Soil Biology & Biochemistry 68 (2014) 252–262

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape☆

Timothy M. Bowles [a,*], Veronica Acosta-Martínez [b], Francisco Calderón [c], Louise E. Jackson [a]

[a] Department of Land, Air and Water Resources, University of California Davis, Davis, CA 95616, USA
[b] USDA-ARS, Cropping Systems Research Laboratory, Wind Erosion and Water Conservation Unit, Lubbock, TX 79415, USA
[c] USDA-ARS, Central Great Plains Research Station, 40335 County Road GG, Akron, CO 80720, USA

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form
13 September 2013
Accepted 2 October 2013
Available online 17 October 2013

Keywords:
Soil enzyme activities
Soil microbial communities
FAME analysis
Microbial biomass
Organic agriculture
Soil organic matter
Landscape heterogeneity

ABSTRACT

Variability in the activity and composition of soil microbial communities may have important implications for the suite of microbially-derived ecosystem functions upon which agricultural systems rely, particularly organic agriculture. An on-farm approach was used to investigate microbial communities and soil carbon (C) and nitrogen (N) availability on 13 organically-managed fields growing Roma-type tomatoes, but differing in nutrient management, across an intensively-managed agricultural landscape in the Central Valley of California. Soil physicochemical characteristics, potential activities of nine soil enzymes involved in C, N, phosphorus (P), and sulfur (S) cycling, and fatty acid methyl esters (FAMEs) were measured during the growing season and evaluated with multivariate approaches. Soil texture and pH in the 0–15 cm surface layer were similar across the 13 fields, but there was a three-fold range of soil C and N as well as substantial variation in inorganic N and available P that reflected current and historical management practices. Redundancy analysis showed distinct profiles of enzyme activities across the fields, such that C-cycling enzyme potential activities increased with inorganic N availability while those of N-cycling enzymes increased with C availability. Although FAMEs suggested that microbial community composition was less variable across fields than enzyme activities, there were slight community differences that were related to organic amendments (manure vs. composted green waste). Overall, however, the general similarity among fields for particular taxonomic indicators, especially saprophytic fungi, likely reflects the high disturbance and low complexity in this landscape. Variation in potential enzyme activities was better accounted for with soil physicochemical characteristics than microbial community composition, suggesting high plasticity of the resident microbial community to environmental conditions. These patterns suggest that, in this landscape, differences in organic agroecosystem management have strongly influenced soil nutrients and enzyme activity, but without a major effect on soil microbial communities. The on-farm approach provided a wide range of farming practices and soil characteristics to reveal how microbially-derived ecosystem functions can be effectively manipulated to enhance nutrient cycling capacity.

1. Introduction

Agricultural landscapes exhibit a high degree of spatial variability, including variation in soil physicochemical characteristics and agroecosystem management (Drinkwater et al., 1995; Vasseur et al., 2013), which can affect the activity and composition of the soil biota (Acosta-Martínez et al., 2008; Schipanski and Drinkwater, 2012). Soil microbes mediate the biochemical transformations of organic matter that underpin essential ecosystem functions, including decomposition, mineralization of plant available

☆ The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable. USDA-ARS is an equal opportunity provider and employer.

* Corresponding author.
E-mail addresses: tmbowles@ucdavis.edu (T.M. Bowles), Veronica.Acosta-Martinez@ars.usda.gov (V. Acosta-Martínez), Francisco.Calderon@ars.usda.gov (F. Calderón), lejackson@ucdavis.edu (L.E. Jackson).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.10.004

nutrients, and nutrient retention. Organic production relies on these microbially-derived ecosystem functions and thus may be a model system for ecological intensification of agriculture (Jackson et al., 2012). By focusing on building and utilizing soil organic matter (SOM) as opposed to using synthetic fertilizers, organic production systems differ greatly from conventional systems; organic management in many research station trials has been shown to improve soil fertility (Burger and Jackson, 2003; Gattinger et al., 2012), reduce nutrient losses (Drinkwater and Wagoner, 1998; Kramer et al., 2006; Syswerda et al., 2012), and reduce global warming potential (Burger et al., 2005; Cavigelli et al., 2013) while supporting similar crop yields in certain contexts (Seufert et al., 2012).

Yet, such research station-based experiments may belie the challenge of evaluating multiple ecosystem services on working organic farms across actual landscapes that vary in topography, soil type, commodities, and motivations of farmers for making the organic transition (Darnhofer et al., 2005; Williams and Hedlund, 2013). Organic farms also use many different nutrient management strategies (Guthman, 2000; Darnhofer et al., 2010) even when growing the same crop in the same region (e.g. Drinkwater et al., 1995; García-Ruiz et al., 2008). While this heterogeneity could help explain some of the ambiguous results of landscape-scale comparisons of organic and conventional farms relative to site-specific experiments (e.g. Williams and Hedlund, 2013), we lack basic understanding of how heterogeneity affects soil microbial activity and community composition and the implications for soil ecosystem functions and agroecosystem management.

The quantity and quality of SOM and carbon (C) and nitrogen (N) inputs are the overriding controls on soil microbial biomass and activity (Fierer et al., 2009; Kallenbach and Grandy, 2011). Thus, distinct organic amendments (e.g. manure, leguminous cover crops, and composted materials) can stimulate microbial biomass differently through increases in labile organic matter (Marriott and Wander, 2006; Smukler et al., 2008; Kallenbach and Grandy, 2011) and/or total soil C on time frames from months to decades (Drinkwater and Wagoner, 1998; Kong et al., 2005). However, little is known about how the quantity and composition of SOM and nutrient inputs (e.g. C:N ratio) affect microbial communities and their enzyme activities, and in turn, transformations of C, N, phosphorus (P), and sulfur (S) on organic farms. The total enzymatic activity of soil, derived from active microorganisms and the stabilized pool in clay–humus complexes (Tabatabai, 1994; Burns et al., 2013), plays a major role in the depolymerization of structurally diverse polymeric macromolecules, which is considered the rate-limiting step in decomposition and nutrient mineralization potential of soil (Schimel and Bennett, 2004).

Organic management increases overall enzyme activity (Mäder et al., 2002; García-Ruiz et al., 2008; Moeskops et al., 2010), but activities of specific enzymes may change depending on the composition of the amendments and the relative availability of nutrients, as well as other factors, such as soil type and its unique characteristics, e.g. pH and texture (Acosta-Martínez et al., 2007; Sinsabaugh et al., 2008; Štursová and Baldrian, 2010). Given the relatively constrained C:N:P ratios of microbial biomass (Cleveland and Liptzin, 2007), enzymatic activity might be expected to enhance the availability of the most limiting nutrients in order to meet microbial metabolic demands (Sinsabaugh et al., 2008; Allison et al., 2011). For instance, in grassland and forest soils, long-term N fertilization increased the activity of soil enzymes involved in labile C breakdown (Ajwa et al., 1999; Saiya-Cork et al., 2002; Tiemann and Billings, 2010) with similar trends in conventionally-managed agricultural soils (Bandick and Dick, 1999; Piotrowska and Wilczewski, 2012).

Properties of SOM and organic amendments may also influence microbial community composition and in turn, microbial activity and associated ecosystem processes (Fraterrigo et al., 2006; Reed and Martiny, 2013). Increases in the fungal:bacterial ratio have been linked to increases in soil C and the C:N ratio across landscapes (Fierer et al., 2009; de Vries et al., 2012) and in response to organic management (Bossio et al., 1998) as well as various organic amendments, such as conifer-based compost (Bernard et al., 2012) and vetch cover-cropping (Carrera et al., 2007). Other studies have shown increases in phospholipid fatty acid biomarkers for arbuscular mycorrhizal fungi (AMF) in response to composted green waste as well as long-term organic management (Bossio et al., 1998; Moeskops et al., 2010, 2012). While management that supports fungal communities has been suggested as a means of increasing agroecosystem N retention and other functions (de Vries and Bardgett, 2012; Jackson et al., 2012), changes in microbial community composition may be relatively constrained in agricultural landscapes with a legacy of intensive agricultural management (Fraterrigo et al., 2006; Culman et al., 2010), even in response to organic management (Williams and Hedlund, 2013). Indeed, in agricultural soils that are intensively managed, microbial activity tends to change more quickly in response to organic management than community composition (Burger and Jackson, 2003).

The overall objective of this study is to examine how soil physicochemical characteristics and nutrient management practices affect soil microbial activity and microbial community composition in organic agricultural systems, using an on-farm approach with several participating farmers. This study is part of a larger project examining plant–soil–microbial interactions and multiple ecosystem functions across a set of organic farms selected to be representative of the local landscape using geographic information system (GIS) techniques (Bowles et al., ms. in preparation). Thirteen organically-managed fields growing Roma-type tomatoes (*Solanum lycopersicum* L.) were selected in Yolo County, part of the Sacramento Valley of California, an agricultural landscape dominated by high-input conventional agriculture with a diverse array of crops. The focus is on the period of maximal tomato nutrient demand when microbial activity is most important for crop productivity. There were two main hypotheses. First, farm fields would differ in soil microbial biomass and enzyme activities, and these differences would depend on the quantity and composition of SOM as well as other factors related to the type of organic amendments. Second, microbial community composition would be influenced by nutrient management practices but with fewer differences across the fields relative to enzyme activities given the overall lack of diversity in the soil biota in this landscape, which appears to be related to high disturbance and low complexity (Culman et al., 2010).

