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Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production

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ABSTRACT

Soil microbes play a key role in soil health, and understanding the functional role of this living component of soil organic matter is critical to developing sustainable systems in major vegetable production regions like Salinas, California. Soil microbial community size and composition was evaluated after six years of commercial-scale production in five organic vegetable systems in a long-term systems experiment. All systems produced lettuce, and spinach or broccoli annually, and differed in yard-waste compost inputs (none or 15.2 Mg ha^{-1} year^{-1}), winter cover crop frequency (annually or every 4th year), and cover crop type (legume-rye, mustard, or rye). The same levels of irrigation, and supplemental fertilizer were applied to all systems. Cumulative organic matter inputs from compost and cover crop shoots over the six years ranged from 7.4 to 136.8 Mg ha^{-1} and caused differences in microbial biomass C (MBC) and N (MBN), and soil organic C (SOC). MBC increased by 40 mg C kg^{-1} soil with compost and infrequent cover cropping, and to levels that were relatively high (200–250 mg C kg^{-1} soil) for a loamy sand soil in systems with annual cover cropping. Changes in SOC between systems were caused primarily by compost while changes in MBC and MBN were more related to cover cropping frequency. Fatty acid methyl ester (FAME) analysis revealed differences in microbial community structure that were consistent with differences between systems in MBC and MBN. Across systems, the ratio of fungal: bacterial FAME indicators decreased over time while indicators of invertebrates, and gram positive bacteria increased. High-throughput sequencing revealed relatively few differences in bacterial phyla between systems, but the increase in cropping intensity across all systems changed the relative abundance of some bacterial phyla (*Bacteroidetes, Deinococcus-Thermus*) and genera (*Flavobacterium*, *Nocardioidetes*). Cover crop type and frequency also influenced the abundance of two bacterial genera (*Pseudomonas, Agromyces*). These results provide evidence that carbon (C) inputs from frequent cover cropping are the primary driver of changes in the soil food web and soil health in high-input, tillage-intensive organic vegetable production systems.
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1. Introduction

Soil health depends on the soil food web which is a complex community of interacting organisms - bacteria, fungi, protozoa, nematodes, arthropods, and earthworms – that rely on inputs of energy-rich plant residues (Ball, 2006; Thies and Grossman, 2006; Ferris and Tuomisto, 2015). Microbial-mediated decomposition of these residues releases energy and nutrients that enables soil organisms to function and provide essential soil ecosystem services (i.e., nutrient transformation and cycling, soil aggregation). Comparisons of organic and conventional management have provided critical insights of soil food web dynamics and the benefits of organic matter inputs (Drinkwater et al., 1995; Ferris et al., 1996; Bossio et al., 1998; Gunapala and Scow, 1998; van Diepeningen et al., 2006; Briar et al., 2007; Birkhofer et al., 2008; Overstreet et al., 2010; Reganold et al., 2010; Cao et al., 2011; Williams and Hedlund, 2013; Henneron et al., 2015). However, there are many ways to farm organically and conventionally for a given cash crop and also many ways to add organic matter to the soil (i.e., compost, cover crops, cash crop residue) which may have different effects on soil health. The terms 'soil health' and 'soil quality' are often used interchangeably (Romig et al., 1995; Karlen et al., 1997) however, in this paper we chose to use 'soil health' because we consider it a more intuitive concept that is akin in many ways to understanding

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the concept of human health (Doran and Parkin, 1996).

To develop more sustainable vegetable systems, long-term research is needed on the effects of specific organic matter inputs and management practices on microbial aspects of soil health. This need is particularly important in high intensity, organic systems in regions such as the central coast of California that provide a large proportion of the organic vegetables that are sold throughout the U.S. (Klonsky and Healy, 2013; USDA-ERS, 2013). Unfortunately, in such systems, 'best management practices' like cover cropping can reduce the number of cash crops produced and complicate management (Hartz, 2006; Klonsky and Tourte, 2011; Brennan, 2017). This helps explain troubling reports (Guthman, 2000; Bowles et al., 2014) of infrequent cover cropping in many organic farms in California.

Vegetable farmers in central coast region of California that practice sustainable soil management typically add large amounts of organic matter to the soil (>5 Mg oven-dry material ha^{-1} annually) from yard-waste compost or frequent cover cropping. These C inputs are important in high-value, intensive vegetable systems because common management practices (i.e., intense tillage, multiple crops annually, frequent irrigation, relatively high nitrogen fertilization rates) may exacerbate C losses from the soil. Furthermore, many leafy vegetables (i.e., lettuce, spinach) return relatively little post-harvest residue to the soil (Mitchell, 1999), and such residues decompose rapidly because of their high nitrogen (N) and moisture content.

Compared with cover cropping, applying compost is a convenient and rapid way to add large amounts of C to the soil. An on-farm survey of row and perennial crop systems in California found positive effects from compost on several soil characteristics (i.e., bulk density, water infiltration, SOC, microbial biomass) (Brown and Cotton, 2011). A two year, vegetable experiment in Salinas, California found that organic matter inputs from compost (made from manure, straw, and vegetable waste) and rye cover crop prolonged soil MBC, and the researchers hypothesized that compost provided a 'slow-release' source of nutrients relative to the more labile (i.e., biologically active) C added by cover crops (Jackson et al., 2004). Labile pools of SOC, specifically the living (i.e., microbial biomass) and non-living fractions (i.e., particulate organic matter), are sensitive indicators of soil quality, whereas total SOC is a relatively crude indicator (Haynes, 2005)

To our knowledge there are no previous reports of the longer-term (>5 years) effects of various combinations of yard-waste compost and cover crops on the soil microbial communities in tillage-intensive, organic or conventional vegetable systems in California. Given the economic importance of agriculture in the Central Coast region of California, there has been considerable research focused on controlling soil borne disease organisms (Koike et al., 1996; Subbarao et al., 2007; Bensen et al., 2009; Njoroge et al., 2009; Fennimore et al., 2014; Muramoto et al., 2014), however, few studies (Klose et al., 2006) on how these agricultural practices affect the soil microbial community size or structure.

An ongoing, long-term study known as the Salinas Organic Cropping Systems (SOCS) experiment began in 2003 to evaluate the effects of cover crops and yard-waste compost on various aspects of high-value organic crop production (Brennan and Boyd, 2012). It includes several organic management systems that differed in the quality, quantity, and frequency of organic matter inputs for the same sequence of vegetable crops, and is the longest running systems study in the U.S. focused on high-value, tillage-intensive, cool-season organic crops. The initial evaluation of soil health in this study was based on nematode community analysis during 8 years of vegetable production and found that cover crop frequency (i.e., annually versus every 4 years) had a greater effect on the soil food web than compost additions (Ferris et al., 2012). In the present paper, we extend this work to characterize bacterial and fungal components of the soil food web to further understand soil health changes during the first 6 years of the experiment. The microbial community size was estimated from soil MBC and MBN. We used fatty acid methyl ester (FAME) indicators to evaluate microbial community composition, and high-throughput sequencing to characterize the bacterial diversity and distribution at different taxonomic levels (phyla, genus). Our objective was to determine the impact of winter cover cropping frequency (i.e., annually versus every 4th year), cover crop type (legume-rye, rye, mustard), and yard-waste compost on microbial community size and composition over a 6 year period.

2. Materials and methods

2.1. Site characteristics, climate, management, experimental design

A detailed description of the field site and ongoing experiment is in Brennan and Boyd (2012) and will only be described here briefly. The experiment is located at the USDA-ARS farm in Salinas, California, on the central coast region of the state. Salinas Valley opens to the Pacific Ocean which moderates the climate such that the average air temperature from 2003 to 2009 was 11 °C from October to March when the cover cropping or winter fallows occurred, and 15 °C during the most typical vegetable production period (April to September) (wwwcimis.water.ca.gov, Station #89, South Salinas). The average annual rainfall from 2003 to 2009 was 285 mm and occurred mostly between October and March. The soil is a Chualar loamy sand (fine-loamy, mixed, thermic Typic Argixerol) with 77% sand, 15% silt, and 8% clay. Prior to the experiment, the field was in hay production, and mixed vegetable and sugar beet trials (1990–1996), and from 1997 to 2003 was frequently fallowed with occasional cover crops and vegetables that included minimal inputs of fertilizers or compost. The field has been certified organic by California Certified Organic Farmers since 1999, and to USDA National Organic Program standards since they were implemented in 2002.

The experimental design was a randomized complete block with 8 systems arranged in each of four blocks in an area 49 m wide and 156 m long. Each system plot was 19.5 m long and 12.2 m wide. Only 5 systems with optimal seeding rates for weed suppression (Brennan, unpublished data) of 8 total systems in the study were included in the current paper (Table 1). These 5 systems of interest were the same ones that were used to evaluate soil phosphorous dynamics (Maltais-Landry et al., 2016), whereas in the analysis of the soil nematode community of the experiment (Ferris et al., 2012), all 8 systems were included with the data pooled across optimal and lower seeding rates.

The experiment began in October 2003 with either a winter fallow (Systems 1 and 2) or winter cover crop (System 3, 4, and 5). The cover crops were planted as a solid stand with a grain drill with 15 cm row spacing; the grain drill included cones (Brennan, 2011) that facilitated planting the different types of cover crops at different seeding rates. During the first 6 years, Systems 1 and 2 were fallow during all winters except the 4th winter (2006–2007), when they were cover cropped with the legume-rye mixture. In contrast, System 3, 4, and 5 were cover cropped every winter with the legume-rye mixture, mustard, or rye, respectively (Table 1). These differences between systems resulted in large differences in organic matter inputs from compost and cover crops over the six years (Fig. 1). Each year, the cover crops were incorporated with a soil spader in February or March to prepare the plots for bed formation; the winter fallowed systems were also spaded at this time. Weeds were controlled during the winter fallow by hand weeding or flaming, and shallow tillage with a rototiller as needed to prevent

Table 1
Description of five systems in the Salinas Organic Cropping Systems experiment that were evaluated for soil microbial changes over six years.

System ID	Compost Rate (Mg ha^{-1} year^{-1})[a]	Cover Crop		
		Type[b]	Seeding rate[c] (kg ha^{-1})	Frequency
1	0	Legume-rye	420	Every 4th Winter
2	15.2	Legume-rye	420	Every 4th Winter
3	15.2	Legume-rye	420	Annually
4	15.2	Mustard	11	Annually
5	15.2	Rye	90	Annually

[a] Yard-waste compost applied on an oven-dry weight basis and split application (7.6 Mg ha^{-1}) prior to each spring and summer vegetable crop.
[b] By seed weight, the legume-rye mixture included 10% Rye ('Merced' *Secale cereale* L.), 35% Faba bean, (*Vicia faba* L.; small-seeded type known as 'bell bean'), 25% Pea, 'Magnus' *Pisum sativum* L., 15% common vetch, *V. sativa* L., and 15% purple vetch, *V. benghalensis* L.; Mustard was mixture of 61% white mustard, 'Ida Gold' *Sinapis alba* L., and 39% India mustard, 'Pacific Gold' *Brassica juncea* L. (Czern.); System 5 used 'Merced' rye.
[c] Seeding rates were those for optimal weed suppression by the cover crops in the experiment (Brennan, unpublished data).

weed seed production. After a cover crop decomposition period of 30–42 days (except during an unusually wet spring in 2006 when the period was 72 days) a tractor with lister plows was used to form peaked beds that were 101.6 cm wide. Yard-waste compost (7.6 Mg ha^{-1} oven-dry weight, C:N ratio ≈22) was applied to the peaked bed tops of all systems except System 1, and supplemental pelleted organic fertilizer at a rate of 56–66 kg N ha^{-1} was injected into all beds. The beds were then shaped down to incorporate the compost and create a flat bed top that was approximately 50 cm wide and ready for transplanted lettuce that was the first of two vegetables each year; additional liquid organic fertilizer was applied to the lettuce by drip irrigation to bring the total N application rate up to 73 kg ha^{-1}. The lettuce was planted in May or June and harvested approximately 45 days later and was followed by a summer crop of either spinach (July–August 2004) or transplanted broccoli (July–September/October 2005 to 2009). Yard-waste compost (7.6 Mg ha^{-1}) was also applied to all systems (except System 1) before the summer vegetable crops, such that the total, annual compost application rate was 15.2 Mg ha^{-1} in System 2, 3, 4, and 5. The N fertilizer rates from supplemental fertilizers did not differ between systems and was 22 kg ha^{-1} for spinach (pelleted fertilizer only) and 134–170 kg N ha^{-1} (pelleted and liquid fertilizer) for broccoli. Beds for broccoli were the same width as for lettuce, but the spinach beds were twice as wide. Irrigation was uniform across all systems and included sprinkle and drip irrigation for lettuce and broccoli, sprinkle only for spinach, and sprinkle to establish the cover crop before the onset of winter rainfall. Depending on field conditions following the commercial harvest of the lettuce, the lettuce beds were either reshaped with a reduced-tillage disc or flattened with a tandem disc or spader before being reformed for the summer crop. Deep tillage with a tractor with ripper shanks to approximately 1 m deep was used as needed after the vegetable crops to break up compaction caused by the commercial harvest operations. Lettuce, and broccoli crops were harvested and marketed by collaborating farms, resulting in export of nutrients from the field; the spinach crop of 2004 and the broccoli crop of 2005 did not meet market standards due disease or insect damage and were not harvested for commercial sale (Brennan and Boyd, 2012).

