium as the carrier gas at 1.4 ml/min constant flow rate. The oven temperature program was 65 °C for 1 min, then 45 °C/min to 170 °C, and then 5 °C/min to 300 °C with a final hold of 15 min. Splitless injection was employed, and peaks were scanned from 50 to 500 amu using electron impact ionization.

### 2.4. Statistical analysis

ANOVA was conducted using the mixed model procedure in SAS software (SAS Institute, Cary, NC, USA). For analysis of marketable yield, cover crop (none, hairy vetch, or rye) and plot treatment within cover crop were analyzed as fixed effects and block and block\*cover crop were random effects. Since the treatment within cover crop effect was significant in all years, means separation among all treatments was performed using the Tukey–Kramer adjustment at *p* < 0.05.

PLFA peak areas were combined into biomarker groups as shown in Table 1. ANOVA was conducted on total PLFA and on each biomarker group in order to see how the total microbial biomass and the biomass of each group of organisms were affected by

**Table 1**  
Fatty acid biomarker groups.

Biomarker	Fatty acids	Reference
Gram-positive bacteria	Iso and anteiso branched fatty acids	(Zelles, 1999)
Gram-negative bacteria	Monounsaturated fatty acids, cyclopropyl 17:0 and 19:0	(Zelles et al., 1997)
Actinomycetes	10-methyl 16:0 and 10-methyl 18:0	(Zelles, 1999)
Fungi	18:2 ω6 cis	(Frostegård and Bååth, 1996)
Protozoa	20:3 ω6 cis and 20:4 ω6 cis	(Ringelberg et al., 1997)
Arbuscular mycorrhizae (AM)	16:1 ω5 cis	(Olsson, 1999)

treatment. Analysis of microbial community composition was conducted using Hellinger-transformed data, which is the square root of the proportional data (Eq. (1)):

$$H_i = \sqrt{\frac{F_i}{\sum_{i=1}^n F_i}} \quad (1)$$

$F_i$  is the  $i$ th fatty acid group,  $n$  is the total number of fatty acid groups, and  $H_i$  is the Hellinger-transformed fatty acid group. This transformation was applied to the biomarker groups for Gram-positive bacteria, Gram-negative bacteria, actinomycetes, fungi, arbuscular mycorrhizal (AM) fungi, and protozoa. Redundancy analysis was conducted using CANOCO (Microcomputer Power, Ithaca, NY, USA). Environmental variables included soil moisture, soil temperature, soil texture, soil pH, month, marketable fruit yield, and cover crop biomass. Variance decomposition of the redundancy analysis was carried out as described (ter Braak and Smilauer, 1998).

### 3. Results

#### 3.1. Cover crop biomass and tomato yield

Rye and hairy vetch cover crops established well and produced abundant biomass by the time of mowing in all years. Rye and hairy vetch above-ground biomass averaged 5940 and 6230 kg ha<sup>-1</sup>, respectively, whereas rye and hairy vetch root biomass averaged 2150 and 460 kg ha<sup>-1</sup>, respectively. The lower root than shoot biomass values, particularly those for hairy vetch roots, are consistent with findings of other researchers (Sainju et al., 2005).

There were few differences in marketable yield among the non-vetch treatments (Table 2). Soils had warmed sufficiently by the time tomatoes were transplanted in late May so that temperature differences among mulching treatments had minimal effect on tomato growth and development. All treatments received sufficient moisture and nutrients so there were minimal differences in availability of these resources among mulching treatments. The hairy vetch treatments with surface vetch residue had similar yields to the highest yielding non-vetch treatments despite receiving only half the fertilizer N (Table 2). This confirms previous research that showed above-ground vetch vegetation contained high levels of N which can meet approximately half of the N requirement of fresh-market tomatoes (Abdul-Baki et al., 1997). However, the hairy vetch root treatment, which lacked this N-rich surface residue but still received only half the rate of fertilizer N as the non-vetch treatments, had lower yields than the other vetch and most other treatments in all years.

**Table 2**

Tomato marketable yield (Mg/ha). Each value is the mean of 4 replicates. Values within a column with the same letter were not significantly different ( $p \leq 0.05$ ).

Treatment	Year		
	2005	2006	2007
Bare	56.0 A	83.9 A	88.0 B
Black Poly	54.2 A	81.6 A	92.1 AB
White Poly	59.0 A	75.5 AB	106.6 A
Rye	59.6 A	80.7 AB	77.8 BC
Rye Roots	55.1 A	70.9 AB	65.6 C
Rye Shoots	57.9 A	84.1 A	82.0 B
Vetch	56.0 A	82.1 A	92.2 AB
Vetch Roots	44.4 B	59.3 B	80.2 BC
Vetch Shoots	62.3 A	82.6 A	93.0 AB

