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Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system

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ABSTRACT

Soil microorganisms play a crucial role in mineralization and breakdown of complex organic compounds in soil. Microbial populations and functional diversity are greatly influenced by quantity and quality of crop residue and other incorporate organic amendments. This study investigated the effect of cover crops (rye or a mixture of rye-vetch) and compost on soil microflora and microfauna under an organic tomato production system. Each cover crop treatment was used in conjunction with or without compost in a split-plot experimental design. Data on soil respiration, microbial biomass, metabolic quotient, and nematode populations were measured at the end of the growing season. Metabolic characteristics of the soil microbial community were determined using 31 C substrates on Biolog-EcoPlate™. Community level physiological profile (CLPP) was assessed by calculating average well color development (AWCD), richness (S), Shannon–Wiener diversity index (H), and evenness (E). Effect of compost was more pronounced on soil respiration than cover crop treatment. Highest microbial biomass was found in the soils amended with rye and compost ($195\text{--}210\ \mu\text{g g dry soil}^{-1}$). Regression analysis between microbial biomass and soil organic matter (SOM) showed strong correlation (R^2 value of $0.68\text{--}0.56$) in two out of the three growing seasons. Calcium, magnesium, and potassium concentrations in soil also positively correlated with microbial biomass. There were significant differences among soils in numbers of plant parasitic, bacterial, and fungal feeding nematodes during the initial years of the study but the differences were not evident later. Shannon–Wiener diversity index was significantly affected by cover crop treatment with rye treatments generally exhibiting higher degree of soil microbial functional diversity. Biolog-EcoPlate™ assay was sensitive to changes in the short-term. Principal component analysis of the Biolog data allowed differentiation of treatments but distribution patterns varied from year to year. We conclude that both rye and rye-vetch mixture can affect the functional diversity of soil microbial community but differences between them are marginal when compared to compost and no-compost treatments. Microbial communities were more responsive to compost applications than cover crop effects.

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1. Introduction

A high and increasing demand for sustainably produced fruits and vegetables have encouraged growers to transition to sustainable and organic production systems (Klonsky, 2004). Such ecologically sound systems have the potential to address a number of ongoing issues in mainstream agriculture namely pollution

due to chemical fertilizers and pesticides, production losses due to pest and disease pressure, soil degradation, loss of soil fertility and productivity. One of the core philosophies of organic production systems is the development of healthy and productive soil that provide essential nutrients for plant growth, supports diverse and active soil biotic communities, and balances the entire farm ecosystem (Insam, 2001; Mäder et al., 2002). Soil biology is directly linked to agricultural sustainability as it is the driving force behind decomposition processes that break down complex organic molecules and substances and convert them to plant available forms (Friedel et al., 2001). Large, stable, and active soil microbial community is an underpinning for sustaining the productivity of soils under sustainable and organic farming systems. To develop such systems growers adopt strategies such as crop rotations, cover cropping, and application of organic amendments (manures and composts)

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that significantly increase soil organic matter (SOM) and improve soil biology and quality (Bending et al., 2002; Buyer et al., 2010).

Cereal rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa* Roth.) (from now on referred to as rye and vetch, respectively) are among the most common cover crops used in regions with temperate climate because of their winter hardiness, the ability to produce considerable biomass, and, in case of vetch, capacity to fix atmospheric nitrogen (Abdul-Baki et al., 1996). When mowed and incorporated these cover crops add SOM, improve soil structure and increase soil biological activity (Carrera et al., 2007; Lundquist et al., 1999). In a three-year study, Buyer et al. (2010) demonstrated that the integration of both rye and vetch cover crop into crop rotations increased soil microbial biomass (SMB) considerably. Along with cover crops, use of compost and manure is considered as an integral component for organic production as it provides essential plant nutrients, adds SOM, and improves soil quality and structure (Russo and Webber, 2007). Application of manure and compost on agricultural lands has been shown to positively increase and enrich soil food web (bacteria, fungi, protozoan and nematode density) and also affect a number of soil characteristics, including SOM, and soil respiration (Carrera et al., 2007; Ferris et al., 2004; Lundquist et al., 1999; Treonis et al., 2010). With increasing number of growers using cover crops and organic amendments in their production systems, it becomes all the more important to better understand the effects of such strategies on soil microorganisms as they are directly involved in organic matter decomposition and nutrient cycling. After soil incorporation, nutrients available in cover crops and organic amendments have to pass through a decomposition pathway which involves a number of soil microorganisms including, bacteria, fungi, and nematodes. Thus, the quality and quantity of plant residues entering the soil can significantly influence soil microorganisms and soil microbial processes (Govaerts et al., 2007). Both crop residue and SOM quality have the potential to increase functional diversity in soil microbial communities (Bending et al., 2002).