2.2. Soil sampling and organic carbon analysis

Soil samples in each of the 4 replicates for each system were all collected from bare, flat plots (i.e., without beds) that had been uniformly tilled to at least 30 cm depth with a disc harrow, chisel or spader to prepare the plot for winter cover crops or fallow. Soil samples for microbial analysis at time zero were collected on October 13, 2003 from six cores that were 6.5 cm in diameter to a

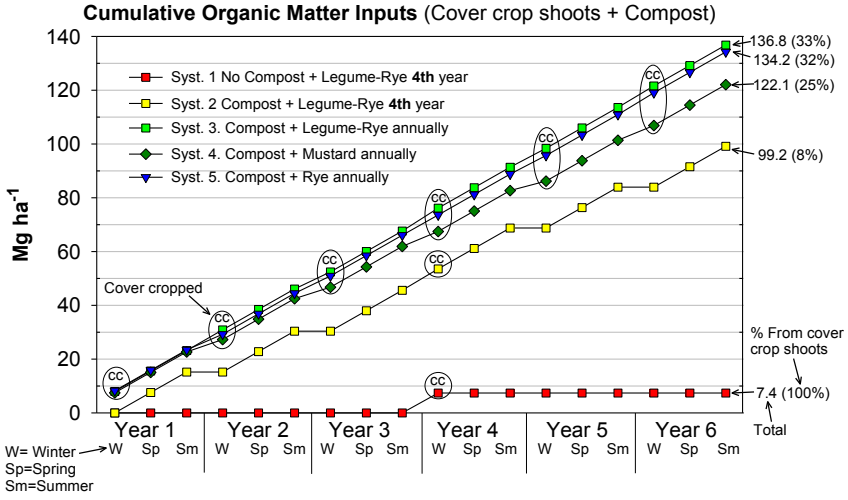


Fig. 1. Cumulative organic matter inputs (oven-dry basis) from cover crop shoots and yard-waste compost in five organic vegetable management systems over 6 years. The systems differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and frequency (annually versus every 4th winter). Winter cover crop inputs were averaged across 4 replicates and only occurred during the winter of the 4th year for Systems 1 and 2. The 'cc' above the circled symbols indicates winters when cover crops were grown for the respective systems. Compost inputs prior to each spring and summer vegetable crop were 7.6 Mg ha^{-1} for all systems except System 1. The numbers on the right of the figure are the total cumulative organic matter inputs from cover crop shoots and compost, with the percentage of this from cover crop shoots in ( ); for example, only 8% of the 99.2 Mg ha^{-1} of cumulative organic matter input to System 2 over the 6 years came from the cover crop that was only grown during the winter of the 3rd to 4th year. The symbols for System 1 and 2 overlap during the winter of year 1 as do the symbols for Systems 3, 4 and 5 for all periods of year 1.

depth of 6.7 cm that were mixed together and frozen at −25 °C; these six cores were evenly distributed within each plot and were 2 m or more from the plot edges. Soil samples for microbial analysis after 6 years were collected on 30 October 2009 to the same depth and using the same sampler used for the 2003 samples, but were taken in an 'x' pattern across each plot to obtain 8 cores that were mixed together and frozen. Soil organic C after 6 years was determined in air-dried samples that had been taken to 30 cm deep and collected in an x pattern across each plot on 26 October 2009 and subjected organic matter analysis using the loss on ignition method (Nelson and Sommers, 1996) at the Analytical Laboratory at the University of California, Davis. To convert organic matter to organic C we used a conversion factor of 0.5 rather than 0.58 as suggested by Pribyl (2010). All microbial analyses were determined for both 2003 and 2009 samples at the same time.

2.3. Assessment of soil microbial community size (microbial biomass)

MBC and MBN were determined in 15-g field moist equivalent soil using the chloroform fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987). Each sample was fumigated in lab duplicates for 24 h in the dark at ambient temperature. The C and N in fumigated and non-fumigated soil samples were extracted by adding 75 ml of 0.5 M K_2SO_4 and shaking for 1 h. Extractants were analyzed using a Shimadzu Model TOC/VCPH-TN C/N analyzer (Shimadzu, Kyoto, Japan). Values for non-fumigated samples were subtracted from fumigated samples, and a K_{EC} of 0.45 for C and K_{EN} 0.54 for N were used (Jenkinson, 1988; Wu et al., 1990; Jenkinson et al., 2004; Joergensen et al., 2011). The percentage of soil microbial C of SOC was calculated by dividing MBC after 6 years by the SOC after 6 years and multiplying by 100; while the MBC samples were from a shallower depth (6.7 cm) than for total organic C (30 cm), we assume that these differences would have relatively little if any effect on the results given the high intensity of tillage in the study.

2.4. Soil microbial community structure with fatty acids methyl esters (FAME) analysis

FAME analysis was performed on a 3-g field-moist equivalent soil sample from each replicate plot using the ester-linked FAME procedure of Schutter and Dick (2000). To redissolve FAMEs, 200 μl of 1:1 methyl *tert*-butyl ether and hexane containing methyl nonadecanoate (19:0) as an internal standard (0.5 mg ml^{-1}) was added. Samples were vortexed and transferred to a 250-μl glass insert in a 2-ml GC vial. FAME analysis was conducted using an Agilent 6890 N gas chromatograph with a 25 m × 0.32 mm × 0.25 μm (5% phenyl)-methylpolysiloxane Agilent HP-5 fused silica capillary column (Agilent, Santa Clara, CA) and flame ionization detector (Hewlett Packard, Palo Alto, CA) with ultra-high purity hydrogen as the carrier gas. The temperature program ramped from 170 °C to 270 °C at 5 °C min^{-1} then ramped to 300 °C for 2 min to clear the column. Peak identification and area calculation was performed using the TSBA6 aerobe program from MIDI (Microbial ID, Inc., Newark, DE). FAMEs are described by the number of C atoms, a colon, the number of double bonds, then the position of the first double bond from the methyl (ω) end of the molecule. Other notations are used for methyl (Me), cis (c) isomers, and iso (i) and anteiso (a) branched FAMEs. Selected FAMEs were used as microbial markers according to previous research (Zelles, 1999), and included: Gram-positive (Gram+) bacteria (i15:0, a15:0, i17:0, a17:0), Gram-negative (Gram−) bacteria (cy17:0, cy19:0), actinomycetes bacteria (10Me16:0, 10Me17:0, 10Me18:0), saprophytic fungi (18:1ω9c, 18:2ω6c) and arbuscular mycorrhizal fungi (AMF) (16:1ω5c); however, Frostegard et al. (2011) pointed out that 18:1ω9c may not be a good indicator of fungi in all soils. A single marker (20:4ω6c) was used for invertebrates (Ruess and Chamberlain, 2010). Absolute amounts of FAMEs (nmol g^{-1} soil) were calculated according to Zelles (1996) using the 19:0 internal standard and these values were subsequently used to calculate mol percent. The fungal:bacterial ratio was calculated by dividing the fungal sum by the bacterial sum.

2.5. Soil bacterial diversity and distribution according to pyrosequencing

DNA was extracted from approximately 0.5 g of soil (oven-dry basis of field-moist soil) from using the Fast DNA Spin Kit for soil (QBIOgene, Carlsbad, CA, USA) following the manufacturer's instructions. Due to funding constraints, this DNA was only extracted from the first three of four replications of the study. The DNA extracted was quantified (2 μL) using Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). The integrity of the DNA extracted from the soils was confirmed by running DNA extracts on 0.8% agarose gel with 0.5 × TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0).

The 16S universal Eubacterial primers 530F (50- GTG CCA GCM GCN GCG G) and 1100R (50- GGG TTN CGNTCG TTG) were used for amplifying the 600 bp region of the 16S rRNA genes. Sample DNA was amplified using a single step reaction (35 cycles) and 1U of HotStart HiFidelity Polymerase was introduced to each reaction (Qiagen). A two-region 454 sequencing run was performed on a 70 × 75 GS PicoTiterPlate (PTP) via Titanium sequencing platform (Roche, Nutley, New Jersey; Research and Testing Laboratory, Lubbock, TX) and the sequences acquired from the FLX sequencing run were further trimmed via a custom scripted bioinformatics pipeline (Handl et al., 2011; Ishak et al., 2011). In summary, each individual sequence was trimmed to a Q25 average, short reads <300 bp depleted, sequences with ambiguous base calls depleted, sequences with homopolymers exceeding 6 bp depleted, until only quality sequence information remained; tags were then extracted from the FLX-created multi-FASTA file while being parsed into individual sample specific files based on the tag sequence. Tags with less than 100% homology, compared to the original sample tag designation, were considered questionable in quality and were not considered. Based upon sequence identity, each bacterium was identified to its closest relative and taxonomic level. Sequence collections were depleted of chimeras using B2C2 software for batch depletion of chimeras from bacterial 16S datasets. Bacterial taxa were identified using Krakenblast (www.krakenblast.com) and compared to a custom highly curated database (Research and Testing Laboratory, Lubbock, TX). After the best hit processing, genus and higher level taxonomic designations were compiled using a secondary post-processing algorithm and relative percentages of bacterial taxa were determined for each individual sample. The sequences data used for this study have been deposited in NCBI under Bioproject PRJNA344674 with accession numbers: SRR4300068, SRR4300077, SRR4300078, SRR4300079, SRR4300080, SRR4300081, SRR4300086, SRR4300087, SRR4300089, SRR4300094, SRR4300095, SRR4300138, SRR4300139, SRR4300140, SRR4300145, SRR4300149, SRR4300150, SRR4300151, SRR4300152, SRR4300153, SRR4300154, SRR4300155, SRR4300156, SRR4300242, SRR4300243, SRR4300244, SRR4300264, SRR4300272, SRR4300284, SRR4300294.

2.6. Statistical analyses

Data for microbial biomass and FAME indicators for different microbial groups were analyzed using SAS (ver. 9.2) (SAS Inst. Cary, NC). The analysis was focused on using 95% confidence intervals (CI) of paired differences (i.e., effect sizes) from time zero to six years later; the difference within each replication was calculated,

followed by the CI of the mean paired difference. This CI based approach to our analysis helped us make practical inferences about our results and their reproducibility (Kirk, 1996; Cumming, 2012). The overlap between CI of independent groups (i.e., systems in our study) can be used as a robust graphical approach to compare independent group means using the 'rule of eye' method (Cumming et al., 2007), whereby the smaller the overlap between confidence intervals, the stronger the evidence of a true difference. Using this method, 95% confidence intervals of independent groups can overlap considerably and still be considered different. For example, where $n \geq 10$, intervals that overlap by half of a confidence interval arm are different at $P \approx 0.05$, and where $n = 3$ (less than in our study), $P \approx 0.05$ if confidence intervals of two groups overlap by one confidence interval arm (Cumming et al., 2007). This confidence interval comparison method is not adjusted to control the family-wise error rate. We chose this statistical approach based on valid criticisms of null-hypothesis significance testing (Anderson et al., 2000; Fidler et al., 2006; Nakagawa and Cuthill, 2007; Hubbard and Lindsay, 2008; Lambdin, 2012; Campbell et al., 2015) that can lead to misinterpretations of results and dichotomous thinking. To help us and readers understand the patterns in our data (variability, skewness and scatter), we plotted the raw data with their CI as suggested by Drummond and Vowler (2011); where CI are reported in the text they are within square brackets [ ]. Exploratory principal component analysis (PCA) was performed on the correlation matrix using the Vegan package (ver. 2.0–2) in R (Oksanen et al., 2011) for all FAMEs. The PCA plot was generated showing 95% confidence interval polygons to compare these five systems according to vectors for 14 FAME markers only (mol percent) as previously done to compare microbial community structure in soils (e.g. Cotton et al., 2013).

3. Results

3.1. Organic matter inputs over 6 years

During the first six years of intensive vegetable production there were large differences between systems in the cumulative organic matter inputs from cover crop shoots and compost (Fig. 1). These differences ranged from 7.4 Mg ha^{-1} in System 1 that never received compost and was only cover cropped once (4th year winter) to 136.8 Mg ha^{-1} in System 3 that was cover cropped every winter and also received compost before each of the two vegetable crops annually. In Systems 3, 4, and 5 that received organic matter inputs from compost and cover crops annually, 25–33% of this came from cover crop shoots. Whereas System 2 that received relatively large inputs of organic matter, obtained only 8% of this from cover crops shoots during the 6 years. Of the three systems that had cover crops every winter, System 4 had lower organic matter inputs because the mustard cover crop was less productive over the six years (30.9 Mg ha^{-1}) than the rye in System 5 (43.1 Mg ha^{-1}) or legume-rye mixture of System 3 (45.6 Mg ha^{-1}).