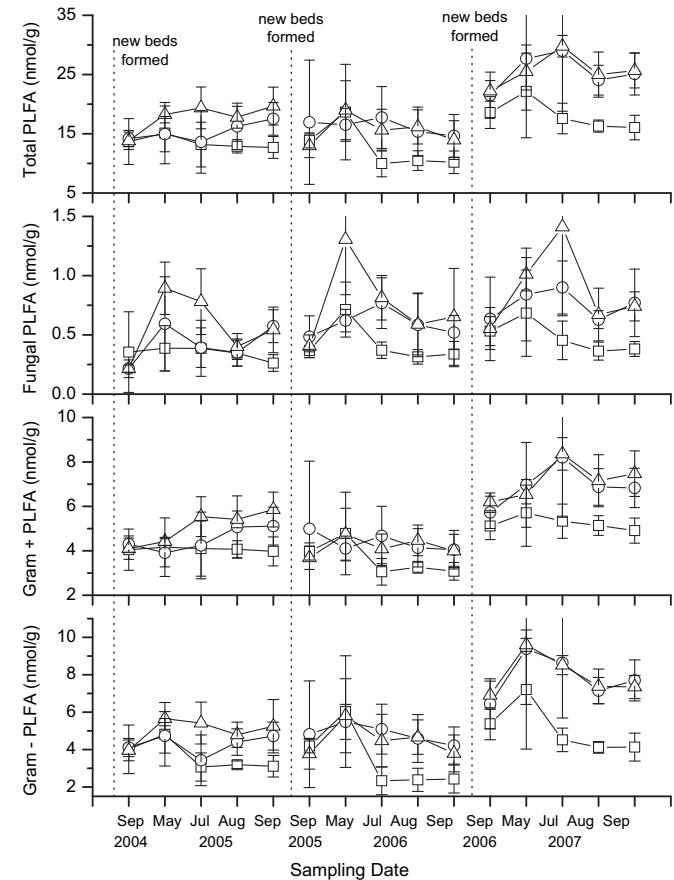
#### 3.2. Total PLFA

Total PLFA was used as a measure of microbial biomass. Changes in bulk soil PLFA concentration with time are shown in Fig. 2A for three treatments. Total PLFA typically increased from September, when new beds were formed, to May, whether or not cover crops were present. Following cover crop incorporation and the laying down of black polyethylene, fatty acid levels declined under black polyethylene and in July, August, and September were lower in the black polyethylene treatment than in the cover crop treatments. Total PLFA was highest in year three, presumably because that field had a large biomass of rye incorporated during the previous rotational year.

PLFA concentrations during July, August, and September are presented in Table 3 for bulk soil. Total PLFA was highest under vetch, vetch root, rye, and rye root treatments. Total PLFA was lower under vetch shoots and rye shoots and lower yet under bare, black polyethylene, and white polyethylene. This result indicates that the effect of cover cropping on bulk soil microbial biomass was due to both above-ground and below-ground portions of the cover crop. In tomato rhizosphere soil (Table 4), total PLFA was increased in the rye, rye root, vetch, and vetch shoot treatments compared to the non-cover cropped treatments.

#### 3.3. Gram-positive bacteria

The Gram-positive (Fig. 2C) bacterial biomarker fatty acids showed little increase from September to May, while the proportion of fatty acids attributed to Gram-positive bacteria decreased



**Fig. 2.** Bulk soil PLFA concentration. Each point represents the mean of 4 replicates. Black polyethylene, □. Rye, ○. Vetch, Δ.

**Table 3**

PLFA concentration (nmol/g) of bulk soil from July to September, 2005–2007. Values are least square means ( $n = 36$ ). Values within a column with the same letter were not significantly different ( $p \leq 0.05$ ).

Treatment	Total	Gram+	Gram-	Actino <sup>a</sup>	Fungi	AM Fungi	Protozoa
Bare	15.07 DE	4.45 CD	3.94 DE	2.17 BC	0.42 BC	0.58 DE	0.07 B
Black Poly	13.27 E	4.10 D	3.28 E	1.87 C	0.36 C	0.48 E	0.04 B
White Poly	15.49 CDE	4.61 BCD	4.04 DE	2.20 BC	0.45 BC	0.59 CDE	0.08 AB
Rye	19.33 AB	5.48 AB	5.58 AB	2.69 A	0.61 AB	0.85 A	0.18 AB
Rye Roots	18.39 ABC	5.26 ABC	5.16 ABC	2.53 AB	0.60 AB	0.73 ABC	0.14 AB
Rye Shoots	16.72 BCD	4.90 BCD	4.51 CD	2.41 AB	0.44 BC	0.66 BCD	0.11 AB
Vetch	20.38 A	5.82 A	5.76 A	2.71 A	0.73 A	0.81 AB	0.20 AB
Vetch Roots	19.04 AB	5.47 AB	5.36 ABC	2.59 AB	0.54 BC	0.72 ABCD	0.27 A
Vetch Shoots	17.39 BCD	5.05 BC	4.77 BCD	2.46 AB	0.55 ABC	0.71 ABCD	0.13 AB

<sup>a</sup> Abbreviations: Actino, Actinomycetes; AM Fungi, arbuscular mycorrhizal fungi.

from September to May and then increased (Fig. 3A). The Hellinger-transformed PLFA data for July, August, and September is presented in Table 5 (bulk soil), and Table 6 (rhizosphere). Black polyethylene increased the proportion of Gram-positive bacteria in both bulk and rhizosphere soil. These results indicate that relative to other microbial groups Gram-positive bacteria responded positively to warmer soil temperatures and negatively to cooler soil temperatures. Also, cover cropping stimulated Gram-positive bacteria less than other components of the soil microbial community.