Soil contains enormous number of diverse living organisms that influence various ecosystem processes, including formation of organic matter, recycling of nutrients, modification of soil physical and chemical properties, and suppression of pests and diseases (Coleman et al., 1978). Biological characteristics of soil play a vital role in defining soil quality and health. Soil quality is an effective indicator of soil fertility and reflects changes in soil properties which are both inherent and anthropogenic. Soil quality can be estimated and quantified through evaluation of physical, biochemical, or microbial parameters (Glover et al., 2000). A number of soil microbial parameters such as SMB, respiration, metabolic quotient (qCO_2), and community profiles have the potential for use as diagnostic indicators of soil quality. Such indicators have been widely used in discerning changes in soil quality and to make comparisons between different soil types and contrasting management systems (Bending et al., 2004; Schloter et al., 2003).

In this study we compared four organic tomato production systems, which differ, based on plant residues and compost inputs. The specific aim of this study was to investigate the impact of cover crop and compost on soil chemical and biological characteristics and tomato yield. Under different cover crop and compost treatments we evaluated parameters such as SMB, nematode community composition, and microbial diversity of aerobic microbial community that rapidly respond to management systems in a short period of time. A simple approach to measure soil microbial functional diversity is to examine the number of different C substrates that are metabolized by the culturable microbial community. This approach of substrate utilization pattern can be obtained using the Biolog-EcoPlate™ system (Garland and Mills, 1991; Zak et al., 1994). The Biolog-EcoPlate™ system assesses the ability of microbial populations to utilize substrates over time and the speed at which

the substrates are utilized. The technique generates a community level physiological profile (CLPP) of aerobic microbial communities (Garland and Mills, 1991). In our study we hypothesized that: (1) a cover crop mixture of rye–vetch would enhance microbial biomass, and affect nematode counts and microbial functional diversity when compared to a rye only cover crop and (2) application of compost would positively influence the abundance of SMB, improve soil microbial functional diversity, and increase tomato yield.

2. Materials and methods

2.1. Field preparation and production techniques

This study was conducted from 2005 to 2009 at the Horticulture Teaching and Research Center (HTRC), Michigan State University, Holt, MI. The soil was a Capac loam with 0–3% slope. Capac loam is a somewhat poorly drained, moderately to moderately slowly permeable soil formed in loamy glacial till on the low parts of moraines and till plains. The soil at the research site was under transition (starting 2005) from a non-organic corn/soybean rotation to an organic tomato production system. Although the study started with the planting of cover crops in the fall of 2005 and vegetable production in 2006, this paper will focus on results from 2007 to 2009 season when more intensive data were collected. Rye is a standard cover crop used by most organic growers in the region and rye alone cover crop treatment was used as a reference in this research. Addition of vetch and/or compost component was to investigate if the rye alone cover cropping system could be improved. The experimental design was a split-plot design with four replications. The main plot treatment was the cover crop treatment, rye or a mixture of rye and vetch, and the split was the subplot with presence or absence of compost (compost or no-compost). For rye alone treatment, the cover crop was drilled at a rate of 78 kg ha⁻¹ in the fall on 18 September, 22 September, and 26 September in 2006, 2007 and 2008, respectively. Rye–vetch treatment was also seeded on same dates at 39–28 kg ha⁻¹ of rye and vetch, respectively. The following spring, dairy compost at the rate of 25 t ha⁻¹ was broadcast applied to compost treatments. The rate of compost was reduced to 12.5 t ha⁻¹ in 2009 due to higher expected availability of nutrients from compost and to avoid phosphorus build up. The N, P, and K content of the compost ranged from 2.1 to 3.0%, 0.7 to 0.9%, and 2.3 to 2.7%, respectively. Each year, in the spring, before soil incorporation of cover crop, four biomass subsamples (shoot and root) from individual 0.25 m² area were collected and dried at 60 °C until constant weight to determine cover crop dry weight. After compost application cover crops were mowed and incorporated using a chisel plow when rye was at Feekes growth Stage 5 (each year usually in the first week of May) (Weisz, 2011).