The specific objectives of this study are to: 1) characterize the variability of soil properties and organic management practices across a number of organically-managed Roma-type tomato fields; 2) determine patterns of soil enzyme activities and fatty acid methyl esters (FAMEs) to indicate microbial community composition and relate them with soil properties and management practices; and 3) consider the implications for microbially-derived ecosystem functions for management of different types of organic farms across this landscape. On 13 organic fields differing in nutrient management practices, soil physicochemical characteristics; microbial biomass C and N; activities of soil enzymes involved in C, N, P, and S cycling; and FAMEs were measured and analyzed with multivariate techniques to model the relationships among these factors. The on-farm approach provided a wide range of farming practices and soil characteristics to reveal how microbially-derived ecosystem functions can be effectively manipulated to enhance nutrient cycling capacity.

2. Materials and methods

2.1. Agroecosystem characteristics

The organically-managed fields in this study were on similar parent material (mixed alluvium) in a 1579 km^2 landscape including all of the arable land in Yolo County, California, which is situated along the western side of the Sacramento Valley. Yolo County has a Mediterranean-type climate with cool, wet winters and hot, dry summers. Annual precipitation in 2011 was 403 mm and the mean maximum and mean minimum temperatures were 21.7 and 7.3 °C, respectively, compared to 462 mm, 23.1 °C, and 8.4 °C for the previous 20 years (CIMIS, 2013). Organic farming has a long history in this area, with roots over 30 years ago (Guthman, 2004), and is relatively widespread and continuing to grow (Jackson et al., 2011). Different land use histories (e.g. history of cultivation, time in organic agriculture) and natural edaphic variability provide a range of soil characteristics.

Roma-type tomatoes are widely grown in this region for conventional and organic markets, and for both processing and direct-marketing to local consumers. The California Certified Organic Farmers (CCOF) directory was used to identify certified organic farming operations growing tomatoes in the study area (CCOF, 2011). CCOF is the primary organic certifier in this region of California (Guthman, 2004). All growers identified from this directory were contacted during winter 2010–11 to assess plans for growing Roma-type tomatoes and to gauge interest in the project. Eight growers expressed interest and we identified a total of 13 fields in which they expected to transplant tomatoes in early April 2011. All fields were transplanted within two weeks of one another. Nutrient inputs varied across farms (Table 1) with two general groups based on primary organic matter amendment (manure or composted green waste). Several farms also used a vetch winter cover crop alone or in conjunction with other amendments and some applied other nutrient sources (e.g. seabird guano, Chilean nitrate, fish emulsion) as a sidedressing or through drip irrigation. Tillage was used on all fields and was of similar intensity. Soil series identified from the SSURGO database are all considered highly productive (Table 1; Soil Survey Staff, Natural Resources Conservation Service, 2011) and had similar mineralogy (Schafer and Singer, 1976).

Table 1
Field management and soil types at the 13 organic tomato fields in Yolo County, California, USA.

Field	Years in organic[a]	Primary organic inputs[b]	Secondary nutrient inputs[c]	Soil type[d]
1	4	Manure	None	Tehama loam
2	8	Manure	None	Tehama loam
3	NA	Manure	None	Capay silty clay
4	3	Vetch	Guano, soluble	Tehama loam
5	8	Manure, vetch	None	Capay silty clay
6	11	Manure, vetch	Guano	Brentwood silty clay loam
7	3	Compost, vetch	Pellets, soluble	Yolo silt loam
8	2	Compost	Pellets, soluble	Yolo silt loam
9	11	Manure, vetch	Guano	Yolo silt loam
10	8	Compost	Chilean nitrate	Yolo silt loam
11	5	Compost	Chilean nitrate	Yolo silt loam
12	26	Compost	Soluble	Yolo silt loam
13	8	Compost	Chilean nitrate	Yolo silt loam

[a] Years since certification (does not include transition years). This information was not available for field 3, but it is certified organic.
[b] Compost and manure were applied in fall 2010, with the exception of field 5, in which manure was applied in early spring prior to tomato transplanting. Winter vetch cover crops were incorporated prior to transplanting. Compost was composted green waste with a C:N ranging from 15 to 18. Manure was poultry manure or poultry litter with a C:N ranging from 9.8 to 15.
[c] Guano refers to seabird guano (12-12-2.5). Pellets were pelletized poultry manure (6-3-2). Chilean nitrate (16-0-0) is $NaNO_3$, a mined mineral product. Soluble refers to solubilized organic fertilizers, especially fish emulsions, which have a range of nutrient concentrations. Guano, pellets, and Chilean nitrate were all applied as a sidedressing close to tomato transplanting. Small amounts (less than 7 kg-N ha^{-1}) of soluble fertilizers are applied through the drip line periodically throughout the growing season.
[d] Tehama loam: fine-silty, mixed, superactive, thermic Typic Haploxeralfs; Capay silty clay: fine, smectitic, thermic Typic Haploxererts; Brentwood silty clay loam: fine, smectitic, thermic Typic Haploxerepts; Yolo silt loam: fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents

2.2. Soil sampling and analyses

Surface soil samples were collected in June 2011. Sampling was timed to coincide with tomato anthesis and early fruit development, a critical phenological and agronomic period in which tomato nutrient demand is high and growers often add supplemental nutrients. Fields were all sampled within two weeks of one another, an average of 68 days after transplanting. In each field, six plots were established at random locations within a 0.25 ha area to monitor soil and plants over the course of the season. An intact soil core (15 cm in diameter, 0–15 cm deep) was removed from each plot in between two tomato plants, situated 15 cm from the centerline of the planting row.

Soil samples were kept on ice until processing within 4 h for different analyses. After thoroughly mixing the soil sample, field-moist soil was used in determination of microbial biomass C (MBC) and N (MBN) within 24 h of sampling (see below). Inorganic N was extracted from moist soils with 2 M KCl and analyzed colorimetrically for ammonium (NH_4^+) and nitrate (NO_3^-; Foster, 1995; Miranda et al., 2001). Olsen P was determined using the methods outlined by Olsen and Sommers (1982) at the University of California Agriculture and Natural Resources (ANR) Analytical Laboratory. Soil pH was determined on air-dried samples using a 1:2.5 soil/water ratio. Gravimetric water content (GWC) was determined by drying at 105 °C for 48 h. Air dried soil samples were sieved to 2 mm, ground, and analyzed for total C and N at the UC Davis Stable Isotope Facility. Particle size was determined by the laser diffraction method according to Eshel et al. (2004). Additionally, a ~50 g subsample was immediately frozen at −80 °C for subsequent analysis of FAME profiles and potential soil enzyme activities (see below).

2.3. Microbial community analyses

MBC and MBN were determined by the chloroform fumigation extraction method (Vance et al., 1987; Wu et al., 1990). Organic C was quantified using a Dohrmann Phoenix 8000 UV-persulfate oxidation analyzer (Tekmar-Dohrmann, Cincinnati, OH) and organic N was quantified using alkaline persulfate oxidation (Cabrera and Beare, 1993). No correction factors were applied. K_2SO_4 extractable organic carbon (EOC) and nitrogen (EON) were calculated as organic C or N, respectively, quantified in non-fumigated samples (Ros et al., 2009).

Soil microbial community composition was characterized using FAME profiles. FAME analysis was performed on a 3-g field-moist equivalent sample using the ester-linked FAME procedure of Schutter and Dick (2000). 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. 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 by dividing each individual

FAME by the total sum of all 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), and actinomycetes (10Me16:0, 10Me17:0, 10Me18:0). Fungal markers included saprophytic fungi (18:1ω9c, 18:2ω6c, and 18:3ω6c) and AMF (16:1ω5c). FAME 20:4ω6c was used as a marker for soil microfauna (protozoa and nematodes) and mesofauna (e.g. Collembola) (Stromberger et al., 2012 and references therein). Bacterial sums were calculated using the Gram+, Gram−, and actinomycete markers; fungal sums were calculated using both saprophytic and AMF fungal markers, and the fungal/bacteria ratio was calculated by dividing the fungal sum by the bacterial sum.

Activities of nine soil enzymes indicative of C-cycling (α-galactosidase, β-glucosidase), C/N-cycling (β-glucosaminidase), N-cycling (aspartase, L-asparaginase, urease), P-cycling (acid phosphatase, alkaline phosphomonoesterase) and S-cycling (arylsulfatase) were evaluated. These enzyme activities were assayed using 1 g of air-dried soil with their appropriate substrate and incubated for 1 h (37 °C) at their optimal pH as described by Tabatabai (1994) and Parham and Deng (2000). Enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation and subtracted from the sample value.

2.4. Statistical analyses

Box plots were graphed using the default settings from the *ggplot2* package in R (Wickham, 2009; R Development Core Team, 2012). The horizontal line is the mean, and upper and lower "hinges" are the first and third quartiles, respectively. Upper and lower "whiskers" extend to the highest or lowest value, respectively, within 1.5 times the inter-quartile range (the distance between the first and third quartiles). Data beyond this range are plotted as points.

Linear relationships between selected soil physicochemical characteristics were tested with the *lm()* function in *R* using field averages (i.e. $n = 13$) in order to examine broad relationships across the fields. Field was considered an explanatory factor (13 levels) in analysis of variance (ANOVA) models with separate analyses for each response variable, including 9 enzyme activities and 9 taxonomic groups compiled from indicator FAMEs. All models were statistically significant at the $p < 0.001$ level. *F*-statistics, i.e. the ratio of variance among fields to variance within fields, derived from these analyses were used to compare the relative magnitude of the field effect for each variable. Hence, greater between field variability for a given enzyme (or taxon) relative to other enzymes (or taxa) is reflected by a larger *F*-statistic.