#### 3.4. Gram-negative bacteria

The Gram-negative (Fig. 2D) bacterial biomarker fatty acids increased from September to May and then decreased, as did the proportion of the total community attributed to Gram-negative bacteria (Fig. 3B). During the summer months in bulk soil Gram-negative bacteria were highest under vetch and generally higher under cover-cropped than non-cover-cropped soils (Table 3). In the tomato rhizosphere Gram-negative bacteria were highest under vetch and vetch shoots (Table 4) and also generally higher in cover-cropped systems than in bare and plastic-mulched systems. Gram-negative bacteria responded positively to both roots and shoots from cover crops, but vetch shoots, alone or in combination with vetch roots, had the greatest positive effect in the tomato rhizosphere.

#### 3.5. Fungi

The fungal PLFA biomarker (Fig. 2B) increased from September to May and then decreased, as did the proportion of the community attributed to fungi (Fig. 3C). In July, August, and September the absolute and relative amounts of fungal PLFA in bulk soil were highest under vetch and lowest under bare and plastic-mulched treatments (Tables 3 and 5). In rhizosphere soil the absolute and relative amounts of fungal PLFA were highest under vetch shoots (Tables 4 and 6). As in the case of Gram-negative bacteria (above),

vetch shoots had a stimulatory effect in the tomato rhizosphere that was not seen with vetch roots or any rye treatment.

The PLFA used as the fungal biomarker, 18:2 ω6 cis, is also found in plants (Zelles, 1997), so it is possible that fine root fragments in our samples could have been partly responsible for the measured concentration of this fatty acid. In order to develop plant root biomarkers we performed PLFA analysis on field-grown vetch and rye roots. In rye, the three major fatty acids were 16:0 (24%), 18:2 ω6 cis (30%), and 18:3 (20%). In vetch, the same three fatty acids were dominant, with 21, 58, and 14%, respectively. While we did not analyze tomato roots, literature values were 25, 54, and 6%, respectively (Ouarihi et al., 1997). The fatty acid 18:3 was found in only 6 out of 862 samples, indicating that there was little or no plant root biomass in the vast majority of the samples. In addition, the ratio of 16:0 to 18:2 ω6 cis averaged 5.98 in bulk samples and 4.12 in rhizosphere samples. Since the ratio of these two fatty acids was 0.36 in vetch, 0.46 in tomato, and 0.8 in rye, it is apparent that plant root fatty acid contributed very little to the fungal biomarker in our study. However, the difference between bulk and rhizosphere samples may include a small contribution from tomato fine roots.

#### 3.6. Actinomycetes

In July, August, and September the concentration of actinomycete PLFA biomarker was higher under cover crops and lower under bare and plastic mulch in both bulk soil and tomato rhizosphere (Tables 3, 4). There was very little variation in the proportion of actinomycetes, but vetch shoots decreased the proportion in tomato rhizosphere soil (Table 6). The stimulatory effect of vetch shoots on Gram-negative bacteria and fungi in the tomato rhizosphere was not seen for actinomycetes.

#### 3.7. Arbuscular mycorrhiza

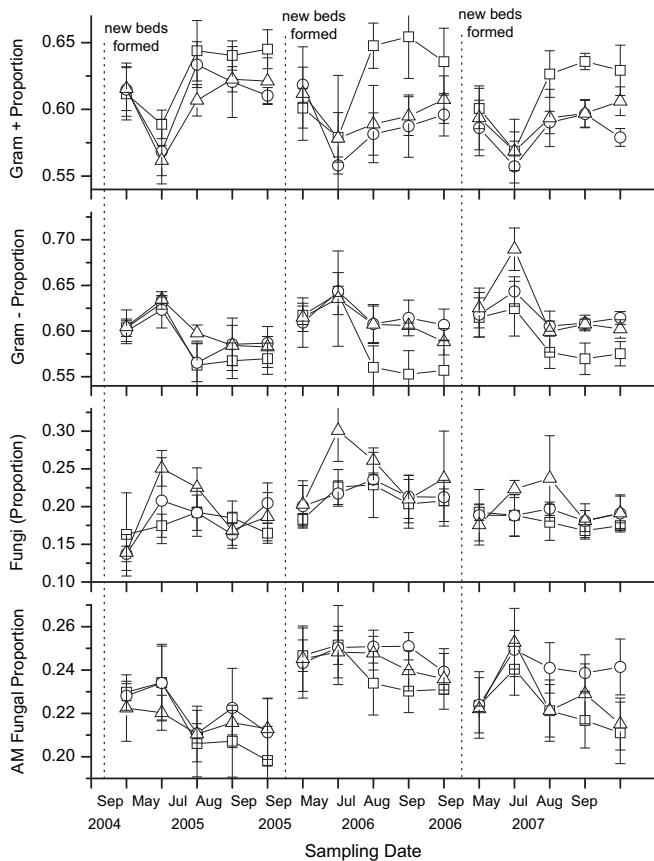
Rye and vetch increased the absolute amount of AM PLFA biomarker in bulk soil (Table 3) in July, August, and September.

**Table 4**

PLFA concentrations (nmol/g) of rhizosphere soil from July to September, 2005–2007. Values are least square means ( $n = 36$ ). Values within a column with the same letter were not significantly different ( $p \leq 0.05$ ).