3.2. Microbial community size (MBC and MBN)

MBC across all systems at the beginning of the experiment in 2003 ranged from an average of 74–92 mg C kg^{-1} soil, and after 6 years of intensive vegetable production with different organic matter inputs from compost and cover crops, the upper range of MBC had more than doubled (Fig. 2A). The changes in MBC over-time were most obvious in Systems 3, 4 and 5 that received compost and cover crop annually, with average increases in these three systems ranging from 87 to 155 mg C kg soil^{-1} (Fig. 2B). In

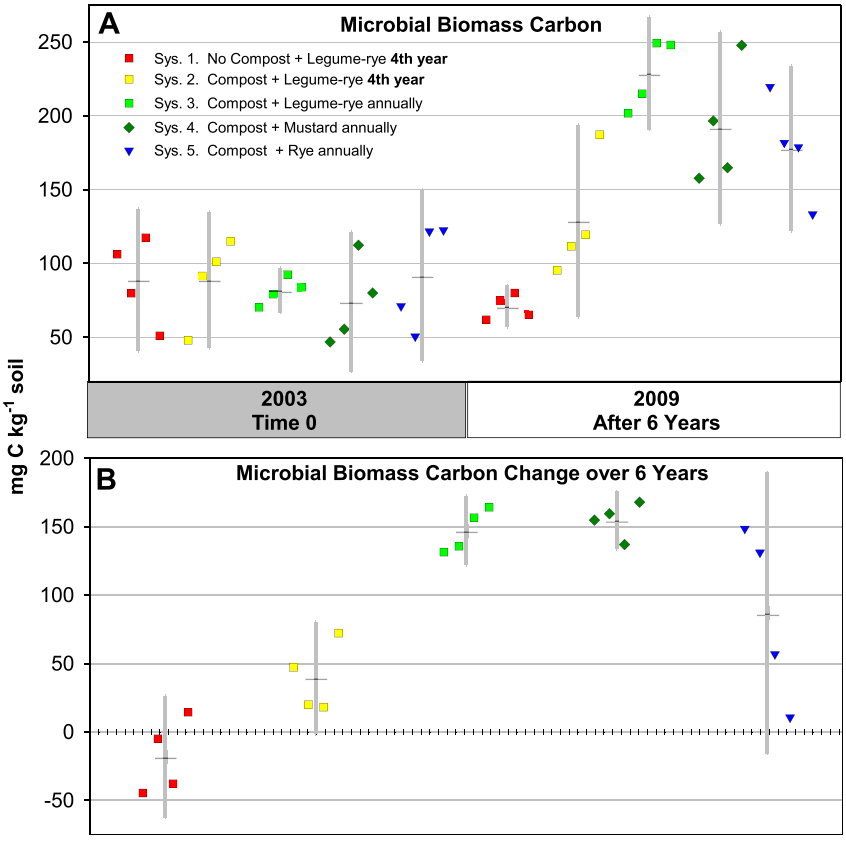


Fig. 2. Microbial biomass carbon at time 0 and after 6 years (A), and the difference (B) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4.

contrast, MBC in System 2 with infrequent cover crops plus compost annually had only increased by an average of 40 mg C kg^{-1} in 6 winters. Whereas MBC declined by an average of 18 mg C kg $soil^{-1}$ in System 1 that had only been cover cropped once without compost (Fig. 2B). The average differences in MBC after 6 years ranged from −18 to 40–147 mg C kg $soil^{-1}$ in the legume-rye cover cropped Systems 1, 2, and 3, respectively. The nearly identical increase in MBC over the six years in System 3 with legume-rye and System 4 with mustard (Fig. 2B) suggests that differences between these systems in cover type, and 14.7 Mg ha^{-1} greater cumulative cover crop shoot biomass in System 3 did not affect soil microbial biomass. System 5, cover cropped with rye, was the only annually cover cropped system where the confidence interval of the difference in MBC after 6 years overlapped with the confidence intervals of System 1 and 2. While the data for System 5 with the rye cover crop tended to cluster with that of the other annually cover cropped systems (Fig. 2A), the difference in MBC of System 5 after 6 years was more variable and overlapped with zero (Fig. 2B).

MBN ranged from an average of 7 to 11 mg N kg^{-1} soil at the start of the experiment and changed in a similar overall pattern to MBC, although there was more overlap of the confidence intervals between systems for MBN (Fig. 3A and B) than occurred with MBC (Fig. 2A and B) suggesting that MBN was less responsive than MBC to differences in management between systems. There was a positive linear relationship between soil MBC and MBN at the beginning of the experiment ($y = 6.4x + 27.9$, $r^2 = 0.64$, $P < 0.001$, where y is MBC and x is MBN) and 6 years later ($y = 5.2x + 43.7$, $r^2 = 0.72$, $P < 0.001$), however, the upper range of the data was much wider after 6 years than at the start of the experiment primarily due to changes in Systems 3, 4, and 5 that were cover cropped annually. There was a general trend across systems for the MBC:MBN ratio to decrease slightly after 6 years (data not shown).

After 6 years of the different management approaches, SOC ranged from an average of 8.1 g kg^{-1} soil (System 1) to 12.3 g kg^{-1} (System 3) and was clearly affected by compost inputs and cover cropping frequency (Fig. 4). For example there was a positive, linear relationship between MBC and SOC across all 5 systems ($r^2 = 0.64$). However, within Systems 1, 2, and 3 that received the legume-rye cover crop, there was a quadratic relationship between MBC and SOC ($r^2 = 0.80$).

There were clear differences between some systems in the ratio of MBC:SOC that ranged from an average of 0.9%–1.9% (Fig. 5). In general, the MBC:SOC ratio increased due to cover crop inputs because they were lowest in System 1 and 2, and highest in System 3, 4, and 5. While the average MBC:SOC was higher for System 2 than 1, the large amount of overlap in the confidence intervals for these systems and the scatter of the raw data, suggest that the annual compost additions in System 2 had relatively little effect on this ratio after 6 years. Within the frequently cover cropped systems (3, 4, 5), there was considerable overlap in the confidence intervals of MBC:SOC which provides strong evidence that differences in cover crop type between the systems had little effect on this ratio.

3.3. Soil microbial community composition via FAME indicators

The biplot of the principle component analysis (PCA) of 13 FAME indicators of bacterial, fungal, and invertebrate groups revealed that the PCA model described 44.8% of variability in the data at Year 6 (Fig. 6). In this plot, the infrequently cover crops systems (1 and 2) clustered together in the negative region of both principal component (PC) axes and were more closely associated with FAME indicators for Gram positive bacteria (*i*15:0, *a*15:0), actinomycetes (10Me16:0, 10Me17:0, 10Me18:0), and one fungi indicator (18:1ω9c; not for all soils as indicated by Frostegard et al., 2010). In contrast, the frequently cover cropped systems (3, 4, and 5) clustered in the positive region of both PC and were more associated with FAME indicators for saprophytic fungi (18:2ω6c), arbuscular mycorrhizal fungi (16:1ω5c), a gram negative bacteria (cy19:0), and invertebrates (20:4ω6c). It is noteworthy that the scatter of the data for System 2 overlapped somewhat with that of System 1, and was situated between System 1 and the other three systems; furthermore, there was considerable overlap in the data for System 3 with legume-rye cover crops and System 5 with rye cover crops, but neither of these overlapped with the data for System 4 that had mustard cover crops. This overall distinction of the five systems in the PCA is relatively consistent with the differences in MBC, MBN and SOC (Figs. 2–5); these patterns are most obvious within the three legume-rye cover cropped systems (1, 2, and 3).

The FAME indicators show some general changes in the soil microbial community structure over time across all systems (Fig. 7). For example, while fungal indicators were most abundant at the beginning of the study, averaged across systems they declined from 29 to 22 mol percent over the 6 years (Fig. 7G). This pattern was relatively consistent across systems although the overlap of the confidence interval of System 5 with zero provides some evidence that the decline was less consistent for System 5. With the arbuscular mycorrhizal fungi (Fig. 7B), the majority of the data suggest an overall decline. In contrast, for saprophytic fungi, most systems indicate no change over time with exception of the System 5 with rye cover crops, where there was an increase from 6.9 to 8.6 mol percent (Fig. 7A). However, there was an overall increase in fungal populations according to FAME profiles (18:2ω6c, 16:1ω5c) and MBC in System 3 to 5 that were cover cropped annually (Figs. 2, 6).

Bacterial indicators showed an average increase in abundance over time (21–23% averaged across systems) across all systems and this increase was most obvious within the Gram positive bacteria (Fig. 7E, H). There were no clear changes in actinomycete or gram negative bacteria across systems (Fig. 7D and F), although there is some evidence of a small increase in gram negative bacteria in System 3. There is strong and consistent evidence that the fungal: bacterial ratio declined over time from approximately 1.4 to 1.0 across all systems (Fig. 7I) due to an overall increase in total bacteria and decrease in total fungi.

The single FAME indicator (20:4ω6c) for invertebrates averaged across systems nearly doubled from 0.39 to 0.82 during the study (Fig. 7C). System 1 that received the least organic matter input was the only system where the confidence interval of the difference of the invertebrate FAME overlapped with zero, whereas Systems 3 and 5 that both include rye cover crop were the only systems where the average change exceeded 0.5. This overall pattern is consistent the overall relative arrangement of the systems for the PCA (Fig. 6) noted above.

3.4. Bacterial population composition with high-throughput sequencing

High-throughput pyrosequencing of the bacterial component of the microbial community at time 0 and 6 years later identified 24 phyla across years including 13 phyla in all system plots (*Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Deferribacteres, Deinococcus-Thermus, Firmicutes, Gemmatimonadetes, Nitrospirae, Planctomycetes, Proteobacteria, TM7, and Verrucomicrobia*), and 11 phyla that were less common (*Aquificae, Chlamydiae, Chrysiogentes, Cyanobacteria, Dictyoglomi, Fibrobacteres, Fusobacteria, Lentisphaerae, Spirochaetes, Synertistetes, Tenericutes*) (Fig. 8). *Proteobacteria* and *Actinobacteria* comprised more that 60% of the phyla followed by *Bacteroidetes* (System 1 and 3), *Firmicutes* (System 2), and *Chloroflexi* (Systems 4 and 5). There were relatively few changes in the relative abundance of the bacterial phyla after 6

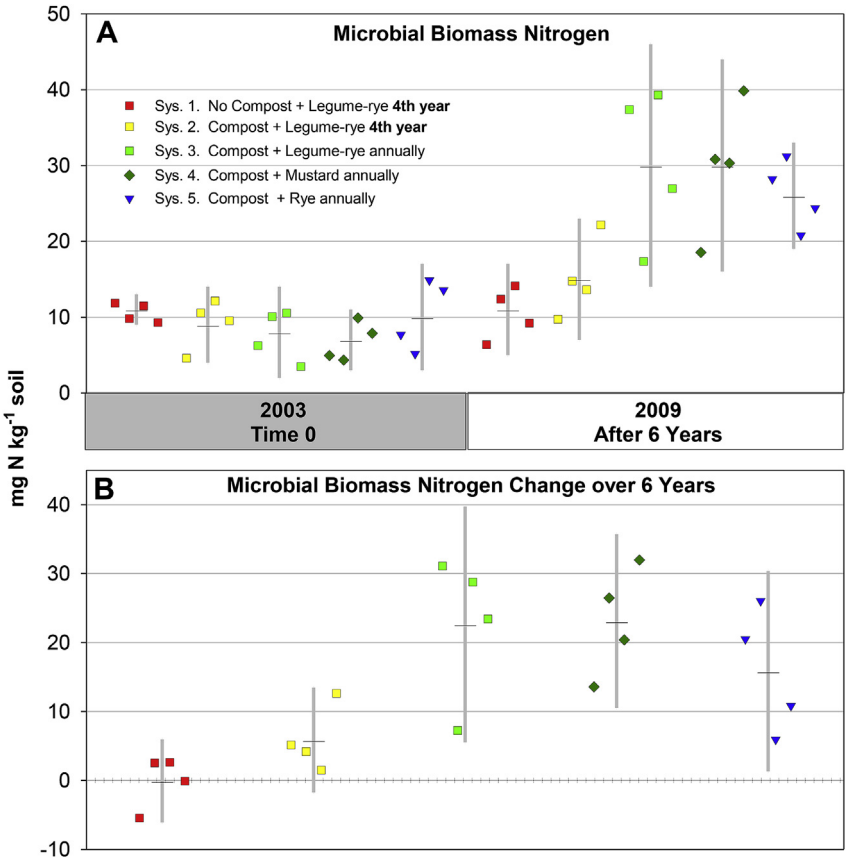


Fig. 3. Microbial biomass nitrogen at time 0 and after 6 years (A), and the difference (B) with 5 organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4.