Principal components analysis (PCA) was performed in the *vegan* package in R (Oksanen et al., 2012) using a correlation matrix. Component scores for each of the six plots within a field were used to generate 95% confidence ellipses around each field using the *ordiellipse()* function within *vegan*. PCA of FAMEs used data expressed as mol percent.

Since the PCA showed patterns among fields for the FAME and soil enzyme data, a constrained ordination technique was then used to evaluate relationships between these data and soil physicochemical factors through redundancy analysis (RDA). RDA combines regression and PCA and allows the direct analysis of how a set of response variables is structured by a set of explanatory variables (Borcard et al., 2011). RDA constrains ordination axes to be linear combinations of explanatory variables. Soil NH_4^+, NO_3^-, and Olsen P were $\ln(x + 1)$ transformed to help correct positive skewing and all variables were standardized prior to analysis. Forward selection of soil physicochemical factors (i.e. 15 variables: soil C and N, soil C:N, clay, silt, sand, pH, Olsen P, MBC, MBN, EOC, EON, NH_4^+ − N, NO_3^- − N, and GWC) was performed independently for each set of response variables (either enzymes or FAMEs) to derive a parsimonious set of explanatory variables based on a double stopping rule of both alpha level ($p < 0.05$) and adjusted R^2 (Blanchet et al., 2008). RDA was performed with the *rda()* function in the *vegan* package.

Canonical variation partitioning (Borcard et al., 1992) was used to determine the relative importance of soil physicochemical properties and FAMEs in explaining variation in soil enzyme activities using adjusted R^2 values to obtain unbiased estimates (Peres-Neto et al., 2006). Soil factors were the same as used in RDA, as identified in the forward selection procedure. The same selection procedure was used to derive a parsimonious set of indicator FAMEs (mol percent) to explain enzyme activities. The analysis was performed in *R* using the *varpart()* function in the *vegan* package. Significance of the fractions (i.e. explained fractions of variation accounted for by the sum of the canonical axes) was tested by partial redundancy analyses and permutational significance tests (1000 permutations).

3. Results

3.1. Soil properties and C and N pools

The 13 organically-managed Roma-type tomato fields had similar soil texture; measurements classified three fields as loams and ten as silt loams (Table 2). Clay content ranged from 9.7 to 21.4% and had a coefficient of variation (CV) of only 20.2 (Table 2). A three-fold range of soil organic C (6.7–20.0 g C kg^{-1} soil) and N (0.8–2.1 g N kg^{-1} soil) occurred across the set of fields, and soil C:N ranged from 8.1 to 9.8 (Table 2). Soil C and N were highly correlated with one another ($p < 0.0001$, $r^2 = 0.95$). Soil pH was near neutral (6.3–7.2) and varied the least of all measured soil properties with a CV of 3.7. Soil NH_4^+, NO_3^-, and Olsen P had the most variation of all measured variables with CVs of 70.8, 127.5, and 76.7, respectively, and were positively skewed, especially NO_3^- (Table 3). Field 4 had the highest level of NO_3^- (44.9 μg-N g^{-1} soil) while field 1 had the lowest (0.2 μg-N g^{-1} soil).

MBC ranged from 67.7 μg-C g^{-1} soil in field 1, to 165.8 μg-C g^{-1} in field 13, but it did not consistently increase across the C gradient (Table 3). Overall, MBC had a positive relationship with soil C ($p = 0.03$, $R^2 = 0.36$), soil N ($p = 0.02$, $R^2 = 0.41$), and silt ($p = 0.05$, $R^2 = 0.31$), although the low R^2 values indicate that none of these variables was highly associated with the variation in MBC. MBN was not significantly related to any soil variable other than MBC ($p = 0.003$, $R^2 = 0.56$) and showed more variability than MBC with a CV of 39.8 vs. 21.9 for MBC. Total FAMEs, which can be considered an alternative measure of microbial population size, had a positive but weak relationship with MBC ($p = 0.05$, $R^2 = 0.31$) and similar associations with soil C and N as MBC. Total FAMEs also had positive relationships with EOC ($p = 0.02$, $R^2 = 0.41$) and EON ($p = 0.02$, $R^2 = 0.40$).

3.2. Patterns of soil enzyme activities

The activities of nine soil enzymes showed different trends across the 13 fields (Fig. 1). Phosphodiesterase activity showed the strongest field effect (*F*-statistic = 27.3); higher *F*-statistic values indicate the relative magnitude of the "field effect", i.e., a higher ratio of variance among fields to variance within a field (see methods section). Six out of nine enzyme activities had an *F*-statistic greater than 20. β-glucosaminidase activity was the least variable of the measured enzymes (*F*-statistic = 8.2), followed by the activities of L-asparaginase (*F*-statistic = 14.1) and urease (*F*-statistic = 19.1). The geometric mean of enzyme activities (Table 2),

Table 2
Soil properties measured at the 13 organic tomato fields in Yolo County, California, USA in the 0–15 cm surface layer (se = standard error).

Field	Total C (g kg^{-1})		Total N (g kg^{-1})		C:N		pH		Clay (%)		Silt (%)		Sand (%)		Soil texture
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	
1	6.7	0.17	0.8	0.03	8.2	0.16	6.7	0.11	13.9	0.80	47.1	2.77	39.1	3.57	Loam
2	9.6	0.23	1.2	0.02	8.3	0.08	6.8	0.10	21.4	0.27	59.3	0.36	19.3	0.56	Silt loam
3	10.7	0.17	1.3	0.03	8.2	0.07	6.7	0.08	19.4	0.12	55.6	0.45	25.1	0.40	Silt loam
4	11.1	0.22	1.4	0.03	8.1	0.09	6.6	0.06	17.6	0.36	61.4	1.30	21.0	1.35	Silt loam
5	11.2	0.22	1.4	0.03	8.1	0.06	6.3	0.06	18.1	0.45	60.1	0.67	21.9	1.04	Silt loam
6	12.5	0.53	1.4	0.07	9.2	0.14	6.3	0.04	16.2	0.19	63.5	0.45	20.3	0.52	Silt loam
7	12.8	0.52	1.4	0.05	9.0	0.10	7.2	0.04	14.6	0.29	52.1	0.59	33.3	0.86	Silt loam
8	13.2	0.38	1.5	0.04	9.0	0.07	6.8	0.02	9.7	0.21	48.4	0.43	41.9	0.49	Loam
9	13.9	0.24	1.6	0.03	9.0	0.15	6.4	0.04	14.0	0.06	58.1	0.26	27.8	0.31	Silt loam
10	16.5	0.33	1.7	0.04	9.7	0.24	6.6	0.06	15.7	0.16	63.8	0.48	20.6	0.62	Silt loam
11	17.1	0.48	1.8	0.04	9.8	0.12	6.9	0.04	17.5	0.16	67.3	0.34	15.3	0.45	Silt loam
12	18.1	0.76	2.0	0.08	9.3	0.05	6.8	0.04	11.2	0.21	47.8	0.40	41.0	0.52	Loam
13	20.0	0.56	2.1	0.05	9.7	0.10	6.5	0.04	16.4	0.38	66.4	1.15	17.3	1.41	Silt loam
Mean	13.3		1.5		8.9		6.7		15.8		57.7		26.4		
CV	28.0		22.1		7.2		3.7		20.2		12.2		35.3		

an indicator of the overall metabolic potential, showed a strong positive relationship with MBC ($p < 0.001$, $R^2 = 0.72$) but weaker relationships with MBN ($p = 0.02$, $R^2 = 0.40$) and soil C ($p = 0.02$, $R^2 = 0.40$).

When soil physicochemical factors were used to constrain the ordination of all nine enzyme activities with RDA (Fig. 2), the model accounted for 65% of the total variation ($p < 0.001$). Based on permutation tests, all explanatory variables retained in the model were significant ($p < 0.05$) in constraining enzyme activities.

Increases in all enzyme activities along axis 1 (42% of total variation, 60% of fitted variation) were positively correlated with MBC, which was more highly associated with this axis than any other variable (Fig. 2a), as indicated by the length and direction of its vector. Axis 2 (14% of the total variation and 20% of the fitted variation) largely differentiated C- vs. N-cycling enzymes, with P- and S-cycling enzymes falling intermediate. Activities of C-cycling enzymes β-glucosidase and α-galactosidase and C/N-cycling enzyme β-glucosaminidase loaded positively on axis 2 and were associated with soil inorganic N, especially NO_3^-, and MBN. Activities of N-cycling enzymes aspartase and L-asparaginase loaded negatively along axis 2 and were associated with soil C and N, soil C:N, and EOC and EON.

Non-overlapping confidence ellipses for most of the fields indicated a unique suite of enzyme activities within each field (Fig. 2b). Clusters of fields reflected the primary organic amendment used. Fields 1 and 2, with the lowest soil C and N and receiving manure, had the lowest values along axis 1 while field 13, with the highest soil C and N and receiving composted green waste, had the highest values. Other fields had similar, positive values along axis 1 (except field 7) but were strongly differentiated by axis 2. Fields 10, 11, 12, and 13, in which composted green waste was applied, had negative values along axis 2, such that increases in N-cycling enzyme activities corresponded with indicators of higher SOM pools. The remaining fields, in which manure or vetch was used (with the exception of field 8), had positive values along axis 2, such that increases in C-cycling enzyme activities corresponded with higher inorganic N and MBN. The pattern of sites and enzyme activities in the RDA resembled that of an ordination by PCA (Supplemental Fig. 1).