Six week-old tomato seedlings (*Lycopersicon esculentum* Mill. 'Mountain Fresh', Seedway, Elizabethtown, PA) were transplanted 28 d after cover crop incorporation on raised beds covered with black plastic mulch and drip irrigation. Each bed was 7.6 m long, 0.6 m wide and 0.2 m high with one row of tomato. Tomato was grown using standard practices for fresh market tomato production in Michigan (staking and tying using the Florida basket weave system). Tomato was harvested five times each year with 3–5 d interval between harvests. Fruits were graded as marketable (U.S. #1, U.S. Combination, U.S. #2, and U.S. #3) or non marketable (deformed, small, cracked, or damaged by cuts, scars, disease, or insects) (USDA, 1991).

2.2. Soil sampling and nutrient analysis

Soil samples were collected during the growing season (August) each year. Four soil cores (15 cm depth) were collected per

treatment from raised beds between individual plants and a composite sample was made. Samples were immediately taken to the lab and stored at 4 °C. Later a part of the sample was dried at 38 °C for 3 d, and ground using a flail grinder for chemical analysis. Soil organic matter was determined by loss of weight-on-ignition method (Combs and Nathan, 1998). A 1N neutral ammonium acetate solution was used to extract calcium, potassium, and magnesium. Analysis of potassium and calcium was carried out by flame emission method and a colorimetric method was used for magnesium (Warncke and Brown, 1998).

2.3. Soil respiration, microbial biomass, metabolic quotient, and nematode population

Soil samples were removed from the cooler (4 °C) and kept at room temperature for 24 h. Soils from each plot were then sieved (2 mm) and visible organic residue and stones were removed. Microbial biomass was determined by chloroform fumigation incubation method (Jenkinson and Powlson, 1976). Six 50 g soil samples from each treatment replication were weighed into beakers. Three of those samples were fumigated with alcohol-free CHCl₃ for 24 h, while the remaining three served as non-fumigated controls. After fumigation, each fumigated sample was inoculated with approximately 1 g of its corresponding non-fumigated soil, thoroughly mixed and brought to 55% water holding capacity. Samples (both fumigated and non-fumigated) were then incubated at 22 °C for 10 d in 1 L air-tight mason jar with rubber septum on the lid. After the incubation period, CO₂ sample was drawn from the septum using 1 mL syringe and injected in to an infrared gas analyzer (Qubit S151 CO₂ analyzer, Qubit System Inc., Kingston, Ontario, Canada). Soil microbial biomass was calculated using the following equation: $1.73F_C - 0.56NF_C$, where F_C and NF_C are the mineralized carbon from fumigated and non-fumigated soil samples (Horwath et al., 1996). Metabolic quotient was derived by dividing soil respiration by SMB.

Nematodes were extracted from 100 g soil using centrifugal floatation technique (Jenkins, 1964). Nematode identification and counting was done by the Plant Diagnostic Laboratory at Michigan State University. Nematodes were separated into food preference groups; plant parasitic (lesion, spiral, and stunt), predatory, bacterial feeding, or fungal feeding based on morphology of the stoma and esophagus.