Treatment	Total	Gram+	Gram-	Actino <sup>a</sup>	Fungi	AM Fungi	Protozoa
Bare	17.74 B	4.94 BC	4.94 BCD	2.23 BCD	0.71 D	0.67 BC	0.15 D
Black Poly	16.63 B	4.76 C	4.46 D	2.02 D	0.71 D	0.59 C	0.16 CD
White Poly	17.54 B	4.98 BC	4.78 CD	2.17 CD	0.73 D	0.62 C	0.17 CD
Rye	24.03 A	6.32 A	7.25 AB	2.90 A	1.05 ABCD	1.03 A	0.35 ABC
Rye Roots	24.14 A	6.36 A	7.04 ABC	2.84 A	1.16 ABC	1.00 A	0.31 ABCD
Rye Shoots	22.30 AB	6.01 AB	6.54 ABCD	2.64 ABC	0.96 BCD	0.92 AB	0.31 ABCD
Vetch	26.89 A	6.78 A	8.38 A	2.76 AB	1.29 AB	1.05 A	0.42 AB
Vetch Roots	22.50 AB	6.07 AB	6.58 BCD	2.72 ABC	0.90 CD	0.91 AB	0.26 BCD
Vetch Shoots	25.30 A	6.46 A	7.53 AB	2.59 ABC	1.38 A	1.08 A	0.46 A

<sup>a</sup> Abbreviations: Actino, Actinomycetes; AM Fungi, arbuscular mycorrhizal fungi.



**Fig. 3.** Bulk soil microbial community composition. Each point represents the mean of 4 replicates. Results are expressed as the Hellinger-transformed proportional values. Black polyethylene, □. Rye, ○. Vetch, △.

In the tomato rhizosphere (Table 4) levels were highest under all of the cover-cropped treatments and lowest under black and white polyethylene. In bulk soil the proportion of AM was highest under rye and lowest under black polyethylene (Table 5), while in tomato rhizosphere the proportion of AM was highest under rye and vetch shoots and lowest under plastic mulch (Table 6). The positive effect of rye, in both bulk and tomato rhizosphere soil, may be readily explained by AM colonization of rye (Wang and Qiu, 2006), but the effect of vetch shoots in the tomato rhizosphere cannot be explained by AM colonization of vetch.

The AM biomarker, 16:1 ω5, is also found in Gram-negative bacteria. In soil Gram-negative bacteria contribute 30–60% of the total amount of 16:1 ω5 measured (Olsson, 1999). This may

**Table 5**

Hellinger-transformed PLFA concentrations of bulk soil from July to September, 2005–2007. Values are least square means ( $n = 36$ ). Values within a column with the same letter were not significantly different ( $p \leq 0.05$ ).

Treatment	Gram+	Gram-	Actino <sup>a</sup>	Fungi	AM fungi	Protozoa
Bare	0.62 BC	0.58 BC	0.43 A	0.19 AB	0.22 BC	0.04 CD
Black Poly	0.64 A	0.57 C	0.43 A	0.19 AB	0.22 C	0.03 D
White Poly	0.63 AB	0.57 BC	0.43 AB	0.20 AB	0.22 BC	0.04 CD
Rye	0.62 BCD	0.60 A	0.42 ABC	0.20 AB	0.23 A	0.09 AB
Rye Roots	0.61 CDE	0.59 A	0.42 ABC	0.20 AB	0.23 BC	0.08 ABC
Rye Shoots	0.62 BCD	0.59 AB	0.43 A	0.18 B	0.23 BC	0.06 BCD
Vetch	0.60 DE	0.60 A	0.41 C	0.21 A	0.23 BC	0.11 A
Vetch Roots	0.61 CDE	0.60 A	0.41 BC	0.19 AB	0.23 AB	0.10 AB
Vetch Shoots	0.61 BCDE	0.59 AB	0.42 ABC	0.19 AB	0.23 AB	0.08 ABC

<sup>a</sup> Abbreviations: Actino, Actinomycetes; AM Fungi, arbuscular mycorrhizal fungi.

**Table 6**

Hellinger-transformed PLFA concentrations of rhizosphere soil from July to September, 2005–2007. Values are least square means ( $n = 36$ ). Values within a column with the same letter were not significantly different ( $p \leq 0.05$ ).

Trt	Gram+	Gram-	Actino <sup>a</sup>	Fungi	AM Fungi	Protozoa
Bare	0.61 AB	0.60 BCD	0.40 A	0.23 B	0.22 BC	0.09 BC
Black Poly	0.62 A	0.58 D	0.40 A	0.23 B	0.21 C	0.08 C
White Poly	0.61 A	0.59 CD	0.40 A	0.24 AB	0.21 C	0.08 C
Rye	0.58 C	0.62 A	0.39 A	0.23 B	0.23 AB	0.13 A
Rye Roots	0.59 BC	0.61 ABC	0.39 AB	0.24 AB	0.23 AB	0.12 A
Rye Shoots	0.59 BC	0.61 AB	0.38 AB	0.23 B	0.23 AB	0.13 A
Vetch	0.58 C	0.62 A	0.37 AB	0.24 AB	0.23 AB	0.14 A
Vetch Roots	0.59 BC	0.61 AB	0.39 A	0.22 B	0.23 AB	0.12 AB
Vetch Shoots	0.58 C	0.62 A	0.36 B	0.26 A	0.23 A	0.14 A

<sup>a</sup> Abbreviations: Actino, Actinomycetes; AM Fungi, arbuscular mycorrhizal fungi.

have caused the AM biomarker responses to resemble the Gram-negative responses more than they would have if the AM biomarker was truly specific for AM fungi.