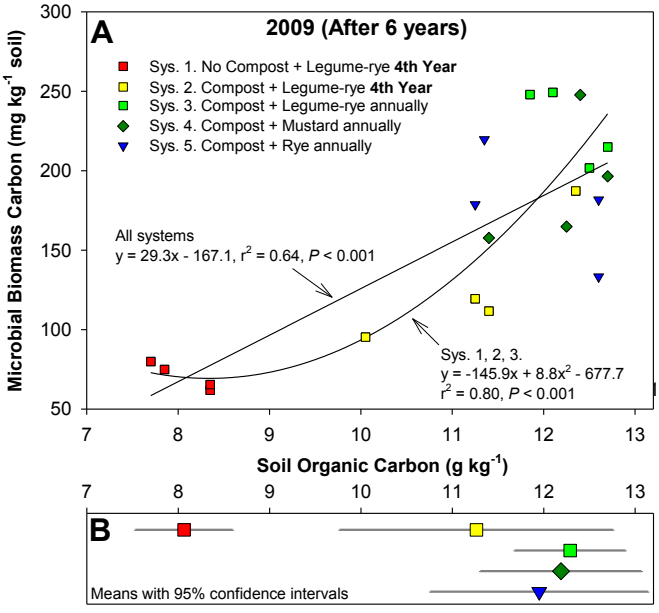


Fig. 4. Microbial biomass carbon versus soil organic carbon after 6 years (A) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). Means and 95% confidence intervals (error bars) for soil organic carbon are in the lower plot (B). The raw data points for the four replicates are shown in the upper plot (A).

years of intensive vegetable production with a few noteworthy exceptions (Fig. 9). For example, among the top six phyla, averaged across all systems, the relative abundance of *Bacteroidetes* increased from 6 to 8.5% and the confidence interval for this change did not include zero which provides strong evidence for this increase. For the three replicates evaluated, this pattern was most consistent in System 3 (2.8–11.9% increase) and System 4 (2.0–3.3% increase), whereas in the other three systems there was one replicate with a

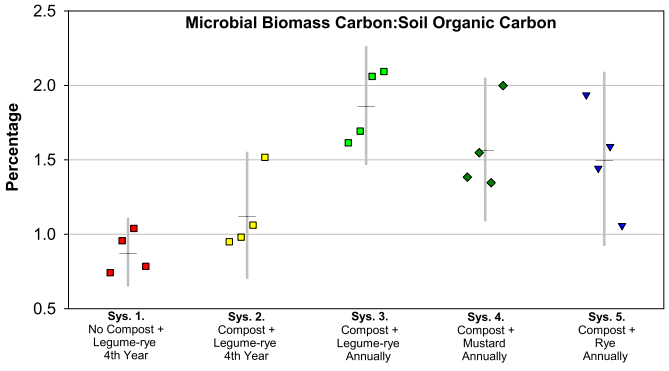
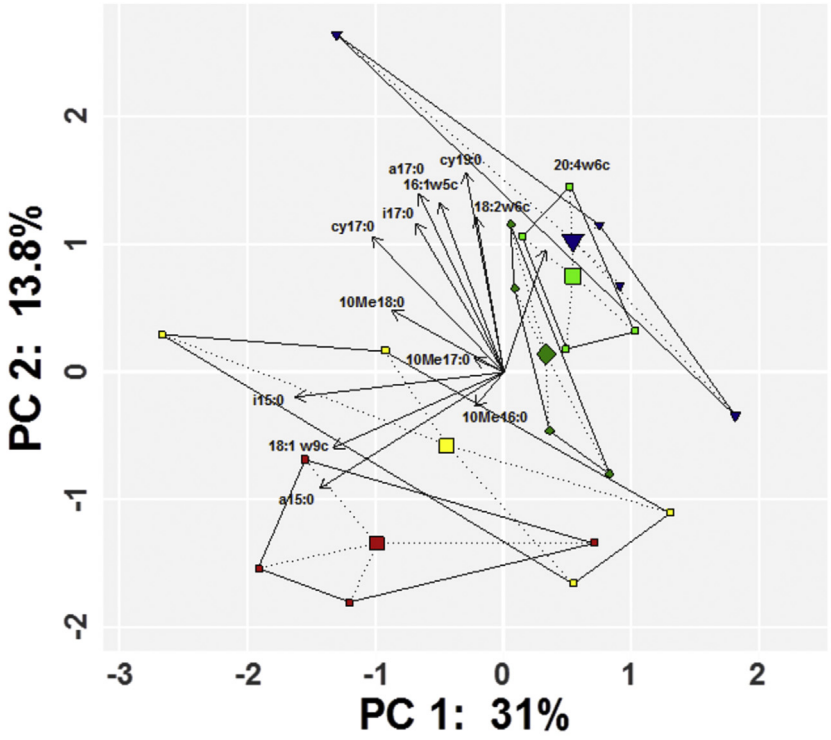


Fig. 5. Microbial biomass carbon in soil organic carbon after 6 years (B) in five organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 4.



Microbial group	FAME indicator
Gram positive bacteria	i15:0, a15:0, i17:0, a17:0
Gram negative bacteria	cy17:0, cy19:0
Actinomycetes bacteria	10Me16:0, 10Me17:0, 10Me18:0
Fungi	18:2ω6c
Saprophytic fungi	18:1ω9c
Arbuscular mycorrhizal fungi	16:1ω5c
Invertebrates	20:4ω6c

- Sys. 1. No Compost + Legume-rye **4th Year**
- Sys. 2. Compost + Legume-rye **4th Year**
- Sys. 3. Compost + Legume-rye annually
- Sys. 4. Compost + Mustard annually
- Sys. 5. Compost + Rye annually

Fig. 6. Principal component analysis of the soil microbial fatty acid methyl ester (FAME) profiles after six years of 5 different organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). For each system, the larger symbol in the center (bigger size) represents the centroid of the 95% confidence interval polygon of the four replicates. Arrows indicate the location of scores for selected FAME indicators of microbial groups (see Materials and methods for further details).

negative change. There is also some evidence supporting an overall decline in the abundance of *Actinobacteria* particularly with System 3 that ranged from a 3–6% decline. Within the less abundant bacterial phyla (*Planctomycetes, Gemmatimonadetes, Nitrospirae, Verrucomicrobia, Deinococcus-Thermus*) there was strong evidence of an overall average decline with *Deinococcus-Thermus* from 0.6 to 0.3% and this pattern was most consistent with System 3 and 5 that both included rye cover crop. While detecting consistent patterns in the relative abundance of the bacterial phyla is difficult given that only three replicates were analyzed for each system, it is interesting to note that within *Gemmatimonadetes* there was a consistent increase across all replicates in System 1 and 2 that were seldom cover cropped, versus a consistent decline in System 4 with mustard cover crop. A decline across all replicates of System 4 was also apparent in *Nitrospirae* and *Verrucomicrobia*.

We identified 501 bacterial genera in this loamy sand soil at the beginning of the experiment including 86 genera that were in all three of the tested replicate plots for all systems. After 6 years of intensive vegetable production, 493 genera were detected including 109 in the three tested replicates of all systems. *Acidobacteria* was the dominant genus and averaged across systems there was no evidence that its abundance changed over time as was the case with two other abundant genera (*Anthrobacteria, Chloroflexus*) (Fig. 10). In contrast, there is strong evidence across most systems of a decrease in the abundance of *Nocardiodes*, *Balneimonas*, and *Streptomyces*, while others (*Agromyces*, *Flavobacterium*) increased. It is important to note that the magnitude of these changes differed somewhat by systems as is well illustrated with *Agromyces* where all three replicates increased by approximately 1.5–2% in System 3 compared with the replicates for System 2 that were below 1%. Furthermore with *Streptomyces*, among the frequently cover cropped systems, all three replicates declined in System 1 and 3, while there was not a consistent pattern with the other three systems.

4. Discussion

4.1. Unique features of this study

There are several features of this systems study that can contribute to improving our knowledge of soil food web dynamics in high-input vegetable production systems: (1) The relatively long-time frame of our study can provide insights into the complexity and resilience of the soil food web and expand on the information from shorter-term studies of microbial changes in Salinas vegetable production soils that ranged from 2 to 6 weeks (Lundquist et al., 1999; Calderon et al., 2000, 2001; Jackson et al., 2003), 5–9 months (Wyland et al., 1995, 1996; Jackson, 2000),

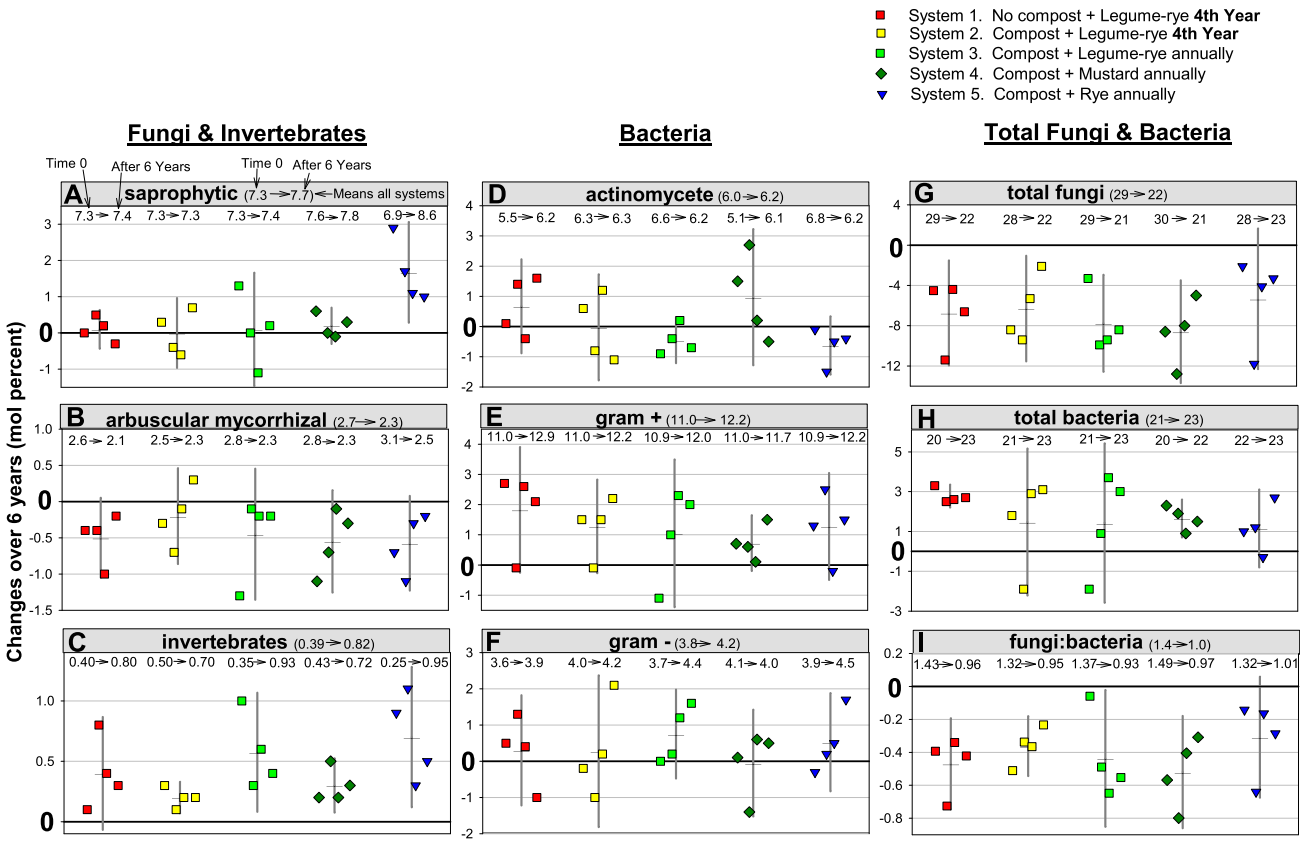


Fig. 7. Changes in the relative abundance of FAME indicators of fungi, bacteria and invertebrates in soil in 5 organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Raw data points are clustered around the mean in order of replicates 1 to 4. The two numbers separated by an arrow error above each data cluster are the mean mol percent of the FAME indicators for each group at the beginning of the experiment (i.e., Time 0) and six years later.

and 2–3 years (Fennimore and Jackson, 2003; Jackson et al., 2004; Smukler et al., 2008). While short-term studies can indicate changes that may occur soon after adoption of a practice, we believe the results of longer-term studies (>5 years) on soil microbial changes like those presented here and previously (Winter et al., 1990; Berkelmans et al., 2003; Oehl et al., 2004; Ros et al.,

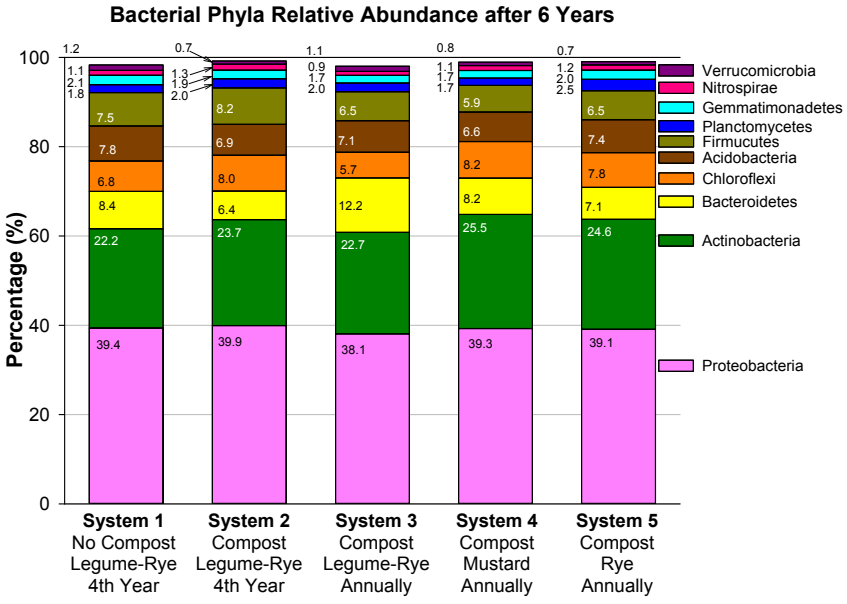


Fig. 8. Bacterial phyla relative abundance after 6 years of 5 different organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). The numbers in the bars are the mean percentage averaged across 3 replicates; mean percentages for the 4 least abundant phyla are to the left of each bar as illustrated for System 2.