3.3. Patterns of FAMEs

Overall, the relative abundance of indicator FAMEs (Fig. 3) was less variable across fields than soil enzyme activities, as reflected by smaller *F*-statistics. Based on relative abundance, markers for Gram+ bacteria showed more variation across fields (*F*-statistic = 11.5) than that of Gram− bacteria (*F*-statistic = 5.4) or actinomycetes (*F*-statistic = 8.2). Markers of saprophytic fungi were generally similar across the fields (*F*-statistic = 3.7) while that of AMF showed more variation (*F*-statistic = 15.2) with the highest

Table 3
Soil nutrient and biological properties measured at the 13 organic tomato fields in Yolo County, California, USA in the 0–15 cm surface layer (se = standard error).

Field	MBC (μg g^{-1})		MBN (μg g^{-1})		Total enzyme activity[a]		Total FAME (nmol g^{-1} soil)		EOC[b] (μg g^{-1})		EON[b] (μg g^{-1})		$NH_4^+ - N$ (μg g^{-1})		$NO_3^- - N$ (μg g^{-1})		Olsen P (μg g^{-1})	
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
1	67.7	4.95	7.2	0.67	28.5	1.26	80.5	3.63	22.9	1.96	2.9	0.45	0.28	0.02	0.19	0.13	20.0	1.25
2	76.1	3.03	4.6	0.79	38.2	1.02	80.6	7.38	32.9	0.94	5.0	0.20	0.38	0.03	0.54	0.08	30.3	2.65
3	142.7	4.91	14.0	1.73	67.3	2.73	114.5	6.48	48.9	4.23	9.5	0.72	0.37	0.03	4.07	1.25	56.5	5.57
4	126.9	11.25	19.2	2.77	61.3	1.83	105.9	5.39	36.6	2.50	4.8	0.37	1.16	0.26	44.91	12.76	29.2	1.11
5	119.5	5.67	9.8	1.22	77.5	3.53	137.6	4.67	44.3	2.16	7.2	0.34	1.19	0.07	10.54	1.56	28.3	3.36
6	122.3	9.41	15.3	0.94	66.5	3.95	118.6	16.09	40.3	4.14	5.8	1.10	0.51	0.03	18.45	8.12	32.9	4.15
7	108.5	4.59	11.7	2.24	49.2	2.87	86.5	5.55	41.4	2.18	8.2	0.65	1.43	0.18	13.12	3.19	29.2	1.78
8	121.6	4.44	23.0	1.43	73.1	3.81	92.7	9.73	41.6	1.75	4.1	0.18	0.34	0.06	4.27	1.81	4.9	0.21
9	122.9	6.53	16.1	0.70	62.6	2.02	100.6	5.37	43.2	2.03	5.8	1.06	0.54	0.12	18.13	7.00	18.3	0.86
10	132.2	7.26	13.5	1.27	62.5	1.23	159.4	8.53	57.8	1.77	10.9	0.29	0.44	0.03	1.48	0.26	16.1	1.80
11	120.4	6.84	12.5	1.38	59.8	2.50	118.5	7.53	57.0	3.37	12.5	0.43	0.24	0.05	2.89	0.55	12.8	1.41
12	100.1	8.88	7.9	1.60	66.8	3.75	132.0	10.76	91.9	4.07	18.0	0.83	0.18	0.03	2.94	0.36	93.1	2.70
13	165.8	11.92	20.0	1.14	83.8	3.39	126.3	10.45	73.0	2.40	12.7	0.52	0.42	0.04	4.21	0.57	15.8	1.03
Mean	117.5		13.4		61.3		111.8		48.6		8.2		0.57		9.67		29.8	
CV	21.9		39.8		24.7		21.2		37.1		52.3		70.8		127.5		76.6	

[a] Total enzyme activity is calculated as the geometric mean of activities of all nine enzymes tested here (García-Ruiz et al., 2008).
[b] EOC: K_2SO_4 extractable organic C; EON: K_2SO_4 extractable organic N.

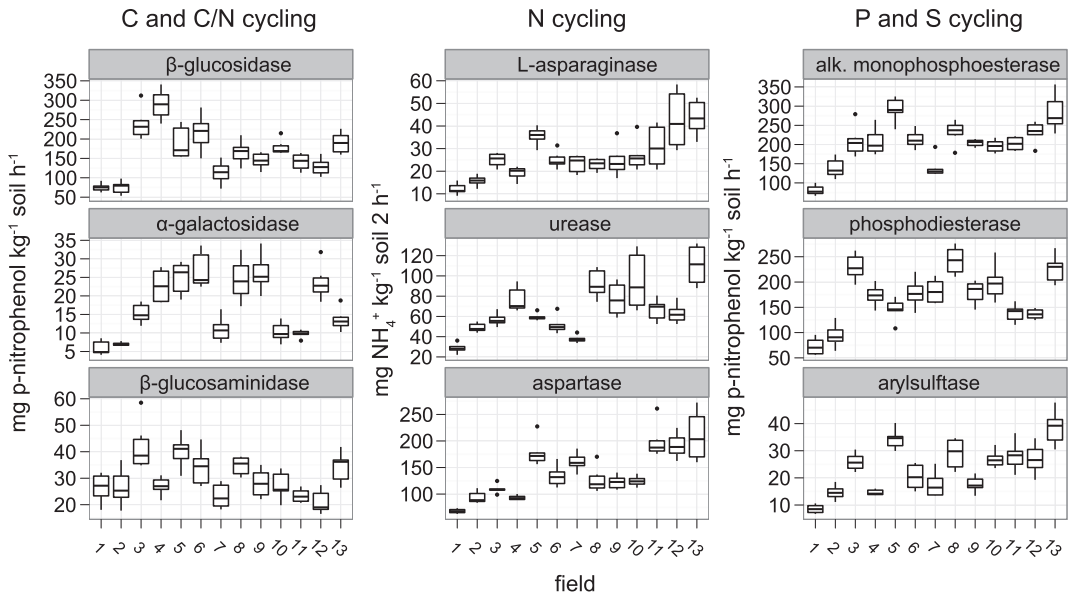

Fig. 1. Box plots of soil potential enzyme activities at the 13 organic tomato fields in Yolo County, California, USA in the 0–15 cm surface layer.

relative abundance in fields 8, 10, 11, 12, and 13. A marker for soil micro/mesofauna showed the most variation of the FAME indicators (F-statistic = 19.4). The sum of all biomarkers for fungi (i.e. total fungi) was relatively consistent across the fields (F-statistic = 3.5), while the sum of all markers for bacteria (i.e. total bacteria) was more variable (F-statistic = 16.9). Biomarkers for total fungi and total bacteria accounted for a mean of 30.1 and 32.2% of the total FAMEs, respectively, which reflected a fungi:bacteria FAME ratio close to one (mean = 0.94) for most fields, except in fields 12 and 13, where it was appreciably lower.

When soil physicochemical factors were used to constrain the ordination of the relative abundance of 14 indicator FAMEs with RDA (Fig. 4), the full RDA model accounted for only 29.3% of the variation ($p < 0.001$). The first two RDA axes accounted for 13.1 and 11.5% of the overall variation and 36.7 and 32.1% of the fitted model (Fig. 4a). All explanatory variables retained in the model were significant ($p < 0.05$) in constraining indicator FAMEs based on permutation tests. Olsen P, GWC, and pH played a role in the dispersion of fields along the first axis, while soil texture variables (clay and silt) and EOC varied mainly along the second axis (Fig. 4b). While it was retained in the selection procedure and significant, MBC did not appear to play a strong role in the ordination of indicator FAMEs, based on the length of its vector. Higher Olsen P was associated with bacterial markers while higher GWC and pH were associated with fungal and micro/mesofaunal markers. EOC was correlated with the gram-positive bacterial marker *i*17:0.

The first axis of a PCA of relative abundances of these indicator FAMEs showed a similar pattern as the RDA, with three out of four fungal markers as well as the micro/mesofaunal marker grouping away from all other markers, while axis 2 did not yield any clear pattern (Supplemental Fig. 2). In general, the fields were more dispersed in the RDA ordination relative to the PCA, reinforcing a role of soil physicochemical factors in explaining variation among fields in the microbial community.

The pattern of FAME biomarkers was also somewhat related to the type of primary organic amendment that was applied in the past year in both the RDA and PCA (Fig. 4b and Supplemental Fig. 2). Fields using manure as the primary organic amendment and several fields using composted green waste formed separate clusters (except field 12), with both groups containing fields that also used a vetch cover crop. The only field using a vetch cover crop without compost or manure clustered with compost.

3.4. Relative influence of soil physicochemical factors and FAMEs on enzyme activities

Canonical variation partitioning showed the relative influence of soil physicochemical factors and microbial community composition on potential enzyme activities by quantifying both the unique and shared proportion of variability accounted for by each set of explanatory factors (Table 4). Soil physicochemical factors and the relative abundance of indicator FAMEs together explained 64.9% of the total variation in soil potential enzyme activities, compared to 37.7% for soil factors alone ($p < 0.001$). Indicator FAMEs uniquely explained only 6.1% of the variation ($p < 0.001$). The remaining variation, which cannot be attributed uniquely to either explanatory dataset, totaled 27.2%.