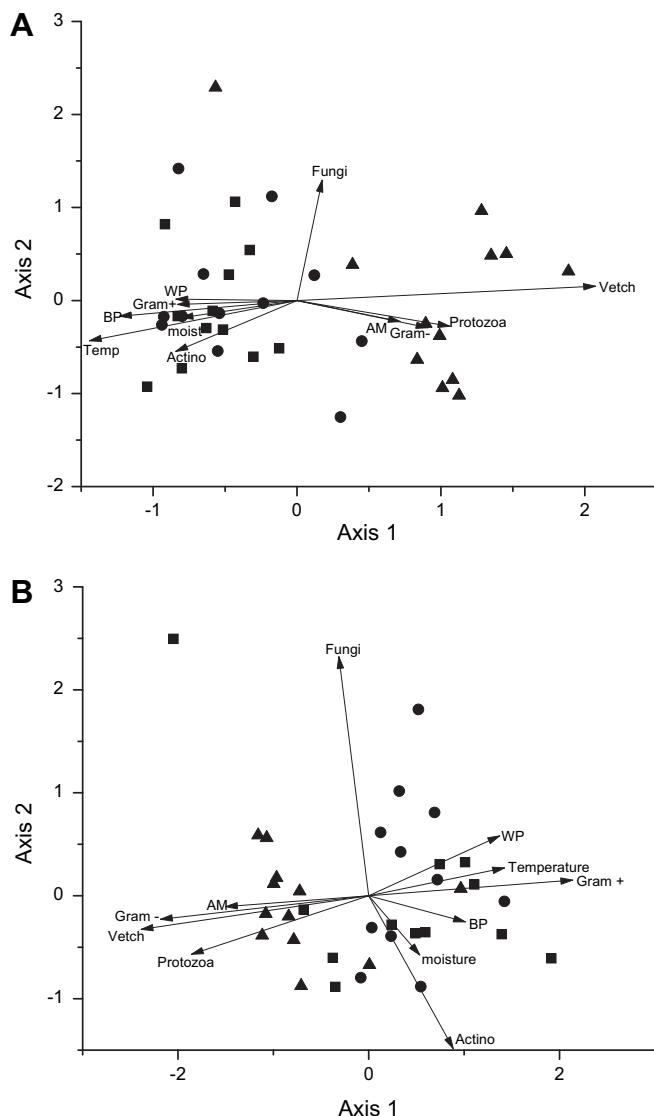
### 3.8. Protozoa

Vetch roots increased the absolute amount of protozoan biomarker compared to bare and black polyethylene treatments in bulk soil during the summer months (Table 3). In the tomato rhizosphere levels were increased by vetch and vetch shoots compared to bare and polyethylene treatments (Table 4). The proportion of PLFA due to protozoans in bulk soil was increased by rye, vetch, and vetch roots when compared to bare and polyethylene treatments (Table 5). In the tomato rhizosphere all cover-cropped treatments had higher proportional levels of protozoan PLFA biomarker when compared to all non-cover cropped treatments (Table 6).

### 3.9. Differences in community composition between cover cropping and plasticulture are not fully explained by soil temperature or moisture

The multivariate (redundancy) analysis of community composition in July, August, and September is presented in Fig. 4 for 2006 and Fig. 5 for 2007. This analysis was performed on three treatments, black polyethylene, white polyethylene, and vetch, because we had measured soil temperature in those treatments. In both years in bulk soil, plasticulture was associated with higher proportions of Gram-positive bacteria and actinomycetes, while vetch was associated with higher levels of Gram-negative bacteria, AM fungi, fungi, and protozoa. In the tomato rhizosphere in both years Gram-negative bacteria were associated with vetch while Gram-positive bacteria and actinomycetes were associated with plasticulture. Fungi, AM fungi, and protozoa were associated with vetch in the tomato rhizosphere in one year but not in the other year.

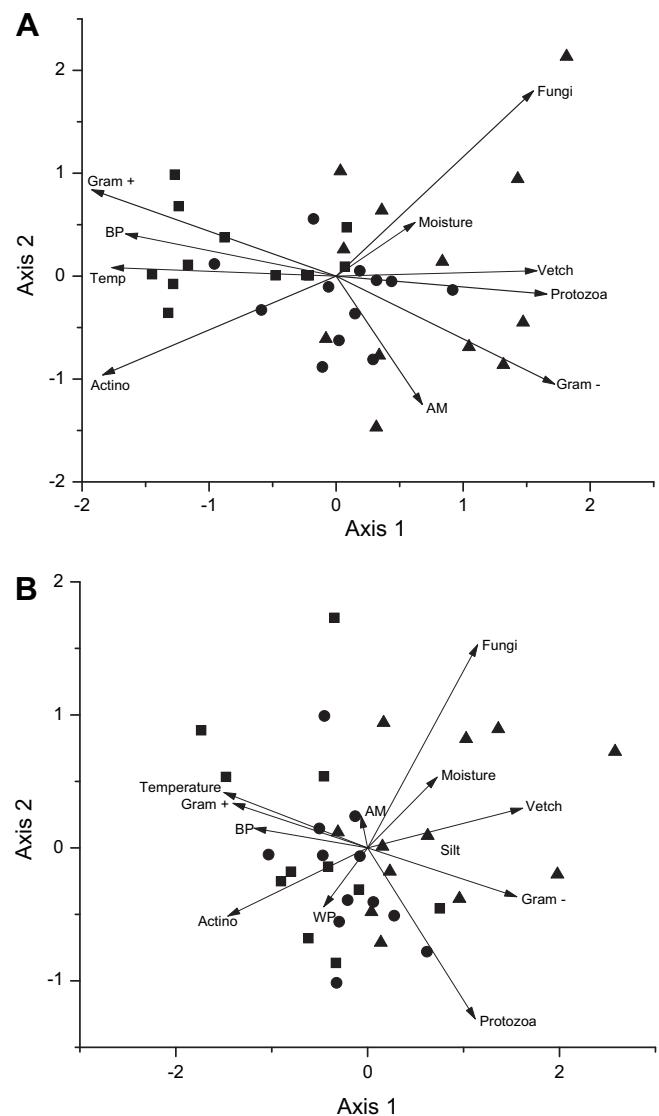
The mean soil temperature was 25.9 °C for black polyethylene, 24.6 °C for white polyethylene, and 24.1 °C for vetch. The temperature vectors in Figs. 4 and 5 aligned with the polyethylene treatments, which is logical since they were warmer than the vetch treatment. The largest difference in community composition was observed between vetch and the two plasticulture treatments. This is consistent with the results previously discussed and presented in Tables 5 and 6. While there were often significant differences between vetch and the two plasticulture treatments, there were never significant differences between black and white polyethylene. Similar results were found in MANOVA, where overall community composition was significantly different ( $p < 0.05$ ) between vetch and either of the two polyethylene treatments,