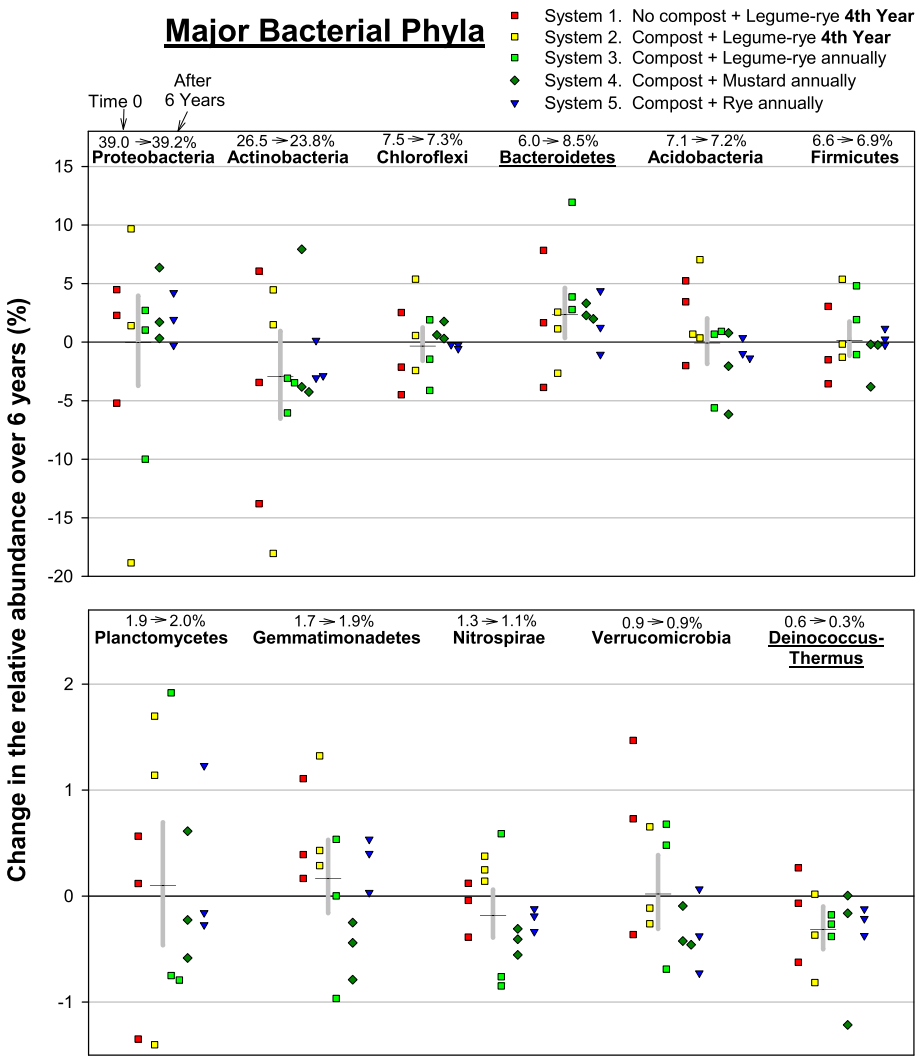


Fig. 9. Changes in the relative abundance of bacterial phyla after 6 years for the top 11 phyla in the soil in 5 different organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Raw data points are clustered around the mean in order of replicates 1 to 3. The percentages separated by an arrow above the labels for each phyla are the relative abundance at the beginning of the experiment (time 0) and 6 years later. Phyla where the confidence interval of the change does not include zero have underlined labels.

2006; Schjonning et al., 2007; Smith et al., 2008; Overstreet et al., 2010; Briar et al., 2011; Ferris et al., 2012; Williams et al., 2013; Murugan et al., 2014; Henneron et al., 2015; Ito et al., 2015; Mbuthia et al., 2015; Montecchia et al., 2015; Jimenez-Bueno et al., 2016; Quist et al., 2016) are critical to identify changes that may accumulate as the practice continues over multiple years. (2) In contrast to previous multi-year studies in this region (Fennimore and Jackson, 2003; Jackson et al., 2004; Smukler et al., 2008) that occurred on loam soils, our study is unique in that it evaluated changes in a sandy soil. This is important because soil texture is known to affect MBC (Franzluebbers et al., 1996), and decomposition rates of organic materials. Decomposition rates are generally higher in sandy than clay soils (Ladd et al., 1995; Sakamoto and Hodono, 2000). This suggests that it would be more difficult to detect changes in microbial biomass in a loamy sand soil like in our study than in a finer textured soil. (3) Another important distinction is that our study represents a transition to increased cropping intensity within an organically managed soil. Thus, although the land on the research farm where the study occurred had been in cultivation with regular tillage for decades, and certified organic for several years before the study began, it had not been intensively managed to produce two, commercial-scale, for-profit vegetable crops annually until the study began in 2003. Despite this, it is interesting and somewhat surprising that the soil MBC at the beginning of our study (i.e., ≈50–125 mg C kg^{-1} soil, Fig. 2A) was within the range of those for sandy loam (151 mg C kg^{-1} soil) and silt loam (75–125 mg C kg^{-1} soil) soils in Salinas with a long history of intensive vegetable production (Jackson et al., 2004; Steenwerth et al., 2005). Steenwerth et al. (2005) found that MBC was approximately 6 times greater in sandy loam soils in this region that were in annual grass pasture than under intensive vegetable production. These differences highlight the profound negative impact on the soil food web that occurs when rainfed pasture is converted to vegetable production. (4) To our knowledge, our study is the first, field-based experiment in California to evaluate the effects of compost made only from yard-waste (i.e., municipal solid waste) in tillage-intensive systems. Compost in previous vegetable studies in California was made primarily from animals manure (Fennimore and Jackson, 2003; Jackson et al., 2004), or a mixture of household organic and green waste (Suddick and Six, 2013). The C:N ratios of compost in those studies was considerably lower (i.e., 10–17) than the compost in our study (22), and unfortunately the

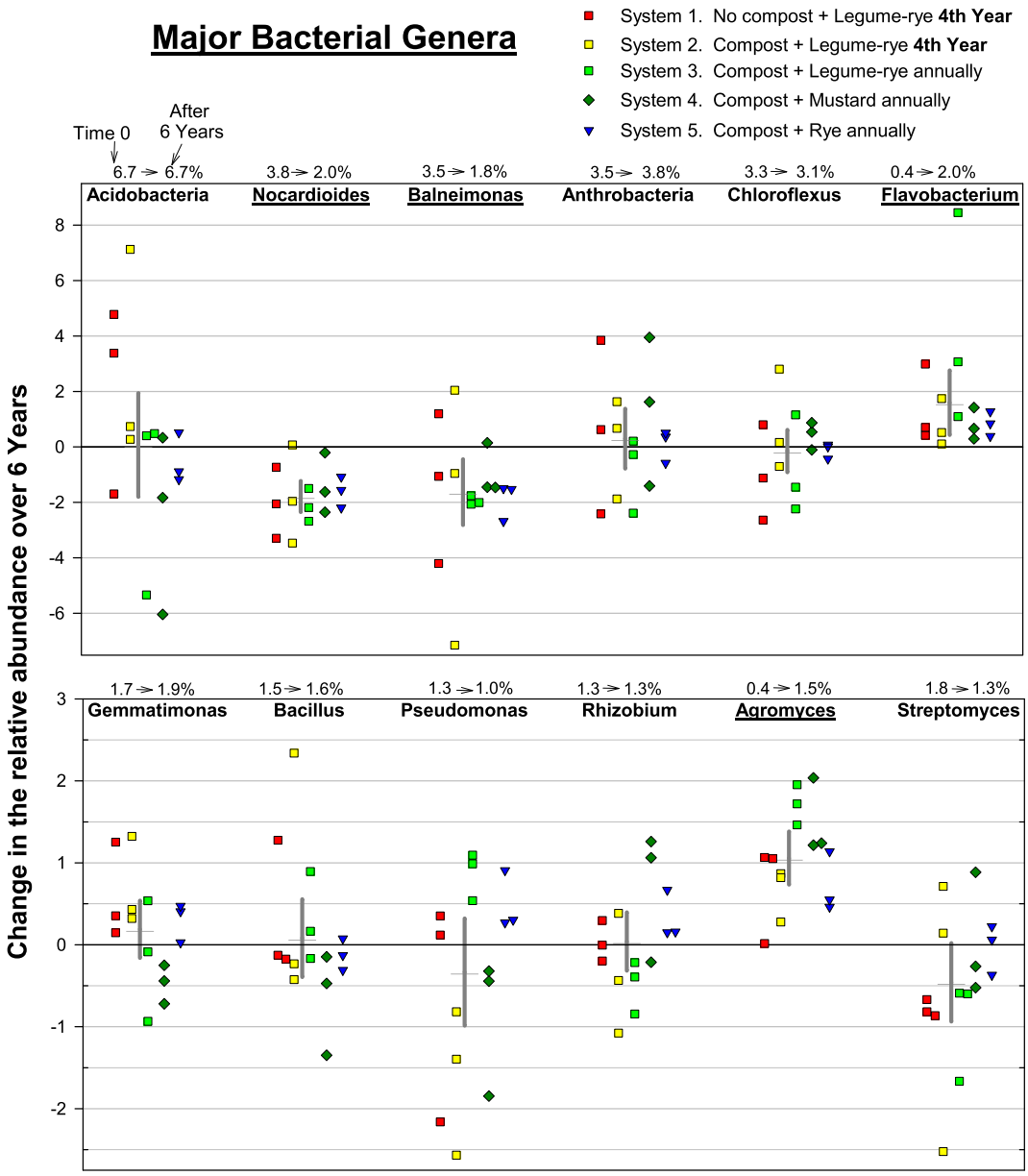


Fig. 10. Changes in the relative abundance of bacterial genera after 6 years for the top 12 genera in the soil in 5 organic vegetable management systems that differed in annual compost additions (0 versus 15.2 Mg ha^{-1} annually), and cover crop type (legume-rye, mustard, or rye) and cover cropping frequency (annually versus every 4th winter). Error bars are 95% confidence intervals with the mean at the central horizontal line. Data points are clustered around the mean in order of replicates 1 to 3. The percentages separated by an arrow above the labels for each genus are the relative abundance at the beginning of the experiment (time 0) and 6 years later. Genera where the confidence interval of the change does not include zero have underlined labels.

compost application rates, on an oven-dry basis, were not reported. These differences make it difficult to compare the effects of compost in these previous studies with our results. Prior to our study, the only data on the long-term impacts of yard-waste compost in California agricultural systems came from a farm survey that provided weak evidence that compost affected soil C levels in vegetable systems at typical application rates after more than nine years of application (Brown and Cotton, 2011).

4.2. Microbial changes across systems during the transition to increased cropping intensity

Several changes occurred to microbial communities across all systems over the 6 years that are likely reflective of the increased tillage, nutrient inputs, and irrigation that were required for commercial-scale vegetable production. This management intensity helps to explain why soils under vegetable production often show dramatic reductions in soil physical, chemical, and biological attributes over long time periods (Haynes and Tregurtha, 1999). Perhaps the most consistent change in our study was the reduction in the fungal FAME indicators (Fig. 7G), compared with the bacterial indicators (especially for Gram +) that tended to increase (Fig. 7E), and consequently reduce the fungal:bacterial ratios substantially (1.4–1.0 averaged across systems, Fig. 7I). The final ratio of fungal:bacterial FAMEs, averaged across systems, are similar to those in an organic on-farm survey with less tillage intensive tomato systems in a warmer region of California (Bowles et al., 2014). Fungal:bacterial ratios are ecologically interesting because they may be related to agricultural system sustainability, C storage, and ecosystem self-regulation (Bardgett et al., 1996; Bardgett and

McAlister, 1999; Frey et al., 1999; Bailey et al., 2002; de Vries et al., 2006; Six et al., 2006; Malik et al., 2016). Furthermore, a global analysis across a range of biomes found a positive linear relationship between soil C:N ratios and the fungal:bacterial ratios (Fierer et al., 2009). The ratio of fungal to bacterial biomass was higher in systems receiving cover crops in California (Bossio et al., 1998), and it has been hypothesized that systems with greater fungal dominance may be better at storing C (Six et al., 2006). It is known that standard tillage practices cause large reductions in fungal biomarkers in cover cropped vegetable systems in California (Minoshima et al., 2007). It is interesting to note the differential response of the fungi indicators in our study - AMF declined across all systems (Fig. 7B) whereas saprophytic fungi showed little change or a slight increase (i.e., System 5, discussed below, Fig. 7A). Allison et al. (2005) found that the response of saprophytic fungi to tillage was not as strong as the response of AMF to tillage which is consistent with our results. A survey of organic tomato farms in California reported slightly higher levels of AMF (≈3–7 mol percent) and several times higher levels of saprophytic fungi (≈20–30 mol percent) than in our study (Fig. 7A), and found a negative relationship between AMF indicators and plant available P (Bowles et al., 2014). Research on AMF colonization of lettuce roots in the central coast region of California where our study occurred found greater colonization in organic than conventional fields, and that colonization was positively correlated to the number of AMF hosts in the rotation (Miller and Jackson, 1998). While cover crops can increase AMF levels in the soil (Kabir and Koide, 2002; White and Weil, 2010; Lehman et al., 2012), the similarity in AMF levels across all systems in our study after six years indicates that the presence of AMF hosts (i.e. rye, legumes, lettuce), non-hosts (broccoli, mustards, spinach), or bare fallow did not affect AMF levels. Although vegetable production soils in the Salinas Valley have a history of excessive P fertilization (Johnstone et al., 2005), there was a gradual increase in AMF colonization of summer and winter vegetables (but not spring or fall vegetables) during a three year transition to organic certification (Smukler et al., 2008). Over fertilization with P can be an important and serious problem in organic systems in California, particularly when there is a heavy reliance on organic fertilizers that typically supply far more P than is needed to replace what was removed in harvests (Maltais-Landry et al., 2015, 2016).

The increased cropping intensity had clear effects on the bacterial population composition with increases in the relative abundance of several taxa (phyla *Bacteroidetes; Agromyces, Flavobacterium*) and decreases with others (phyla *Deinococcus-Thermus; Nocardioides, Balneimonas*) (Figs. 9 and 10). These patterns provide evidence that in this soil type, these taxa may act as useful indicators of changes in cropping intensity across a range of management scenarios represented by the five systems in our study.