4. Discussion

This research approach provides insight into how microbial community function and composition respond to the variation that exists across organic farm fields where farmers are growing the same crop in a single landscape. The two hypotheses were supported in the following ways. First, distinct profiles of soil potential enzyme activities indicated unique potential metabolic capacities across the fields, such that C-cycling enzyme activity increased with inorganic N availability while N-cycling enzyme activity increased with C availability. Second, although FAMEs suggested that microbial community composition was less variable across fields than potential enzyme activities, there were slight community differences that were related to the use of compost vs. manure as the primary organic amendment. Overall, however, the general similarity among fields for particular taxonomic indicators, such as saprophytic fungi, is consistent with another nearby study in this intensively managed landscape (Young-Mathews et al., 2010). These patterns suggest that differences in organic agroecosystem management have strongly influenced soil nutrients and potential

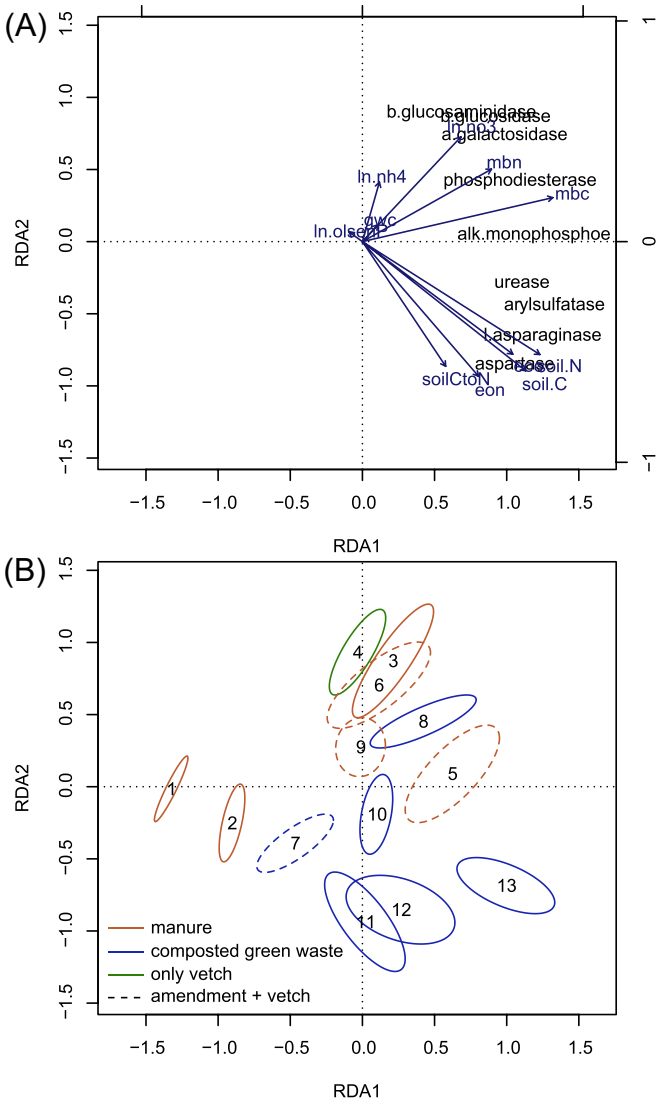

Fig. 2. Redundancy analysis of soil potential enzyme activities constrained by soil physicochemical properties (0–15 cm) at the 13 organic tomato fields in Yolo County, California, USA. A parsimonious set of explanatory factors was generated using a forward selection procedure (see methods; Blanchet et al., 2008). (a) Vectors represent selected soil physicochemical properties. Axes 1 and 2 represent 42 and 14% of the total variation, respectively, with the whole model accounting for 65% of the variation. (b) 95% confidence ellipses (see methods) for RDA field scores (6 per field), numbered by field and colored by primary organic amendment. Dotted lines indicate fields where a vetch cover crop was grown in addition to the organic amendment (either manure or composted green waste) used.

enzyme activity, but without a major effect on soil microbial communities in this landscape. Development of better indicators of microbial functions in organic systems may help farmers evaluate and discover management options that continue to improve the nutrient cycling capacity of the soil.

4.1. Patterns and determinants of soil potential enzyme activity

In this landscape, the majority of the variation in potential enzyme activities could be explained by soil characteristics related to nutrient availability and microbial biomass, which are well-known to be strongly influenced by management on relatively short times scales, as well as soil properties well-known to be influenced by both management and soil type at longer time scales (e.g. soil C and N). Soil variables determined by soil type (e.g. texture) did not contribute to explaining variation in enzyme activities, which may be partly a result of the similar soil types and relatively narrow range of soil textures sampled. MBC was positively correlated with increases in the potential activity of most enzymes, as reflected in the first axis of the RDA, and strongly related to the geometric mean of enzyme activity, an indicator of overall microbial metabolic capacity (García-Ruiz et al., 2008). Most fields showed fairly similar values along this axis, except for fields at opposite ends of the SOM gradient. At the low extreme are fields 1 and 2 with soil C below 10 g kg^{-1} and much lower MBC and enzyme activities than other fields. Below a certain level of MBC, microbial functioning may be reduced. At the high extreme is field 13, with high soil C, MBC, and potential enzyme activities, especially for those involved in nutrient release. The other fields, with mid-range values for soil C and MBC, suggest that a diverse set of soil conditions and nutrient management strategies result in similar overall soil metabolic capacity, albeit with differences in the activity of certain enzymes related to C vs. nutrient cycling processes. Interestingly, this may also be reflected in tomato yields. Nine of the 13 fields had similar yields (104.0 ± 3.6 tons ha^{-1}, mean ± SE) that were above the Yolo County average in 2011 (86 tons ha^{-1}), which included both conventional and organic Roma-type tomatoes (Bowles et al. ms. in preparation).

More subtle patterns in the potential activity of C vs. N cycling enzymes were apparent along the second RDA axis. Microbes regulate extracellular enzyme production to acquire limiting nutrients, so changes in enzyme activities may reflect patterns of microbial nutrient limitations and hence nutrient availability (Allison et al., 2007, 2011; Sinsabaugh et al., 2008; Burns et al., 2013). The strong association among soil inorganic N, particularly NO_3^-, and the activities of C-cycling enzymes (β-glucosidase, α-galactosidase) and a C/N-cycling enzyme (β-glucosaminidase) suggest a shift toward increased C acquisition as N becomes readily available. Other studies have shown increased activity of cellulases (i.e. enzymes that catalyze degradation of cellulose, including β-glucosidase and α-galactosidase in this study) in response to N fertilization (Bandick and Dick, 1999; Sinsabaugh et al., 2005; Piotrowska and Wilczewski, 2012). Reduced activity of enzymes involved directly in N mineralization (e.g. urease and amidase) with higher inorganic N availability has also been shown in agricultural systems (Dick et al., 1988; Bandick and Dick, 1999) and agree with our results of reduced potential activity of L-asparaginase and aspartase in several fields with higher NO_3^- (e.g. field 4). Higher levels of soil NO_3^- were typically found in fields with intermediate levels of soil C and N in conjunction with application of a labile N source (e.g. seabird guano), which was likely rapidly mineralized and nitrified.

In contrast, greater potential activity of two N-cycling enzymes (L-asparaginase and aspartase) but lower activity of C-cycling enzymes occurred in fields with higher soil C and N where composted green waste was applied as a primary organic matter source. In such situations, an abundant supply of diverse C sources may have resulted in N limitation for the microbial community and hence, greater production of enzymes to mineralize N. The high concentrations of EOC and EON and the low concentrations of soil NH_4^+ and NO_3^- in fields 10, 11, 12, and 13 support this hypothesis. EOC and EON are comprised of a diverse array of organic molecules, including free amino acids (Yu et al., 2002; Paul and Williams, 2005) that would include substrates for L-asparaginase and aspartase (Frankenberger and Tabatabai, 1991; Senwo and Tabatabai, 1996). Furthermore, we hypothesize that rapid microbial and plant uptake of mineralized N likely kept soil NH_4^+ and NO_3^- concentrations low, even while the supply rate may have been high, given the activity of these enzymes. High rates of both gross

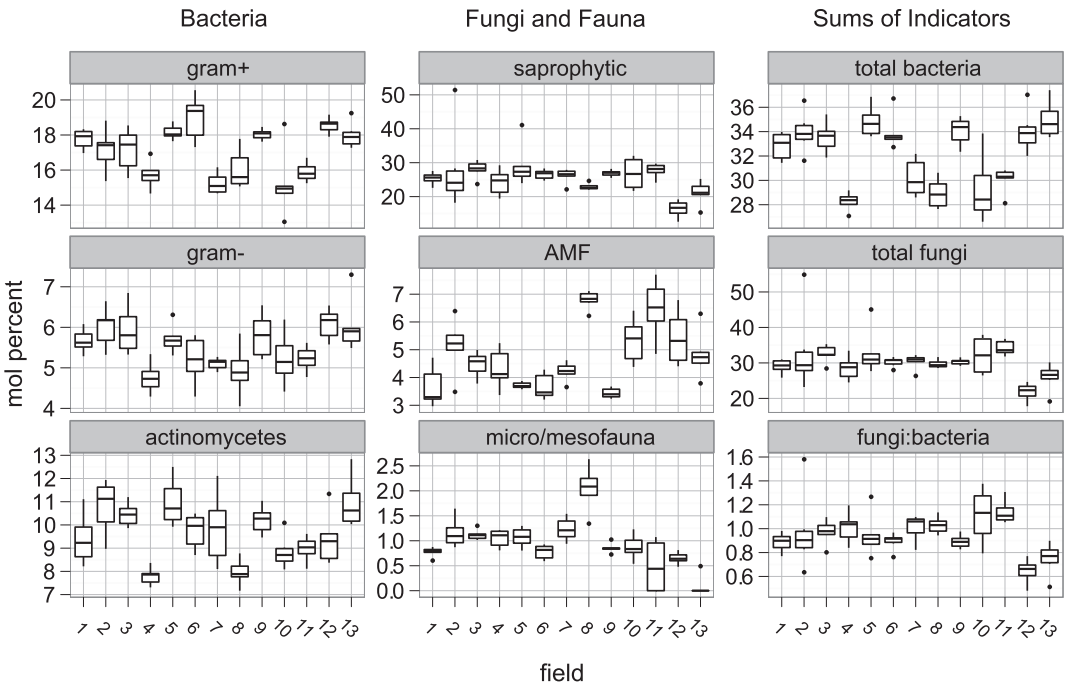

Fig. 3. Box plots of relative abundance of indicator FAMEs in soil from the 0–15 cm surface layer at the 13 organic tomato fields in Yolo County, California, USA. Each group is composed of one or more FAMEs shown to be associated with the particular taxon (see methods).