**Fig. 4.** Redundancy analysis of soil microbial community composition, July–September, 2006. A) bulk soil. B) tomato rhizosphere soil. Black polyethylene, □. White polyethylene, ○. Vetch, △. Vectors indicate the degree of correlation between each factor and the axes. While vectors for species were plotted directly, vectors for treatment and environmental factors were scaled 3:1. For the sake of clarity very small species vectors were eliminated, and of the environmental variables only treatment, moisture, and temperature vectors are plotted. BP, black polyethylene; WP, white polyethylene; Actino, actinomycetes; AM, arbuscular mycorrhizal fungi. The analysis included temperature and moisture weekly averages for 1–4 weeks before each harvest, but each set of vectors were very similar to each other, so only one vector each for temperature and moisture are displayed.

but black and white polyethylene were not significantly different from each other, for both bulk soil and rhizosphere soil (data not shown). The lack of difference between black and white polyethylene is inconsistent with the hypothesis that soil temperature is a primary factor in controlling soil microbial community composition in plasticulture and cover-cropped systems.

In 2005 and 2006, soil moisture was higher under plasticulture treatments (15.7% in 2005, 21.9% in 2006) than under vetch (13.4% in 2005, 13.6% in 2006), while in 2007 soil moisture was higher under vetch (14.5%) than under plasticulture (12.5%). This may be explained by the difference in rainfall at the beginning of the season for each of the three years of the experiment. For the period three weeks before polyethylene mulch was laid to two weeks after



**Fig. 5.** Redundancy analysis of soil microbial community composition, July–September, 2007. A) bulk soil. B) tomato rhizosphere soil. Black polyethylene, □. White polyethylene, ○. Vetch, △. Vectors indicate the degree of correlation between each factor and the axes. While vectors for species were plotted directly, vectors for treatment and environmental factors were scaled 3:1 in Fig. 5A and 4:1 in Fig. 5B. For the sake of clarity very small species vectors were eliminated, and of the environmental variables only treatment, moisture, and temperature vectors are plotted. BP, black polyethylene; WP, white polyethylene; Actino, actinomycetes; AM, arbuscular mycorrhizal fungi. The analysis included temperature and moisture weekly averages for 1–4 weeks before each harvest, but each set of vectors were very similar to each other, so only one vector each for temperature and moisture are displayed.

(approximately when transplanting took place), rainfall was 94, 85, and 28 mm in 2005, 2006, and 2007, respectively. In 2007 the polyethylene mulched beds were much drier when they were covered, and drip irrigation was not adequate to make up this difference. Furthermore, overall soil moisture was lower in 2007 (13.0%) than in 2005 (13.8%) or 2006 (15.2%). In bulk soil, the vectors for soil moisture in 2006 (Fig. 4A) aligned with polyethylene and in 2007 (Fig. 5A) aligned with vetch, undoubtedly due to the treatment effect on soil moisture. However, due to the treatment effect on microbial community composition, in 2006 the moisture vectors aligned with Gram-positive bacteria and actinomycetes, while in 2007 the moisture vectors aligned with Gram-negative bacteria, fungi, and AM fungi. Since it is extremely unlikely

that higher soil moistures could favor different taxonomic groups from one year to the next, we conclude that soil moisture was not significantly affecting microbial community composition.

The relative importance of treatment and various environmental factors was further examined through variance decomposition of the redundancy analysis (Table 7). We analyzed the total variance due to treatment, rather than the variance due purely to treatment, because (1) treatments were imposed over any environmental gradients present and (2) the imposed treatments strongly affected the environmental gradients, as discussed above. This analysis showed that treatment was most important, explaining 23–32 percent of the observed variance, and was statistically significant ( $p \leq 0.01$ ). Soil moisture and temperature each explained 4–17 percent of the observed variance, and were rarely significant at the 95% level. Factors such as month, soil texture, marketable fruit yield, cover crop shoot and root biomass, and soil pH explained less variance and were never statistically significant.