4.3. Importance of cover cropping frequency versus yard-waste compost

Understanding the importance of organic matter inputs from cover cropping versus compost in this study is complex because the experimental design was not a full factorial design. For example, there was no system with annual cover crops without compost inputs. Despite this limitation, within the three systems (1, 2, 3) receiving the same cover crop (legume-rye) at different frequencies, the MBC, MBN and FAME data from the first 6 years of the study provide strong evidence that frequent cover cropping during the winter had a far greater impact than yard-waste compost additions on the soil food web in these intensive vegetable systems. This conclusion is consistent with the results of the nematode community analysis from the first 8 years of our study (Ferris et al., 2012). For example, if C inputs from compost and cover crop were equally important for soil food web dynamics, we would expect more similarities between System 2 and 3 that both received frequent and relatively similar amounts of C inputs, primarily from compost (Fig. 1). However, the results show far more similarities, with a variety of parameters reported here (i.e., MBC, MBN, MBC:SOC, FAMEs; Figs. 2–6) and elsewhere (i.e., vegetable yields, Ferris et al., 2012; Maltais-Landry et al., 2015) between systems 1 and 2 that were seldom cover cropped, than between systems 2 and 3 that received compost. This suggests that the frequent inputs of compost that were the most likely cause of the increased SOC in System 2 (Fig. 4) had a relatively minimal effect on the soil food web, presumably because the C added from compost was not readily accessible to soil organisms. This reasoning is supported by the relatively small increase in MBC (Fig. 2B) over the 6 years in System 2 (40 mg C kg^{-1} soil, 95% CI [-1, 80]) compared with System 3 (147 [122, 172]). The overlap in confidence intervals of SOC in Systems 2 and 3 after 6 years (Fig. 4B) suggests that compost had a greater effect than cover crops on SOC; this reasoning is based on the fact that compost inputs were the same for these systems (91.2 Mg ha^{-1}) while cover crop shoot inputs differed by more than 5 fold (System 2, 7.9 Mg ha^{-1}; System 3, 45.6 Mg ha^{-1}, Fig. 1). However, despite the overlap in confidence intervals of SOC for System 2 and 3, the majority of the replicates in System 2 had lower SOC levels than occurred in System 3 indicating that frequent cover cropping in System 3 also contributed to the higher SOC levels. Comparing the results of our study with previous research evaluating municipal solid waste compost is complex due to a variety of factors that differ between our study and others (i.e., crop rotation, tillage intensity, compost application rate, compost application frequency, supplemental fertilizer inputs, compost composition, etc.). For example, a study with barley that applied municipal solid waste compost with a relatively low C:N ratio (9) at 20 Mg ha^{-1} annually during 5 of the years found that soil MBC increased by 10% after 9 years (Garcia-Gil et al., 2000). Interestingly, that study also found a 29% increase in soil MBC with cow manure (C:N, 16) applied at the same application rate. It is important to acknowledge that a weakness of our study is the absence of a no compost, frequently cover cropped system. Without such as system, we are unable to definitively separate the potentially synergistic effects of compost from cover crop.

The nematode analysis on soil taken at end of several of the vegetable crops during the first 8 years of this study, found a greater abundance of three colonizer-persister (cp) structural guilds of nematodes (cp1, cp2, cp3) in systems that received cover crops annually than in the infrequently cover cropped systems (Ferris et al., 2012). In all systems, short-lived opportunist guilds (cp1, bacteriovores; cp2, bacteriovores or fungivores) that are less sensitive to environmental disturbance were the most abundant nematodes followed by cp3 to cp5 guilds that are longer lived, more sensitive to disturbance, and include a variety of feeding habits (Bongers and Bongers, 1998; Bongers and Ferris, 1999). The data from our study on nematodes (Ferris et al., 2012) and soil microbial biomass from the present paper together provide strong evidence that the soil food webs in System 1 and 2 that were infrequently cover cropped were relatively limited by lower inputs of fresh C compared with the System 3, 4 and 5 that were cover cropped annually. This apparent C limitation in System 2 is remarkable when you consider the large amount of compost that added to this system annually (15.2 Mg ha^{-1}) that clearly increased the SOC levels (Fig. 4) but had a relatively small impact on MBC and MBN. This suggests that C inputs from compost were not as important for soil food web dynamics as were C inputs from cover crops.

Belowground biomass from cover crops (roots and root exudates) are also an important C input from cover crops that was not included in the cumulative organic matter inputs for the various

systems (Fig. 1). Based on estimates for small grains and soybean (Bolinder et al., 2007), we assume that shoot biomass from the cover crops in our systems accounted for approximately 80% of the total cover crop C inputs to the soil. Roots exudates represent a major flow of energy from plants into the soil that have a large effect on soil food web dynamics, soil health, and interactions between plants and soil microbes (Fustec et al., 2009; Hartmann et al., 2009; Chaparro et al., 2012). Based on Bolinder et al. (2007) we estimate that the cumulative, below-ground C inputs (i.e., roots and root exudates) in the frequently cover cropped systems over the six years represent the equivalent amount of C added by cover crop shoots alone during 1.5 winter cover cropping periods with approximately 40% of the below ground input coming from root exudates. We speculate that this substantial input of labile C that occurred when cover crops were grown was a primary driver of the increases in microbial biomass in our study.

Inspection of changes in the relative abundance of the major bacterial phyla and genera of Systems 1 and 2 that differed in compost inputs, provides little evidence that compost alone, with infrequent cover cropping with the legume-rye mixture, had any impact on the composition of the soil bacterial population (Figs. 8–10). This lack of effect of compost on soil bacterial population structure in our study is consistent with 5–6 year studies using municipal solid waste compost of different quality (C:N, 10–29) (Crecchio et al., 2004; Sharifi et al., 2014).

By comparing System 2 and 3 that received the same compost rates, we can see strong evidence that frequent cover cropping as occurred in System 3 increased the relative abundance of *Pseudomonas* and *Agromyces* (Fig. 10). *Pseudomonas* spp. are a diverse bacterial group that has received considerable research attention and includes plant pathogens, beneficial species that can act as biological control agents and plant growth promoting rhizobacteria (Mercado-Blanco and Bakker, 2007; Lugtenberg and Kamilova, 2009). Interestingly the relative abundance of *Pseudomonas* also increased in System 5 with the rye cover crop but decreased with mustard. We speculate the rye component that dominated the legume-rye mixture may have caused this increase because recent work with carrots that found greater abundance of *Pseudomonas* spp. following rye cover crops and also more isolates of antagonisitic bacteria and fungi following rye (Patkowska et al., 2016). The greater increase in the relative abundance of *Agromyces* in the frequently cover cropped legume-rye system (3) than occurred in the System 1 and 2 that were usually bare during the winter, is somewhat consistent with research of bacterial population structure across forest and grasslands (Nacke et al., 2011). These researchers reported a greater abundance of *Agromyces* in grasslands than forests, particularly in an intensively managed and fertilized grassland. While all systems in our study received the same supplemental fertilizer inputs during vegetable production, System 3 had the largest inputs of N from biological fixation over the 6 year period that may explain the greater abundance of *Agromyces* in System 3. For example, based on the shoot biomass production of the total legume-rye shoot biomass over the 6 years (Brennan and Boyd, 2012; Brennan, unpublished data) and the estimate that 30–40 kg N ha^{-1} is fixed for 1 Mg ha^{-1} shoot dry biomass (Peoples et al., 2009), we estimate that cumulative N inputs from biological fixation over the 6 years would have been several times greater in System 3 ($\approx$320 kg N ha^{-1}) than in Systems 1 and 2 ($\approx$80 kg N ha^{-1}).

4.4. Cover crop type effects on the soil microbial component

The systems that were cover cropped annually and all received the same compost inputs, but differed only in cover crop type (Systems 3, 4, 5) showed similarly trends in microbial community size and composition with a few exceptions. When the cover crops were incorporated into the soil in the spring, their shoot C:N ratios averaged across the 6 years were higher in System 5 with rye (C:N = 31) than System 4 with mustard (C:N = 22) or System 3 with the legume-rye mixture (C:N = 21) (Brennan et al., 2013). Decomposition of lower quality materials (i.e., higher C:N) is favored by fungi, whereas bacteria are better at decomposing higher quality material (Bossuyt et al., 2001). Therefore, the higher C:N of the rye in System 5 would presumably make this material more accessible to fungal than bacterial decomposition. This may explain why the FAME indicators for saprophytic fungi (Fig. 7A) increased over the 6 years from 6.9 to 8.6 compared in System 5, but changed little with the other systems (i.e., the confidence intervals of the difference for saprophytic fungi were centered near to zero for Systems 1 to 4). This reasoning is also consistent with the closer proximity of System 5 to the saprophytic fungal indicator (18:2ω6c) than occurred with System 3 or 4 (Fig. 6). Furthermore, System 5 was the only system where the confidence interval of the difference over time in total fungi and the fungal:bacterial ratio overlapped with zero (Fig. 7G, I). Rye biomass in the legume-rye mixture in System 3 comprised an average of 80% of the total mixture biomass over the six years, but the C:N of rye in the mixture was only 24, presumably because of N transfer from the legume component. This lower C:N of the rye in the mixture in System 3 than in rye in System 5 (C:N=31) suggests that rye in the mixture would decompose more readily than rye growing alone. This is supported by the lower N mineralization of the rye residue in System 5 than occurred in System 3 and 4 during the second year of the study (Brennan et al., 2013).

The data provides no evidence that the relatively large differences (i.e., $\approx$12–15 Mg ha) in cumulative cover crop shoot biomass between mustard (30.5 Mg ha^{-1}), legume-legume rye (45.1) or rye (42.9) (Fig. 1) had any apparent impact on microbial biomass (Figs. 2 and 3), or SOC (Fig. 4) after 6 years. This is surprising given that these differences are similar to the amount of shoot biomass that a vigorous rye or legume rye cover crop would produce during two winter periods. One explanation for the lack of relationship between cover crop shoot biomass and the microbial biomass or SOC is that all of the cover crops provided a minimum threshold level of fresh C input that was needed to drive changes in the soil food web. The SOC similarities between Systems 2 to 5 that all received compost but different amounts of cover crop shoots inputs provides strong evidence that C inputs from compost, not cover crop, was the primary factor driving soil organic matter changes over time. Furthermore, it is surprising that the three annually cover cropped systems were similar in terms of MBC, MBN, and SOC despite the greater input of N from biological fixation that occurred in System 3 (i.e., $\approx$53 kg N ha^{-1} annually, see calculation details in above section).

The different types of cover crops had few effects on the relative abundance of soil bacterial phyla. For example, the abundance of the phyla *Gemmatimonadetes* increased in System 5 with rye cover crops but declined with mustard (System 4) (Fig. 10). DeBruyn et al. (2011) studied the possible link between soil moisture and the abundance of this phyla and provided evidence that *Gemmatimonadetes* is adapted to low soil moisture. This is somewhat consistent with measurements of moisture in the top 46 cm of soil during the winter in our study that were lower in the rye than in the mustard and the legume-rye systems (Cahn and Miyao, 2011). We speculate that the consistent reduction in the abundance of *Gemmatimonadetes* in the Systems 1 and 2 may be related to the bare surface in these plots that could result in relatively dry conditions in the top several cm of this well-drained sandy soil particularly during prolonged dry periods that occurred some winters (Brennan and Boyd, 2012); the cover crops were primarily rainfed other than to establish them early in the fall before the rains began.

The different cover crops also showed some interesting differences in the relative abundance of major bacterial genera after 6 years of management (Fig. 10). *Pseudomonas* tended to decline with mustard cover crops but increased in System 3 with the legume-rye and System 5 with rye. This similarity in rye and legume-rye systems may be related to the presence of rye. Furthermore, the increase in the relative abundance of *Agromyces* was approximately twice as great in System 3 (legume-rye) than in System 5 (rye); possible reasons for the effect of cover crop type on *Agromyces* and *Pseudomonas* are discussed above in section 4.3.

4.5. Practical implications for soil health

The microbial biomass data presented here combined with the nematode community analysis (Ferris et al., 2012) from this ongoing systems study provide compelling evidence of the potential benefits of frequent cover cropping and compost inputs on the soil health in high-value, tillage-intensive vegetable production systems. While C input from yard-waste compost increased SOC levels by approximately 3 g kg^{-1} soil after 6 years (Fig. 4), our data indicate that improvements in the soil food web (i.e., increases in MBC and MBN) in these systems was primarily driven by inputs of fresh plant material from frequent cover cropping (Figs. 2 and 3). We acknowledge that this conclusion is somewhat speculative because the experimental design lacked systems with frequent cover cropping without compost inputs, however, if compost was a major driver of the soil food web we would have expected to more similarities in microbial biomass in systems that all received compost (i.e. Systems 2, 3, 4, 5). These results help to explain why vegetable yields in this our study were greater in the frequently cover cropped systems compared with yields in the systems that were infrequently cover cropped regardless of compost inputs (Ferris et al., 2012; Maltais-Landry et al., 2015); more details of marketable yields over the first 8 years of vegetable production will be presented in future papers from the study.

Although this study occurred in a certified organic context, we believe that the results are equally applicable in conventional vegetable rotations in this region. Given the importance of frequent cover cropping on soil microbial activity, compared with the surprisingly minimal benefits from yard waste compost, we suggest that organic and conventional vegetable farms in this region work to develop soil health management programs that place more emphasis on frequent cover cropping to add biologically active forms of organic matter to the soil and less on emphasis on the use of off-farm sources of organic matter such as yard-waste compost. This approach would likely improve the sustainability of these systems in several ways by (1) adding diversity of the crop rotation, (2) increasing N use efficiency by minimizing N leaching losses during the winter, (3) increasing soil health with regular inputs of C from cover crop roots and shoots, (4) achieving more balanced phosphorous (P) budgets, (5) reducing winter runoff and erosion potential, and (6) maximizing the on-farm capture of solar energy to fuel the soil food web. The issue of excessive P application is an important problem in organic systems in California and one that could be reduced by minimizing the use of off-farm organic inputs (compost and fertilizers) that resulted in more than 400 kg ha^{-1} of excess P inputs in our cover cropped systems over 8 years of vegetable production (Maltais-Landry et al., 2016).