mineralization and microbial immobilization have been observed in an organic tomato system on similar soil in the same landscape (Burger and Jackson, 2003). An alternative hypothesis is that increased C availability increased denitrification and subsequently lowered soil NO_3^-; however, N_2O emissions over two growing seasons were negligible in a separate case study of an organic Roma tomato field managed by one of the growers involved in this research (Smukler et al., 2010) as well as in other organic tomato systems in this area (Burger et al., 2005). Thus, denitrification was probably low.

The lack of association between P availability, as indicated by Olsen P, and the potential activity of P-cycling enzymes, phosphodiesterase and alkaline phosphomonoesterase, is in contrast with previous work that demonstrates a negative relationship between phosphatase activity and P availability in non-agricultural systems (Olander and Vitousek, 2000; Allison et al., 2007). Across the 13 fields, these enzyme activities appear related to microbial biomass; for instance, phosphodiesterase activity had the strongest positive relationship with MBC of any enzyme ($p < 0.001$ $R^2 = 0.727$), suggesting that soil microbial biomass was more important than P availability in regulating investments in phosphatases across these fields.

The relative importance of microbial community composition vs. environmental factors in regulating enzyme expression remains unclear (Sinsabaugh et al., 2005; Allison et al., 2007; Frossard et al., 2012; Reed and Martiny, 2013). In this study, microbial community composition explained little unique variation in potential enzyme activities relative to soil physicochemical properties. The plasticity of the resident microbial community to respond to environmental conditions may be high, as suggested by the relatively large fraction of variation in potential enzyme activities explained by soil physicochemical characteristics (37.7%). Moreover, a large fraction of variation was also explained jointly by soil factors and FAMEs (~27% of the total variation in the canonical variance partitioning analysis), indicating that microbial communities did influence activity under specific environmental conditions, despite the low variation in community composition across this landscape (see below).

4.2. Patterns and determinants of microbial community biomass and composition

Soil microbial community composition, as measured by FAMEs, was not as strongly differentiated among individual fields as soil potential enzyme activities, based on *F*-statistics that were predominately lower for FAMEs. Rather, FAMEs formed weak clusters in both the PCA (Supplemental Fig. 2) and RDA (Fig. 4) that were associated at least in part with the primary organic amendment used (manure, composted green material, and/or vetch cover crop). The clusters in the PCA and the RDA, as well as the relatively low proportion of FAME variation accounted for in the RDA with the measured soil physicochemical factors (<30%), suggests that unmeasured attributes of the organic amendments may exert strong effects on the microbial community, or that past management is still having effects. Microbial communities unique to the type of organic amendment may also have an inoculating effect and contribute to the differentiation of the microbial community composition (Marschner et al., 2003; Lazcano et al., 2008).

Microbial community composition was associated with factors determined by parent soil type (e.g. clay and silt) as well as those influenced by a combination of soil characteristics and management (e.g. Olsen P and pH). Similarly, Bossio et al. (1998) showed that soil type followed by specific management operations (e.g. cover crop incorporation or manure application) were the primary factors in governing the composition of microbial communities in a cropping system experiment comparing organic and conventional management on similar soils in the same landscape. Of these 13 fields, those that used manure as a primary organic amendment clustered together in the RDA and were associated with increased Olsen P and increases in Gram+ and Gram− bacteria and with decreases in fungal and micro/mesofaunal markers. Increased P availability has been shown to negatively affect fungi in other

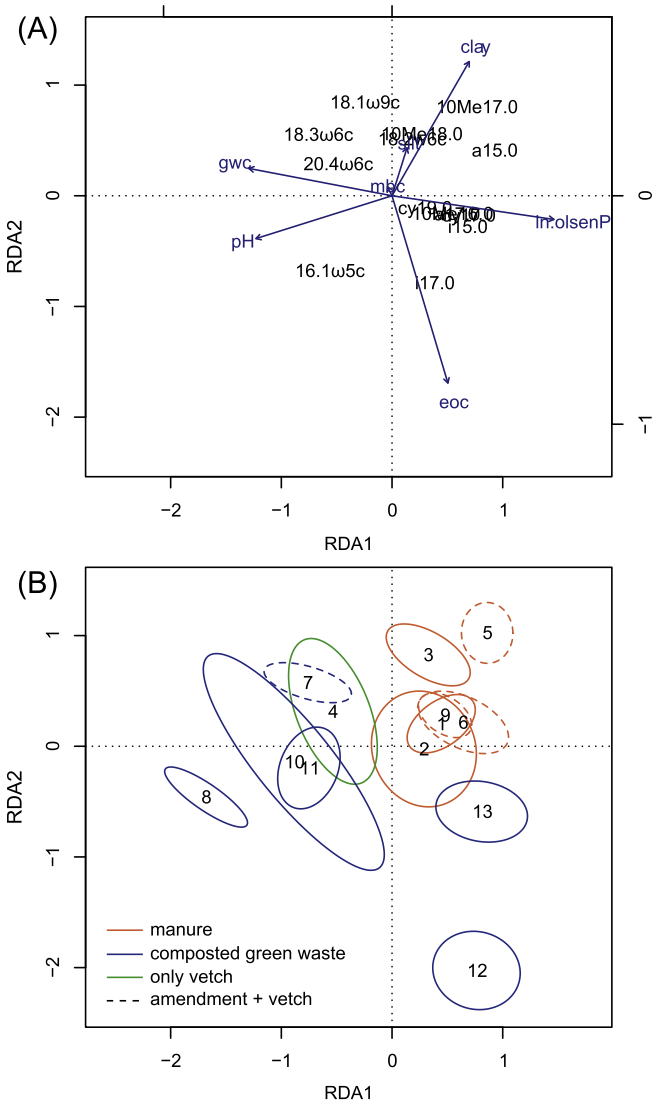

Fig. 4. RDA of indicator FAMEs in soil from the 0–15 cm surface layer at the 13 organic tomato fields in Yolo County, California, USA, constrained by soil physicochemical properties. A parsimonious set of explanatory factors was generated using a forward selection procedure (see methods; Blanchet et al., 2008). (a) Vectors represent selected soil physicochemical properties. Axes 1 and 2 represent 13.1 and 11.5% of the total variation, respectively, with the whole model accounting for 29.3% of the variation. (b) 95% confidence ellipses (see methods) for RDA field scores (6 per field), numbered by field and colored by primary organic amendment. Dotted lines indicate fields where a vetch cover crop was grown in addition to the organic amendment (either manure or composted green waste) used.

agricultural landscapes (Lauber et al., 2008; Williams and Hedlund, 2013) and can result from manure application over time (Clark et al., 1998). Another example of possible management effects is field 12, in which the microbial community was strongly differentiated from other fields. It had been in organic management more than twice as long (26 years) as any other field in the study. High EOC and EON in this field may be indicative of a diversity of organic moieties built up over time with organic management (Aranda et al., 2011) and supportive of a more unique microbial community composition (Giacometti et al., 2013).

Despite differences in management, the relative abundance of FAMEs indicative of saprophytic fungi and the fungi:bacteria ratio was much more consistent across fields than bacterial FAMEs. Disturbance intensity and frequency appear to be lower in landscapes where studies have shown positive relationships between the fungi: bacteria ratio and soil C and soil C:N (Fierer et al., 2009; de Vries et al., 2012). Saprophytic fungi are well-known to be particularly sensitive to certain management practices, especially tillage and fertilization (Minoshima et al., 2007). Tillage intensity was similar across the 13 fields, since these organically-managed farms rely on cultivation as a means of weed control and for incorporating organic amendments. While organic management may increase fungi relative to conventional management (Bossio et al., 1998), routine soil disturbance may represent a strong filter for fungi, such that only a resistant subset persist in arable soils in this area (Calderón et al., 2000; Young-Mathews et al., 2010). In contrast to saprophytic fungi, the relative abundance of a FAME biomarker for AMF, 16.1ω5c, was distinctly different across fields. A significant negative relationship ($p = 0.017$, $R^2 = 0.45$) was found between the relative abundance of this marker and Olsen P when field 12, which has high Olsen P, is excluded from the analysis. This is in line with other studies in agricultural landscapes (Williams and Hedlund, 2013) and reflects the sensitivity of AMF to P availability.