ANOVA was used to analyze soil pH separately from other factors. While there were no statistically significant treatment effects on soil pH, there was a significant block effect. In each year one block was significantly higher in pH than the remaining blocks. Thus, in 2005, block 4 had a pH of 6.2, while the other three blocks had pH values ranging from 5.1 to 5.3. In 2006, block 1 had a pH of 6.7, while the other blocks had pH values of 5.6–5.9. In 2007, block 4 had a pH of 6.7, while the other blocks had pH values of 6.1–6.2. Despite this statistically significant pH gradient there was very little effect of pH on the microbial community composition.

#### 4. Discussion

In this study our goal was to identify and prioritize some of the factors that contributed to soil and rhizosphere microbial biomass and community composition in a tomato production agro-ecosystem. This task is difficult due to interactions, correlations, and shared variance between treatments and environmental variables. Despite these complexities we were able to assign significant proportions of shared variance to particular mechanisms by which cover cropping and plasticulture affect soil and rhizosphere microbial communities. Cover cropping and plasticulture induced small changes in soil moisture and temperature. In addition, there were gradients in pH and soil texture across the field. However, our statistical analysis clearly indicates that cover cropping was far more important than the measured environmental variables in controlling soil microbial community structure.

**Table 7**  
Decomposition of variance.

	2006		2007	
	Bulk	Rhizosphere	Bulk	Rhizosphere
Total Explained Variance	0.649*	0.704*	0.714**	0.802***
Total Variance due to Treatment	0.325***	0.251***	0.269***	0.234**
Variance Due Purely to:				
Moisture	0.061	0.121	0.129	0.161
Temperature	0.04	0.116	0.118	0.167*
Texture	0.046	0.037	0.018	0.017
Month	0.007	0.03	0.067	0.071
Tomato Fruit Yield	0.011	0.048	0.037	0.013
Vetch Shoot Biomass	0.017	0.009	0.024	0.002
Vetch Fruit Biomass	0.012	0.004	0.029	0.009
Soil pH	0.027	0.021	0.015	0.002
Total Shared Variance	0.425	0.296	0.239	0.328
Total Unexplained Variance	0.351	0.296	0.286	0.198

Significance of results: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.005$ .

Microbial biomass, as measured by total PLFA, was increased by both vetch and rye cover cropping. Both rye and vetch roots increased microbial biomass more than shoots, but shoots also had a positive effect on microbial biomass. This experiment was not designed to determine if the increase in biomass with cover crop roots was due to release of nutrients through root exudates or due to degradation of roots after the cover crops were killed. However, the biomass under vetch was significantly greater in May than that under bare soil, indicating that vetch root turnover and exudation during the winter and early spring had a positive effect on soil microbial biomass. In other studies cover cropping has been observed to increase soil microbial biomass after cover crop incorporation (Doran et al., 1987; Wang et al., 2007b). In the current study we show that a management system in which cover crops are not tilled into the soil, but instead are mowed and left in place on the soil surface to suppress weeds and conserve water, nevertheless results in increased soil microbial biomass. Similarly, cover crops increased microbial biomass whether disked in or left untilled (Ingels et al., 2005; Ndiaye et al., 2000).

Our results were somewhat different than those from the Oregon Long-Term Soil Quality Project (Schutter et al., 2001), where it was found that cover crop effects were not observed until after incorporation of the cover crop in the spring. We observed effects of the cover crop on microbial biomass and community composition before the cover crop was mowed. This discrepancy may be due to differences in cover crop, climate, and the exact timing of the spring sampling. In the Oregon study, as well as in other studies (Bossio et al., 1998; van Diepeningen et al., 2006), it was also observed that soil texture differences were more important than agricultural management system in determining soil microbial community structure. However, there was much wider variation in soil texture in those studies than in ours.

Gram-positive bacteria behaved differently than members of other taxonomic groups. While they did increase under cover cropping, presumably because they acquired carbon from cover crops, they increased less than other groups, indicating that they were less active in utilizing cover crop-derived carbon. Similar results were found in previous studies using straw incorporation (Bossio and Scow, 1998) or dairy manure (Peacock et al., 2001) to increase C availability. In grassland soils, Gram-positive bacteria and actinomycetes were less involved in processing of rhizodeposits than other microbial groups (Denef et al., 2009). Also, in the current study the proportion of Gram-positive bacteria increased with the high soil temperatures occurring under black polyethylene, which may be attributed to the more rapid exhaustion of readily available carbon at higher temperatures (Feng and Simpson, 2009; Frey et al., 2008). Thus, under polyethylene, there are no cover crop rhizodeposits or carbon from the mulch layer, and there are higher soil temperatures which increase the turnover of any readily available carbon, resulting in higher proportional levels of Gram-positive bacteria. When comparing bare or white polyethylene-covered soils to cover-cropped soils, there are no cover crop rhizodeposits, so even though soil temperatures are not increased as much as under black polyethylene the proportion of Gram-positive bacteria is still increased relative to the cover-cropped treatment. The increase in readily available carbon due to cover crop roots and shoots has more impact on soil microbial community composition than the increase in soil temperature under polyethylene.

In previous studies some iso and anteiso PLFAs were found to be labeled with  $^{13}\text{C}$  derived from rye and clover crops (McMahon et al., 2005; Williams et al., 2006). This apparent contradiction to our results may be explained by the broad taxonomic and phylogenetic diversity of the Gram-positive bacteria (Sneath et al., 1986). It would be unrealistic to expect every Gram-positive organism,

or every organism with iso and/or anteiso PLFAs, to respond identically to carbon input. Furthermore, copiotrophy and oligotrophy represent a continuum rather than two mutually exclusive states, and not all phyla are readily classified as one or the other (Fierer et al., 2007). The results of our study indicate that as a group the Gram-positive bacteria, as defined by iso and anteiso PLFAs, were less copiotrophic than other groups.