Considering the need for a mixture of winter vegetable production, and access to bare winter fallowed fields for early spring plantings, it is unreasonable to expect that most farms in the Salinas region could grow standard, high biomass winter cover crops like those in the current study on every field during the winter. However, several alternative and novel cover cropping approaches for this region were recently proposed that may increase cover cropping here (Brennan, 2017). We believe that farmers would be more likely to integrate important best management practices such as cover cropping into their rotations if they had access to reliable tests that measure soil biological activity as proposed by Franzluebbers (2016). These tests could indicate short-term improvements in soil health from practices like cover cropping. For example, within the three systems that received the legume-rye cover crops (System 1, 2, 3), the measurement of SOC from a standard soil test suggests that systems 2 and 3 are relatively similar to each other, and are both substantially different from System 1 (Fig. 4). In contrast, a biologically-based soil test such as MBC indicates far more similarities between Systems 1 and 2 that were infrequently cover cropped (Fig. 2A).

5. Conclusions

This relatively long-term assessment of the soil microbial changes in tillage-intensive, high-input organic vegetable systems provides important insights into the effects of increasing cropping intensity on soil food web dynamics and how these can be altered by the use of winter cover crops and yard-waste compost. Frequent cover cropping had more impact than compost on the soil microbial community and this effect was relatively similar regardless of whether the cover crop was a legume-rye mixture, mustard, or rye. The benefits of cover cropping to the soil food web were most likely the result of inputs of labile C from above and below ground production. The bacterial population composition was relatively unaffected by management differences between the organic systems. However there is evidence that cover crop type and frequency altered the relative abundance of some bacteria such as *Pseudomonoas* that includes species that are important biological control agents and plant growth promoting rhizobacteria. Reductions in the fungal:bacterial ratio across all systems highlight the need to develop reduced tillage options for high-value vegetable systems. This study provides clear examples of large differences in soil heath within organic production systems for the same vegetable crops. These results are relevant to organic and conventional systems that are working to improve soil health and develop more sustainable rotations.

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Appendix A Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.01.014.

References

Allison, V.J., Miller, R.M., Jastrow, J.D., Matamala, R., Zak, D.R., 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Science Society of America Journal 69, 1412–1421.

Anderson, D.R., Burnham, K.P., Thompson, W.L., 2000. Null hypothesis testing: problems, prevalence, and an alternative. Journal of Wildlife Management 64, 912–923.
Bailey, V.L., Smith, J.L., Bolton, H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology & Biochemistry 34, 997–1007.
Ball, A.S., 2006. Energy inputs in soil systems. In: Uphoff, N., Ball, A.S., Fernandes, E., Herren, H., Husson, O., Laing, M., Palm, C., Pretty, J., Sanchez, P., Sanginga, N., Thies, J. (Eds.), Biological Approaches to Sustainable Soil Systems. CRC Press, Boca Raton, pp. 79–89.
Bardgett, R.D., Hobbs, P.J., Frostegard, A., 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biology and Fertility of Soils 22, 261–264.
Bardgett, R.D., McAlister, E., 1999. The measurement of soil fungal : bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils 29, 282–290.
Bensen, T.A., Smith, R.F., Subbarao, K.V., Koike, S.T., Fennimore, S.A., Shem-Tov, S., 2009. Mustard and other cover crop effects vary on lettuce drop caused by sclerotinia minor and on weeds. Plant Disease 93, 1019–1027.
Berkelmans, R., Ferris, H., Tenuta, M., van Bruggen, A.H.C., 2003. Effects of long-term crop management on nematode trophic levels other than plant feeders disappear after 1 year of disruptive soil management. Applied Soil Ecology 23, 223–235.
Birkhofer, K., Bezemer, T.M., Bloem, J., Bonkowski, M., Christensen, S., Dubois, D., Ekelund, F., Fliessbach, A., Gunst, L., Hedlund, K., Mader, P., Mikola, J., Robin, C., Setala, H., Tatin-Froux, F., Van der Putten, W.H., Scheu, S., 2008. Long-term organic farming fosters below and aboveground biota: implications for soil quality, biological control and productivity. Soil Biology & Biochemistry 40, 2297–2308.
Bolinder, M.A., Janzen, H.H., Gregorich, E.G., Angers, D.A., VandenBygaart, A.J., 2007. An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. Agriculture Ecosystems & Environment 118, 29–42.
Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Applied Soil Ecology 10, 239–251.
Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution 14, 224–228.
Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology 36, 1–12.
Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16, 195–208.
Bowles, T.M., Acosta-Martinez, V., Calderon, F., Jackson, L.E., 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biology & Biochemistry 68, 252–262.
Brennan, E.B., 2011. A time-efficient scooping method to prepare cover crop seed for cone planters. Agronomy Journal 103, 917–924.
Brennan, E.B., 2017. Can we grow organic or conventional vegetables sustainably without cover crops? Horttechnology (in press).
Brennan, E.B., Boyd, N.S., 2012. Winter cover crop seeding rate and variety affects during eight years of organic vegetables: I. Cover crop biomass production. Agronomy Journal 104, 684–698.
Brennan, E.B., Boyd, N.S., Smith, R.F., 2013. Winter cover crop seeding rate and variety affects during eight years of organic vegetables: III. Cover crop residue quality and nitrogen mineralization. Agronomy Journal 105, 171–182.
Briar, S.S., Fonte, S.J., Park, I., Six, J., Scow, K., Ferris, H., 2011. The distribution of nematodes and soil microbial communities across soil aggregate fractions and farm management systems. Soil Biology & Biochemistry 43, 905–914.
Briar, S.S., Grewal, P.S., Somasekhar, N., Stinner, D., Miller, S.A., 2007. Soil nematode community, organic matter, microbial biomass and nitrogen dynamics in field plots transitioning from conventional to organic management. Applied Soil Ecology 37, 256–266.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil-nitrogen–A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837–842.
Brown, S., Cotton, M., 2011. Changes in soil properties and carbon content following compost application: results of on-farm sampling. Compost Science & Utilization 19, 88–97.
Cahn, M.D., Miyao, G., 2011. Water management and impact on water quality. In: Smith, R.F., Bugg, R.L., Daugovish, O., Gaskell, M., Van Horn, M. (Eds.), Cover Cropping for Vegetable Production: a Grower's Handbook. Univ. California, Oakland, CA, pp. 31–36.
Calderon, F.J., Jackson, L.E., Scow, K.M., Rolston, D.E., 2000. Microbial responses to simulated tillage in cultivated and uncultivated soils. Soil Biology & Biochemistry 32, 1547–1559.
Calderon, F.J., Jackson, L.E., Scow, K.M., Rolston, D.E., 2001. Short-term dynamics of nitrogen, microbial activity, and phospholipid fatty acids after tillage. Soil Science Society of America Journal 65, 118–126.
Campbell, K.G., Thompson, Y.M., Guy, S.O., McIntosh, M., Glaz, B., 2015. Is, or is not, the two great ends of Fate: errors in Agronomic Research. Agronomy Journal 107, 718–729.
Cao, Z.P., Han, X.M., Hu, C., Chen, J., Zhang, D.P., Steinberger, Y., 2011. Changes in the abundance and structure of a soil mite (Acari) community under long-term organic and chemical fertilizer treatments. Applied Soil Ecology 49, 131–138.
Chaparro, J.M., Sheflin, A.M., Manter, D.K., Vivanco, J.M., 2012. Manipulating the soil microbiome to increase soil health and plant fertility. Biology and Fertility of Soils 48, 489–499.
Cotton, J., Acosta-Martinez, V., Moore-Kucera, J., Burow, G., 2013. Early changes due to sorghum biofuel cropping systems in soil microbial communities and metabolic functioning. Biology and Fertility of Soils 49, 403–413.
Crecchio, C., Curci, M., Pizzigallo, M.D.R., Ricciuti, P., Ruggiero, P., 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biology & Biochemistry 36, 1595–1605.
Cumming, G., 2012. Understanding the New Statistics: Effect Sizes, Confidence Intervals, and Meta-analysis. Routledge, New York.
Cumming, G., Fidler, F., Vaux, D.L., 2007. Error bars in experimental biology. Journal of Cell Biology 177, 7–11.
de Vries, F.T., Hoffland, E., van Eekeren, N., Brussaard, L., Bloem, J., 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology & Biochemistry 38, 2092–2103.
DeBruyn, J.M., Nixon, L.T., Fawaz, M.N., Johnson, A.M., Radosevich, M., 2011. Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. Applied and Environmental Microbiology 77, 6295–6300.
Doran, J., Parkin, T.B., 1996. Quantitative indicators of soil quality: a minimum data set. In: Doran, J., Jones, A.J. (Eds.), Methods of Assessing Soil Quality. Soil Science Society of America, Madison, WI, pp. 25–37.
Drinkwater, L.E., Letourneau, D.K., Workneh, F., van Bruggen, A.H.C., Shennan, C., 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. Ecological Applications 5, 1098–1112.
Drummond, G.B., Vowler, S.L., 2011. Show the data, don't conceal them. Clinical and Experimental Pharmacology and Physiology 38, 287–289.
Fennimore, S.A., Jackson, L.E., 2003. Organic amendment and tillage effects on vegetable field weed emergence and seedbanks. Weed Technology 17, 42–50.
Fennimore, S.A., Martin, F.N., Miller, T.C., Broome, J.C., Dorn, N., Greene, I., 2014. Evaluation of a mobile steam applicator for soil disinfestation in California strawberry. HortScience 49, 1542–1549.
Ferris, H., Sanchez-Moreno, S., Brennan, E.B., 2012. Structure, functions and interguild relationships of the soil nematode assemblage in organic vegetable production. Applied Soil Ecology 61, 16–25.
Ferris, H., Tuomisto, H., 2015. Unearthing the role of biological diversity in soil health. Soil Biology and Biochemistry 85, 101–109.
Ferris, H., Venette, R.C., Lau, S.S., 1996. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. Applied Soil Ecology 3, 161–175.
Fidler, F., Burgman, M.A., Cumming, G., Buttrose, R., Thomason, N., 2006. Impact of criticism of null-hypothesis significance testing on statistical reporting practices in conservation biology. Conservation Biology 20, 1539–1544.
Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecology Letters 12, 1238–1249.
Franzluebbers, A.J., 2016. Should soil testing services measure soil biological activity? Agricultural and Environmental Letters 1, 150009.
Franzluebbers, A.J., Haney, R.L., Hons, F.M., Zuberer, D.A., 1996. Active fractions of organic matter in soils with different texture. Soil Biology & Biochemistry 28, 1367–1372.
Frey, S.D., Elliott, E.T., Paustian, K., 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology & Biochemistry 31, 573–585.
Frostegard, A., Tunlid, A., Baath, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology & Biochemistry 43, 1621–1625.
Fustec, J., Lesuffleur, F., Mahieu, S., Cliquet, J.B., 2009. Nitrogen rhizodeposition of legumes. A review. Agronomy for Sustainable Development 30, 57–66.
Garcia-Gil, J.C., Plaza, C., Soler-Rovira, P., Polo, A., 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biology & Biochemistry 32, 1907–1913.
Gunapala, N., Scow, K.M., 1998. Dynamics of soil microbial biomass and activity in conventional and organic farming systems. Soil Biology & Biochemistry 30, 805–816.
Guthman, J., 2000. Raising organic: and agro-ecological assessment of grower practices in California. Agriculture and Human Values 17, 257–266.
Handl, S., Dowd, S.E., Garcia-Mazcorro, J.F., Steiner, J.M., Suchodolski, J.S., 2011. Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. FEMS Microbiology Ecology 76, 301–310.
Hartmann, A., Schmid, M., van Tuinen, D., Berg, G., 2009. Plant-driven selection of microbes. Plant and Soil 321, 235–257.
Hartz, T.K., 2006. Vegetable production best management practices to minimize nutrient loss. Horttechnology 16, 398–403.
Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: an overview. In: Sparks, D.L. (Ed.), Advances in Agronomy, pp. 221–268.
Haynes, R.J., Tregurtha, R., 1999. Effects of increasing periods under intensive arable vegetable production on biological, chemical and physical indices of soil quality. Biology and Fertility of Soils 28, 259–266.
Henneron, L., Bernard, L., Hedde, M., Pelosi, C., Villenave, C., Chenu, C., Bertrand, M., Girardin, C., Blanchart, E., 2015. Fourteen years of evidence for positive effects of conservation agriculture and organic farming on soil life. Agronomy for Sustainable Development 35, 169–181.
Hubbard, R., Lindsay, R.M., 2008. Why P values are not a useful measure of evidence in statistical significance testing. Theory & Psychology 18, 69–88.

Ishak, H.D., Plowes, R., Sen, R., Kellner, K., Meyer, E., Estrada, D.A., Dowd, S.E., Mueller, U.G., 2011. Bacterial diversity in solenopsis invicta and solenopsis geminata ant colonies characterized by 16S amplicon 454 pyrosequencing. Microbial Ecology 61, 821–831.

Ito, T., Araki, M., Higashi, T., Komatsuzaki, M., Kaneko, N., Ohta, H., 2015. Responses of soil nematode community structure to soil carbon changes due to different tillage and cover crop management practices over a nine-year period in Kanto, Japan. Applied Soil Ecology 89, 50–58.