4.3. On-farm approach to microbial community functioning

The on-farm approach used in this study provided a range of SOM characteristics (e.g. a three-fold range of total soil C and N) and organic nutrient management practices to investigate how these factors influence soil microbes and ecosystem functions while controlling for other factors to the extent possible in a real landscape. Narrowing a landscape's extent to a smaller geographic area allowed for sampling generally similar soils, in terms of texture, mineralogy, and parent material, while still encompassing a range of farming strategies. Focusing on the same crop controlled for the effect of plant species and plant functional traits, which also strongly influence the microbial community (Gardner et al., 2011). Since timing (relative to seasonal and agronomic events) can exert a strong influence on enzyme activities and microbial community composition, we carefully sampled during a defined crop phenological period when nutrient demand was maximal (i.e. anthesis to early green fruit stage), which provided insight into soil functions at a crucial time for crop productivity. Furthermore, the fields in this study were planted within a two-week period, and this minimized the differential effects of temperature and rainfall on soil biology and plant growth across fields. Since farmers in this area use irrigation and summers are reliably hot and dry, inter-annual variability may be reduced compared to locations with less predictable summer weather.

Differing nutrient management practices and SOM characteristics across these fields reinforces the need for robust indicators of microbially-derived ecosystem functions to support management decisions, since one-size-fits-all recommendations are not viable in such heterogeneous systems. While MBC would have differentiated fields with apparently compromised soil quality (i.e. fields 1 and 2) from others, it would not have differentiated more subtle variation related to potential activities of C- and N-cycling enzymes, which may have important implications for ecosystem functioning, such as sufficient N availability with low potential for N loss. Differences in enzyme activities in concert with specific C and N pools may eventually be useful to farmers for improving site-specific management to balance these types of tradeoffs. Such research complements nearby research station-based experiments (e.g. Bossio et al., 1998; Kong et al., 2011), which were by design and necessity limited to a relatively narrow set of practices at a single field. In turn, such experiments disentangle the relative effects of individual management practices and examine long-term trends, which can be challenging in a landscape approach. A dynamic interplay between site-specific experimental research and landscape-scale

Table 4
Partitioning variation in soil potential enzyme activities as a function of soil physicochemical factors and indicator FAMEs at the 13 organic tomato fields in Yolo County, California, USA in the 0–15 cm surface layer.

	Percent variation explained[a]	Explanatory variables[b]	Variables retained
Overall model	64.9***	16	
Soil physicochemical factors	37.7***	11	MBC, total N, NO_3^- – N, GWC, total C, MBN, NH_4^+ – N, C:N, Olsen P, DOC, DON
FAME indicators	6.1***	10	20.4ω6c, 16.1ω5c, 10Me18.0, i15.0, a15.0, 10Me16.0, a17.0, cy17.0, cy19.0, 10Me17.0

[a] Variation explained by the full model (i.e. soil factors and FAMEs) consists of both the respective contributions of each set of explanatory variables as well as their covariation. Significance was assessed using 1000 permutations of either the full RDA model or the appropriate partial redundancy analysis model, with $^{***}P < 0.001$.
[b] A parsimonious set of explanatory variables was derived using forward selection based on a double stopping rule of both an alpha criterion and an adjusted R^2 within each set of explanatory variables individually (Blanchet et al., 2008; Borcard et al., 2011).

surveys of working farms may be the most promising route to improving understanding and management of microbial processes for ecological intensification of agriculture.

Acknowledgments

We thank the eight growers in Yolo Co. for collaborating on this project, allowing us to sample their fields, and providing management information. Andrew Margenot, Felipe Barrios-Masias, Amanda K. Hodson, Cristina Lazcano, and Alia Tsang provided field assistance. Steve Culman provided statistical advice. This research was funded by the USDA NIFA Organic Agriculture Research and Education Initiative Award 2009-01415, the UC Davis Graduate Group in Ecology Block Grants, and an NSF Graduate Research Fellowship to T.M. Bowles.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.10.004.

References

Acosta-Martínez, V., Cruz, L., Sotomayor-Ramírez, D., Pérez-Alegría, L., 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. Appl. Soil Ecol. 35, 35–45.
Acosta-Martínez, V., Acosta-Mercado, D., Sotomayor-Ramírez, D., Cruz-Rodríguez, L., 2008. Microbial communities and enzymatic activities under different management in semiarid soils. Appl. Soil Ecol. 38, 249–260.
Ajwa, H.A., Dell, C.J., Rice, C.W., 1999. Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biol. Biochem. 31, 769–777.
Allison, V.J., Condron, L.M., Peltzer, D.A., Richardson, S.J., Turner, B.L., 2007. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. Soil Biol. Biochem. 39, 1770–1781.
Allison, S.D., Weintraub, M.N., Gartner, T.B., Waldrop, M.P., 2011. Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. In: Shukla, G., Varma, A. (Eds.), Soil Enzymology. Springer, Berlin, pp. 229–243.
Aranda, V., Ayora-Cañada, M.J., Domínguez-Vidal, A., Martín-García, J.M., Calero, J., Delgado, R., Verdejo, T., González-Vila, F.J., 2011. Effect of soil type and management (organic vs. conventional) on soil organic matter quality in olive groves in a semi-arid environment in Sierra Mágina Natural Park (S Spain). Geoderma 164, 54–63.
Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471–1479.
Bernard, E., Larkin, R.P., Tavantzis, S., Erich, M.S., Alyokhin, A., Sewell, G., Lannan, A., Gross, S.D., 2012. Compost, rapeseed rotation, and biocontrol agents significantly impact soil microbial communities in organic and conventional potato production systems. Appl. Soil Ecol. 52, 29–41.
Blanchet, F., Legendre, P., Borcard, D., 2008. Forward selection of explanatory variables. Ecology 89, 2623–2632.
Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological variation. Ecology 73, 1045–1055.
Borcard, D., Gillet, F., Legendre, P., 2011. Canonical ordination. In: Numerical Ecology with R. Springer, New York, pp. 153–225.
Bossio, D., Scow, K.M., Gunapala, N., Graham, K., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb. Ecol. 36, 1–12.
Burger, M., Jackson, L.E., 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biol. Biochem. 35, 29–36.
Burger, M., Jackson, L.E., Lundquist, E., Louie, D.T., Miller, R.L., Rolston, D.E., Scow, K.M., 2005. Microbial responses and nitrous oxide emissions during wetting and drying of organically and conventionally managed soil under tomatoes. Biol. Fertil. Soils 42, 109–118.
Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216–234.
Cabrera, M., Beare, M., 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57, 1007–1012.
Calderón, F.J., Jackson, L.E., Scow, K.M., Rolston, D.E., 2000. Microbial responses to simulated tillage in cultivated and uncultivated soils. Soil Biol. Biochem. 32, 1547–1559.
Carrera, L.M., Buyer, J.S., Vinyard, B., Abdul-Baki, A.A., Sikora, L.J., Teasdale, J.R., 2007. Effects of cover crops, compost, and manure amendments on soil microbial community structure in tomato production systems. Appl. Soil Ecol. 37, 247–255.
Cavigelli, M.A., Mirsky, S.B., Teasdale, J.R., Spargo, J.T., Doran, J., 2013. Organic grain cropping systems to enhance ecosystem services. Renew. Agric. Food Syst. 28, 145–159.
CCOF, 2011. Organic Directory. Available at: http://www.ccof.org/members/advanced (accessed 12.01.11.).
CIMIS, 2013. California Irrigation Management Information System. Department of Water Resources. Office of Water Use Efficiency. Available at: http://wwwcimis.water.ca.gov/cimis/welcome.jsp (accessed 14.05.13).
Clark, M.S., Horwath, W.R., Shennan, C., Scow, K.M., 1998. Changes in soil chemical properties resulting from organic and low-input farming practices. Agron. J. 90, 662–671.
Cleveland, C.C., Liptzin, D., 2007. C: N:P stoichiometry in soil: is there a "redfield ratio" for the microbial biomass? Biogeochemistry 85, 235–252.
Culman, S.W., Young-Mathews, A., Hollander, A.D., Ferris, H., Sánchez-Moreno, S., O'Geen, A.T., Jackson, L.E., 2010. Biodiversity is associated with indicators of soil ecosystem functions over a landscape gradient of agricultural intensification. Landsc. Ecol. 25, 1333–1348.
Darnhofer, I., Schneeberger, W., Freyer, B., 2005. Converting or not converting to organic farming in Austria: farmer types and their rationale. Agric. Hum. Values 22, 39–52.
Darnhofer, I., Lindenthal, T., Bartel-Kratochvil, R., Zollitsch, W., 2010. Conventionalisation of organic farming practices: from structural criteria towards an assessment based on organic principles. A review. Agron. Sustain. Develop. 30, 67–81.
de Vries, F.T., Bardgett, R.D., 2012. Plant–microbial linkages and ecosystem nitrogen retention: lessons for sustainable agriculture. Front. Ecol. Environ. 10, 425–432.
de Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H.C., Kattge, J., Bardgett, R.D., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecol. Lett. 15, 1230–1239.
Dick, R.P., Rasmussen, P.E., Kerle, E.A., 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol. Fertil. Soils 6, 159–164.
Drinkwater, L.E., Wagoner, P., 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396, 262–265.
Drinkwater, L.E., Letourneau, D., Workneh, F., van Bruggen, A., Shennan, C., 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. Ecol. Appl. 5, 1098–1112.
Eshel, G., Levy, G., Mingelgrin, U., Singer, M., 2004. Critical evaluation of the use of laser diffraction for particle-size distribution analysis. Soil Sci. Soc. Am. J. 68, 736–743.
Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecol. Lett. 12, 1238–1249.
Foster, J.C., 1995. Soil nitrogen. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, San Diego, pp. 79–87.
Frankenberger, W.T., Tabatabai, M.A., 1991. Factors affecting L-asparaginase activity in soils. Biol. Fertil. Soils 11, 1–5.
Fraterrigo, J.M., Balser, T.C., Turner, M.G., 2006. Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. Ecology 87, 570–579.