Vetch shoots increased the amount and proportion of Gram-negative bacteria, fungi, and mycorrhizal fungi in the tomato rhizosphere. Since mycorrhizal fungi contain the fungal biomarker fatty acid, it is possible that mycorrhizal fungi were the only fungi to respond in this way. There may have been a priming or synergistic effect occurring between nutrients released in the rhizosphere and nutrients released from vetch shoots. Alternatively, vetch shoots may have stimulated the tomato plants to release more nutrients from their roots. A number of genes in the tomato plant are up-regulated by hairy vetch mulch (Kumar et al., 2004), which could provide a mechanism for increased rhizodeposition.

In this experiment moisture was not found to be important in controlling soil or rhizosphere microbial communities. It is important to recognize that irrigation most likely minimized the treatment effects on soil moisture in our study. Similarly, cover crop incorporation increased microbial biomass while irrigation rates did not in a tomato field study (Wang et al., 2007b). In microcosms soil drying did not affect microbial community structure (Griffiths et al., 2003). In other studies, flooding was found to significantly affect PLFA profiles (Bossio and Scow, 1998), but that was a more severe treatment than the variations in soil moisture found in our study. Similarly, flooding affected community composition but changes in soil moisture from air dry to half field capacity did not (Drenovsky et al., 2004). Microbial communities in drought-prone prairie soils did respond to increased water availability (Williams, 2007; Williams and Rice, 2007), but these were long-term treatments with greater differences between irrigated and non-irrigated treatments than the differences in our study. Soil moisture was observed to be a significant factor in Verrucomicrobia abundance (Buckley and Schmidt, 2001b). We do not have a PLFA biomarker for Verrucomicrobia at the present time, and the little data currently available on fatty acid composition of Verrucomicrobia (Op den Camp et al., 2009; Schlesner et al., 2006) does not suggest that PLFA will be useful for measuring Verrucomicrobia abundance in complex ecosystems.

We did not find soil pH to be a significant factor in microbial community composition. In 2006 soil pH explained 2.7% of the variance in bulk soil while the difference between the lowest and highest values was 1.1 pH units. In 2007 soil pH explained 1.5% of the variance in bulk soil while the gradient was 0.6 pH units. While soil pH was previously shown to be a major factor in controlling soil microbial diversity and composition across a wide range of habitats (Fierer and Jackson, 2006) it appears that in our study the imposed treatments were far more significant.

Vetch and rye cover crops had generally similar effects on soil microbial communities. They also gave similar tomato fruit yields, despite the nitrogen-fixing activity of the vetch-*Rhizobium* symbiosis. Nitrogen was provided to all treatments in sufficient quantities to support intensive tomato production, essentially decoupling crop yield from natural ecosystem function. Results on tomato fruit yield, and possibly on soil microbial communities, might well have been very different if less nitrogen fertilizer was provided. However, this experiment was designed to test hypotheses in an agroecosystem representative of current agricultural management practices, and the amount of nitrogen fertilizer used was realistic in that context.

Herbicide, pesticide, and fungicide applications do not appear to have affected our results. While long-term herbicide usage has

been shown to alter soil microbial community structure (el Fantroussi et al., 1999), short-term usage was reported to produce only transient effects (Ratcliff et al., 2006; Fließbach and Mäder, 2004). Furthermore, the rye and vetch shoot treatments had exactly the same herbicide applications as the bare, black polyethylene, and white polyethylene treatments, yet the microbial community composition as measured by PLFA was quite different.

In a literature review it was concluded that plant type, soil type, and soil management all contribute to soil microbial community structure, and that complex interactions were involved (Garbeva et al., 2004). Our results are consistent with that conclusion. The treatment and environmental factors that appear most important in a given study appear to be somewhat site-specific, depending on the cropping system, the factors that are measured, and the amount of variation in each factor found within that study. In the current study, management was found to be most important in determining soil microbial community composition, which suggests that in short time frames microbial community structure can be influenced by management decisions. These findings confirm that the soil microbial community is dynamic and responsive to annual land use management decisions and should not be treated as a black box in theory or process based models. The next step in defining the broader impact of management decisions will be to link the observed changes in microbial community structure to quantification of microbial community functionality. Ultimately, we need a broad model that will predict soil microbial community structure based on a wide variety of physical, chemical, and biological factors. We suggest that interpreting the changes in microbial community structure in terms of the statistical analysis of the effects of likely environmental variables is a valid way to begin to prioritize the complex factors influencing microbial community structure and function.

We conclude that vetch and rye cover cropping increase soil microbial biomass and alter microbial community structure in a vegetable cropping system. Both above-ground and below-ground cover crop biomass contribute to the observed effects. In addition, vetch shoots interact with tomato plants to increase microbial biomass and alter microbial community structure in the tomato rhizosphere. These effects were not explained by the soil temperature and moisture gradients induced by the experimental treatments, and were larger than effects due to observed gradients in soil texture and pH. We attribute these effects to a combination of rhizosphere exudation, nutrients released from decomposing roots, and nutrients leached into the soil from above-ground cover crop biomass.

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