Jackson, L.E., 2000. Fates and losses of nitrogen from a nitrogen-15-labeled cover crop in an intensively managed vegetable system. Soil Science Society of America Journal 64, 1404–1412.

Jackson, L.E., Calderon, F.J., Steenwerth, K.L., Scow, K.M., Rolston, D.E., 2003. Responses of soil microbial processes and community structure to tillage events and implications for soil quality. Geoderma 114, 305–317.

Jackson, L.E., Ramirez, I., Yokota, R., Fennimore, S.A., Koike, S.T., Henderson, D.M., Chaney, W.E., Calderon, F.J., Klonsky, K., 2004. On-farm assessment of organic matter and tillage management on vegetable yield, soil, weeds, pests, and economics in California. Agriculture. Ecosystems & Environment 103, 443–463.

Jenkinson, D.S., 1988. Determination of microbial biomass carbon and nitrogen in soil. In: Wilson, J.R. (Ed.), Advances in Nitrogen Cycling in Agricultural Ecosystems. CAB International, Walling-ford, pp. 368–386.

Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biology & Biochemistry 36, 5–7.

Jimenez-Bueno, N.G., Valenzuela-Encinas, C., Marsch, R., Ortiz-Gutierrez, D., Verhulst, N., Govaerts, B., Dendooven, L., Navarro-Noya, Y.E., 2016. Bacterial indicator taxa in soils under different long-term agricultural management. Journal of Applied Microbiology 120, 921–933.

Joergensen, R.G., Wu, J.S., Brookes, P.C., 2011. Measuring soil microbial biomass using an automated procedure. Soil Biology & Biochemistry 43, 873–876.

Johnstone, P.R., Hartz, T.K., Cahn, M.D., Johnstone, M.R., 2005. Lettuce response to phosphorus fertilization in high phosphorus soils. HortScience 40, 1499–1503.

Kabir, Z., Koide, R.T., 2002. Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake and yield of sweet corn in Pennsylvania USA. Plant and Soil 238, 205–215.

Karlen, D.L., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F., Schuman, G.E., 1997. Soil quality: a concept, definition, and framework for evaluation. Soil Science Society of America Journal 61, 4–10.

Kirk, R.E., 1996. Practical significance: a concept whose time has come. Educational and Psychological Measurement 56, 746–759.

Klonsky, K., Healy, B., 2013. Statistical Review of California's Organic Agriculture. Agricultural Issues Center, University of California.

Klonsky, K., Tourte, L., 2011. Economics. In: Smith, R., Bugg, R.L., Daugovish, O., Gaskell, M., Van Horn, M. (Eds.), Cover Cropping for Vegetable Production: a Grower's Handbook. Univ. California, Oakland, CA., pp. 83–85

Klose, S., Acosta-Martinez, V., Ajwa, H.A., 2006. Microbial community composition and enzyme activities in a sandy loam soil after fumigation with methyl bromide or alternative biocides. Soil Biology & Biochemistry 38, 1243–1254.

Koike, S.T., Smith, R.F., Jackson, L.E., Wyland, L.J., Inman, J.I., Chaney, W.E., 1996. Phacelia, Lana woollypod vetch, and Austrian winter pea: three new cover crop hosts of Sclerotinia minor in California. Plant Disease 80, 1409–1412.

Ladd, J.N., Amato, M., Grace, P.R., Vanveen, J.A., 1995. Simulation of C-14 turnover through the microbial biomass in soils incubated with C-14-labeled plant residues. Soil Biology & Biochemistry 27, 777–783.

Lambdin, C., 2012. Significance tests as sorcery: science is empirical-significance tests are not. Theory & Psychology 22, 67–90.

Lehman, R.M., Taheri, W.I., Osborne, S.L., Buyer, J.S., Douds, D.D., 2012. Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. Applied Soil Ecology 61, 300–304.

Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting Rhizobacteria. Annual Review of Microbiology 541–556.

Lundquist, E.J., Jackson, L.E., Scow, K.M., Hsu, C., 1999. Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. Soil Biology & Biochemistry 31, 221–236.

Malik, A.A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P.G.M., Jehmlich, N., von Bergen, M., Griffiths, R.I., Gleixner, G., 2016. Soil fungal:bacterial ratios are linked to altered carbon cycling. Frontiers in Microbiology 7.

Maltais-Landry, E., Scow, K., Brennan, E.B., Torbert, E., Vitousek, P., 2016. Higher flexibility in input N: P ratios results in more balanced phosphorus budgets in two long-term experimental agroecosystems. Agriculture Ecosystems & Environment 223, 197–210.

Maltais-Landry, E., Scow, K., Brennan, E.B., Vitousek, P., 2015. Long-term effects of compost and cover crops on soil phosphorus in two California agroecosystems. Soil Science Society of America Journal 79, 688–697.

Mbuthia, L.W., Acosta-Martinez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N., 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: implications for soil quality. Soil Biology & Biochemistry 89, 24–34.

Mercado-Blanco, J., Bakker, P.A.H.M., 2007. Interactions between plants and beneficial Pseudomonas spp.: exploiting bacterial traits for crop protection. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 92, 367–389.

Miller, R.L., Jackson, L.E., 1998. Survey of vesicular-arbuscular mycorrhizae in lettuce production in relation to management and soil factors. Journal of Agricultural Science 130, 173–182.

Minoshima, H., Jackson, L.E., Cavagnaro, T.R., Sanchez-Moreno, S., Ferris, H., Temple, S.R., Goyal, S., Mitchell, J.P., 2007. Soil food webs and carbon dynamics in response to conservation tillage in California. Soil Science Society of America Journal 71, 952–963.

Mitchell, J.P., 1999. Organic matter recycling varies with crops grown. California Agriculture 53, 37–40.

Montecchia, M.S., Tosi, M., Soria, M.A., Vogrig, J.A., Sydorenko, O., Correa, O.S., 2015. Pyrosequencing reveals changes in soil bacterial communities after conversion of Yungas forests to agriculture. PLoS One 10.

Muramoto, J., Gliessman, S., Koike, S.T., Shennan, C., Bull, C.T., Klonsky, K., Swezey, S.L., 2014. Integrated biological and cultural practices can reduce crop rotation period of organic strawberries. Agroecology and Sustainable Food Systems 38, 603–631.

Murugan, R., Koch, H.-J., Joergensen, R.G., 2014. Long-term influence of different tillage intensities on soil microbial biomass, residues and community structure at different depths. Biology and Fertility of Soils 50, 487–498.

Nacke, H., Thurmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schoning, I., Schrumpf, M., Daniel, R., 2011. Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. PLoS One 6.

Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biological Reviews 82, 591–605.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), Methods of Soil Analysis. Part 3. Chemical Methods, Number 5 in the Soil Science Society of America Book Series Ed. American Society of Agronomy, Soil Science Society of America, Madison, pp. 1001–1006.

Njoroge, S.M.C., Kabir, Z., Martin, F.N., Koike, S.T., Subbarao, K.V., 2009. Comparison of crop rotation for Verticillium Wilt management and effect on Pythium species in conventional and organic strawberry production. Plant Disease 93, 519–527.

Oehl, F., Sieverding, E., Mader, P., Dubois, D., Ineichen, K., Boller, T., Wiemken, A., 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138, 574–583.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2011. Vegan: Community Ecology Package. R Package Version 2.0-2. http://CRAN.R-project.org/package=vegan.

Overstreet, L.F., Hoyt, G.D., Imbriani, J., 2010. Comparing nematode and earthworm communities under combinations of conventional and conservation vegetable production practices. Soil & Tillage Research 110, 42–50.

Patkowska, E., Blazewicz-Wozniak, M., Konopinski, M., Wach, D., 2016. The effect of cover crops on the fungal and bacterial communities in the soil under carrot cultivation. Plant Soil and Environment 62, 237–242.

Peoples, M.B., Brockwell, J., Herridge, D.F., Rochester, I.J., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Dakora, F.D., Bhattarai, S., Maskey, S.L., Sampet, C., Rerkasem, B., Khan, D.F., Hauggaard-Nielsen, H., Jensen, E.S., 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48, 1–17.

Pribyl, D.W., 2010. A critical review of the conventional SOC to SOM conversion factor. Geoderma 156, 75–83.

Quist, C.W., Schrama, M., de Haan, J.J., Smant, G., Bakker, J., van der Putten, W.H., Helder, J., 2016. Organic farming practices result in compositional shifts in nematode communities that exceed crop-related changes. Applied Soil Ecology 98, 254–260.

Reganold, J.P., Andrews, P.K., Reeve, J.R., Carpenter-Boggs, L., Schadt, C.W., Alldredge, J.R., Ross, C.F., Davies, N.M., Zhou, J.Z., 2010. Fruit and soil quality of organic and conventional strawberry agroecosystems. PLoS One 5.

Romig, D.E., Garlynd, M.J., Harris, R.F., McSweeney, K., 1995. How farmers assess soil health and quality. Journal of Soil and Water Conservation 50, 229–236.

Ros, M., Klammer, S., Knapp, B., Aichberger, K., Insam, H., 2006. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use and Management 22, 209–218.

Ruess, L., Chamberlain, P.M., 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biology & Biochemistry 42, 1898–1910.

Sakamoto, K., Hodono, N., 2000. Turnover time of microbial biomass carbon in Japanese upland soils with different textures. Soil Science and Plant Nutrition 46, 483–490.

Schjonning, P., Munkholm, L.J., Elmholt, S., Olesen, J.E., 2007. Organic matter and soil tilth in arable farming: management makes a difference within 5-6 years. Agriculture Ecosystems & Environment 122, 157–172.

Schutter, M.E., Dick, R.P., 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal 64, 1659–1668.

Sharifi, M., Lynch, D.H., Hammermeister, A., Burton, D.L., Messiga, A.J., 2014. Effect of green manure and supplemental fertility amendments on selected soil quality parameters in an organic potato rotation in Eastern Canada. Nutrient Cycling in Agroecosystems 100, 135–146.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555–569.

Smith, R.G., McSwiney, C.P., Grandy, A.S., Suwanwaree, P., Snider, R.M., Robertson, G.P., 2008. Diversity and abundance of earthworms across an

agricultural land-use intensity gradient. Soil & Tillage Research 100, 83–88.
Smukler, S.M., Jackson, L.E., Murphree, L., Yokota, R., Koike, S.T., Smith, R.F., 2008. Transition to large-scale organic vegetable production in the Salinas Valley, California. Agriculture Ecosystems & Environment 126, 168–188.
Steenwerth, K.L., Jackson, L.E., Calderon, F.J., Scow, K.M., Rolston, D.E., 2005. Response of microbial community composition and activity in agricultural and grassland soils after a simulated rainfall. Soil Biology & Biochemistry 37, 2249–2262.
Subbarao, K.V., Kabir, Z., Martin, F.N., Koike, S.T., 2007. Management of soilborne diseases in strawberry using vegetable rotations. Plant Disease 91, 964–972.
Suddick, E.C., Six, J., 2013. An estimation of annual nitrous oxide emissions and soil quality following the amendment of high temperature walnut shell biochar and compost to a small scale vegetable crop rotation. Science of the Total Environment 465, 298–307.
Thies, J.E., Grossman, J.M., 2006. The soil habitat and soil ecology. In: Uphoff, N., Ball, A.S., Fernandes, E., Herren, H., Husson, O., Laing, M., Palm, C., Pretty, J., Sanchez, P., Sanginga, N., Thies, J. (Eds.), Biological Approaches to Sustainable Soil Systems. CRC Press, Boca Raton, pp. 59–78.
USDA-ERS, 2013. Certified Organic Vegetable Acreage.
van Diepeningen, A.D., de Vos, O.J., Korthals, G.W., van Bruggen, A.H.C., 2006. Effects of organic versus conventional management on chemical and biological parameters in agricultural soils. Applied Soil Ecology 31, 120–135.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703–707.
White, C.M., Weil, R.R., 2010. Forage radish and cereal rye cover crop effects on mycorrhizal fungus colonization of maize roots. Plant and Soil 328, 507–521.
Williams, A., Borjesson, G., Hedlund, K., 2013. The effects of 55 years of different inorganic fertiliser regimes on soil properties and microbial community composition. Soil Biology & Biochemistry 67, 41–46.
Williams, A., Hedlund, K., 2013. Indicators of soil ecosystem services in conventional and organic arable fields along a gradient of landscape heterogeneity in southern Sweden. Applied Soil Ecology 65, 1–7.
Winter, J.P., Voroney, R.P., Ainsworth, D.A., 1990. Soil microarthropods in long-term No-Tillage and conventional tillage corn production. Canadian Journal of Soil Science 70, 641–653.
Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation extraction - an automated procedure. Soil Biology & Biochemistry 22, 1167–1169.
Wyland, L.J., Jackson, L.E., Chaney, W.E., Klonsky, K., Koike, S.T., Kimple, B., 1996. Winter cover crops in a vegetable cropping system: impacts on nitrate leaching, soil water, crop yield, pests and management costs. Agriculture, Ecosystems & Environment 59, 1–17.
Wyland, L.J., Jackson, L.E., Schulbach, K.F., 1995. Soil-plant nitrogen dynamics following incorporation of a mature rye cover crop in a lettuce production system. Journal of Agricultural Science 124, 17–25.
Zelles, L., 1996. Fatty acid patterns of microbial phospholipids and lipopolysaccharides. In: Schinner, F., Ohlinger, R., Kandeler, E., Margesin, R. (Eds.), Methods in Soil Biology. Springer, Berlin, pp. 80–93.
Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111–129.