Frossard, A., Gerull, L., Mutz, M., Gessner, M.O., 2012. Disconnect of microbial structure and function: enzyme activities and bacterial communities in nascent stream corridors. ISME J. 6, 680–691.
García-Ruiz, R., Ochoa, V., Hinojosa, M.B., Carreira, J.A., 2008. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol. Biochem. 40, 2137–2145.
Gardner, T., Acosta-Martínez, V., Senwo, Z., Dowd, S.E., 2011. Soil rhizosphere microbial communities and enzyme activities under organic farming in Alabama. Diversity 3, 308–328.
Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fliessbach, A., Buchmann, N., Mäder, P., Stolze, M., Smith, P., Scialabba, N.E.-H., Niggli, U., 2012. Enhanced top soil carbon stocks under organic farming. Proc. Natl. Acad. Sci. U. S. A. 109, 18226–18231.
Giacometti, C., Demyan, M.S., Cavani, L., Marzadori, C., Ciavatta, C., Kandeler, E., 2013. Chemical and microbiological soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems. Appl. Soil Ecol. 64, 32–48.
Guthman, J., 2000. Raising organic: an agro-ecological assessment of grower practices in California. Agric. Hum. Values 17, 257–266.
Guthman, J., 2004. Agrarian Dreams: the Paradox of Organic Farming in California. University of California Press, Berkeley.
Jackson, L.E., Wheeler, S.M., Hollander, A.D., O'Geen, A.T., Orlove, B.S., Six, J., Sumner, D.A., Santos-Martin, F., Kramer, J.B., Horwath, W.R., Howitt, R.E., Tomich, T.P., 2011. Case study on potential agricultural responses to climate change in a California landscape. Climat. Change 109, 407–427.
Jackson, L.E., Bowles, T.M., Hodson, A.K., Lazcano, C., 2012. Soil microbial-root and microbial–rhizosphere processes to increase nitrogen availability and retention in agroecosystems. Curr. Opin. Environ. Sustain. 4, 517–522.
Kallenbach, C., Grandy, A.S., 2011. Controls over soil microbial biomass responses to carbon amendments in agricultural systems: a meta-analysis. Agric. Ecosyst. Environ. 144, 241–252.
Kong, A.Y.Y., Six, J., Bryant, D.C., Denison, R.F., van Kessel, C., 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. Soil Sci. Soc. Am. J. 69, 1078–1085.
Kong, A.Y.Y., Scow, K.M., Córdova-Kreylos, A.L., Holmes, W.E., Six, J., 2011. Microbial community composition and carbon cycling within soil microenvironments of conventional, low-input, and organic cropping systems. Soil Biol. Biochem. 43, 20–30.
Kramer, S.B., Reganold, J.P., Glover, J.D., Bohannan, B.J.M., Mooney, H.A., 2006. Reduced nitrate leaching and enhanced denitrifier activity and efficiency in organically fertilized soils. Proc. Natl. Acad. Sci. U. S. A. 103, 4522–4527.
Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. 40, 2407–2415.
Lazcano, C., Gómez-Brandón, M., Domínguez, J., 2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. Chemosphere 72, 1013–1019.
Mäder, P.D.A., Fließbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and biodiversity in organic farming. Science 296, 1694–1697.
Marriott, E.E., Wander, M., 2006. Qualitative and quantitative differences in particulate organic matter fractions in organic and conventional farming systems. Soil Biol. Biochem. 38, 1527–1536.
Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biol. Biochem. 35, 453–461.
Minoshima, H., Jackson, L.E., Cavagnaro, T.R., Sánchez-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 Sci. Soc. Am. J. 71, 952.
Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide: Biol. Chem. 5, 62–71.
Moeskops, B., Buchan, D., Sleutel, S., Herawaty, L., Husen, E., Saraswati, R., Setyorini, D., De Neve, S., 2010. Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. Appl. Soil Ecol. 45, 112–120.
Moeskops, B., Buchan, D., Van Beneden, S., Fievez, V., Sleutel, S., Gasper, M.S., D'Hose, T., De Neve, S., 2012. The impact of exogenous organic matter on SOM contents and microbial soil quality. Pedobiologia 55, 175–184.
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., 2012. Vegan: Community Ecology Package. R Package Version 2.0-5. http://CRAN.R-project.org/package=vegan.
Olander, L., Vitousek, P., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49, 175–190.
Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L. (Ed.), Methods of Soil Analysis, Part 2, Agron. Monogr., second ed., vol. 9. ASA and SSSA, Madison, WI, pp. 403–430.
Parham, J., Deng, S., 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biol. Biochem. 32, 1183–1190.
Paul, J., Williams, B., 2005. Contribution of α-amino N to extractable organic nitrogen (DON) in three soil types from the Scottish uplands. Soil Biol. Biochem. 37, 801–803.
Peres-Neto, P.R., Legendre, P., Dray, S., Borcard, D., 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology 87, 2614–2625.
Piotrowska, A., Wilczewski, E., 2012. Effects of catch crops cultivated for green manure and mineral nitrogen fertilization on soil enzyme activities and chemical properties. Geoderma 189–190, 72–80.
R Development Core Team, 2012. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Reed, H.E., Martiny, J.B.H., 2013. Microbial composition affects the functioning of estuarine sediments. ISME J. 7, 868–879.
Ros, G.H., Hoffland, E., van Kessel, C., Temminghoff, E., 2009. Extractable and dissolved soil organic nitrogen – a quantitative assessment. Soil Biol. Biochem. 41, 1029–1039.
Saiya-Cork, K., Sinsabaugh, R.L., Zak, D., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol. Biochem. 34, 1309–1315.
Schafer, W., Singer, M., 1976. Influence of physical and mineralogical properties on swelling of soils in Yolo County, California. Soil Sci. Soc. Am. J. 40, 557–562.
Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.
Schipanski, M.E., Drinkwater, L.E., 2012. Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. Plant Soil 357, 147–159.
Schutter, M.E., Dick, R.P., 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Sci. Soc. Am. J. 64, 1659–1668.
Senwo, Z.N., Tabatabai, M.A., 1996. Aspartase activity of soils. Soil Sci. Soc. Am. J. 60, 1416–1422.
Seufert, V., Ramankutty, N., Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. Nature 485, 229–232.
Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75, 201–215.
Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264.
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. Agric. Ecosyst. Environ. 126, 168–188.
Smukler, S.M., Sánchez-Moreno, S., Fonte, S.J., Ferris, H., Klonsky, K., O'Geen, A.T., Scow, K.M., Steenwerth, K.L., Jackson, L.E., 2010. Biodiversity and multiple ecosystem functions in an organic farmscape. Agric. Ecosyst. Environ. 139, 80–97.
Soil Survey Staff, Natural Resources Conservation Service, USDA, 2011. Soil Survey Geographic (SSURGO) Database for Yolo Co., California. Available at: http://soildatamart.nrcs.usda.gov (accessed 06.03.11.).
Stromberger, M.E., Keith, A.M., Schimdt, O., 2012. Distinct microbial and faunal communities and translocated carbon in *Lumbricus terrestris* drilospheres. Soil Biol. Biochem. 46, 155–162.
Šťursová, M., Baldrian, P., 2010. Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. Plant and Soil 338, 99–110.
Syswerda, S.P., Basso, B., Hamilton, S.K., Tausig, J.B., Robertson, G.P., 2012. Long-term nitrate loss along an agricultural intensity gradient in the Upper Midwest USA. Agric. Ecosyst. Environ. 149, 10–19.
Tabatabai, M., 1994. Soil enzymes. In: Weaver, R., Angle, J., Bottomley, P. (Eds.), Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 775–833.
Tiemann, L.K., Billings, S.A., 2010. Indirect effects of nitrogen amendments on organic substrate quality increase enzymatic activity driving decomposition in a mesic grassland. Ecosystems 14, 234–247.
Vance, E., Brookes, P., Jenkinson, D., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Vasseur, C., Joannon, A., Aviron, S., Burel, F., Meynard, J.-M., Baudry, J., 2013. The cropping systems mosaic: how does the hidden heterogeneity of agricultural landscapes drive arthropod populations? Agric. Ecosyst. Environ. 166, 3–14.
Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis. Springer, New York.
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. Appl. Soil Ecol. 65, 1–7.
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 Biol. Biochem. 22, 1167–1169.
Young-Mathews, A., Culman, S.W., Sánchez-Moreno, S., Toby O'Geen, A., Ferris, H., Hollander, A.D., Jackson, L.E., 2010. Plant–soil biodiversity relationships and nutrient retention in agricultural riparian zones of the Sacramento Valley, California. Agrofor. Syst. 80, 41–60.
Yu, Z., Zhang, Q., Kraus, T., Dahlgren, R., Anastasio, C., Zasoski, R.J., 2002. Contribution of amino compounds to dissolved organic nitrogen in forest soils. Biogeochemistry 61, 173–198.
Zelles, L., 1996. Fatty acid patterns of microbial phospholipids and lipopolysaccharides. In: Schinner, F., Öhlinger, 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. Biol. Fertil. Soils 29, 111–129.