2.4. Community-level physiological profile (CLPP)

Substrate utilization patterns of culturable soil microbial population was determined using Biolog-EcoPlate™ (BIOLOG Inc., CA, USA) by a procedure adapted from Garland and Mills (1991). Soil samples (10 g field-moist weight) were shaken with 90 mL of sterilized saline solution (0.85% NaCl, w/v) for 60 min and then pre-incubated for 18 h to allow microbial utilization of any soluble organic compound from the soil (Dick et al., 1996). Samples were brought to 10³ final dilution before inoculation. Each Biolog-EcoPlate™ (96-well) consists of three replications, each one comprising 31 sole carbon sources and a blank (control well with water). A 150 µL aliquot was inoculated into each microplate well. The rate of utilization of C sources is indicated by the reduction of tetrazolium dye which changes from colorless to purple. The plates were incubated at 25 °C, and color development in each well was recorded as optical density (OD) at 590 nm with a plate reader (Bio-Rad 680, Bio-Rad Laboratories, Hercules, CA, USA) over a 7 d period (24, 48, 72, 96, 120, 144, and 168 h). Readings obtained soon after inoculation (day 0) were subtracted from subsequent readings to eliminate background color of substrates and the bacterial suspension. Additionally, color response of the control well was subtracted

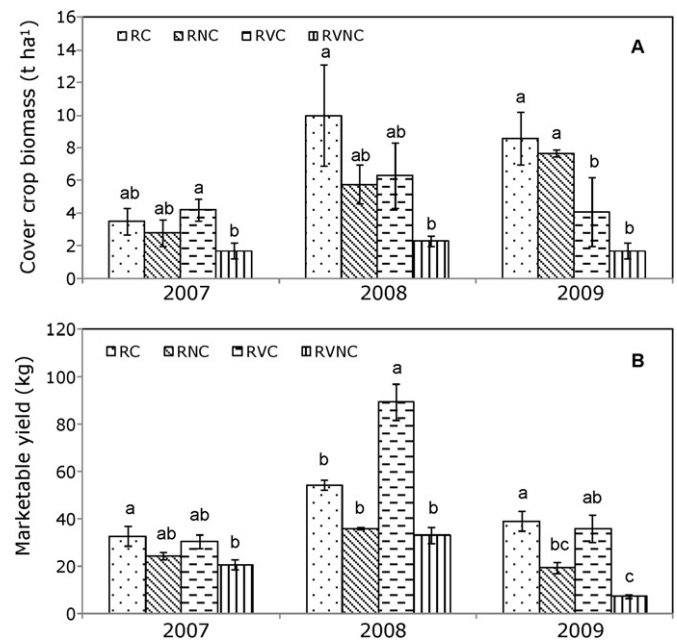


Fig. 1. Cover crop biomass (A) and tomato yields (B) for 2007–2009 growing season. Error bars indicate standard error ($n=4$). RC, rye-compost; RNC, rye-no-compost; RVC, rye-vetch-compost; RVNC, rye-vetch-no-compost.

from the color response of each response well. The average well color development (AWCD) value was calculated for each sample at each time point by using the following equation:

$$AWCD = \frac{\sum OD_i}{31}, \quad (1)$$

where OD_i is the optical density value from each well. Substrate richness (S) was calculated by counting total number of C substrates oxidized by individual treatments on Biolog-EcoPlate™ (counting all positive OD readings). Diversity parameters, such as Shannon–Wiener diversity index (H) and Evenness (E) were calculated using the following equations (Shannon and Weaver, 1969; Zak et al., 1994):

$$H = -\sum p_i (\ln p_i) \quad \text{and} \quad E = \frac{H}{\log S}, \quad (2)$$

where p_i is the ratio of the corrected absorbance value of each well to the sum of absorbance value of all wells. Subsequently, well color responses were normalized by dividing the absorbance values by AWCD to reduce bias between samples due to differences in inoculum densities (Garland, 1997).

2.5. Statistical analysis

Crop yield, soil physical, chemical and biological data were analyzed by PROC MIXED procedure of SAS (Statistical Analysis Systems Institute Inc., Version 9.2; Cary NC). Significant differences among treatment means were separated by 'lsmeans' and 'pdiff' statement in SAS ($P \leq 0.05$ level). Color response data, based on substrate utilization, was further analyzed using principal component analysis (PCA) (XLSTAT Statistical Software, New York, NY). Substrate utilization assay data were analyzed after substrates were divided into six groups and the average absorbance per category was calculated (Zak et al., 1994). All meaningful loadings (>0.5) were included and considered significant in the interpretation of principal components (Manly, 1994). In most cases principal component 1 (PC1) and principal component 2 (PC2) would account for most of the variance. Biplots were constructed to interpret the

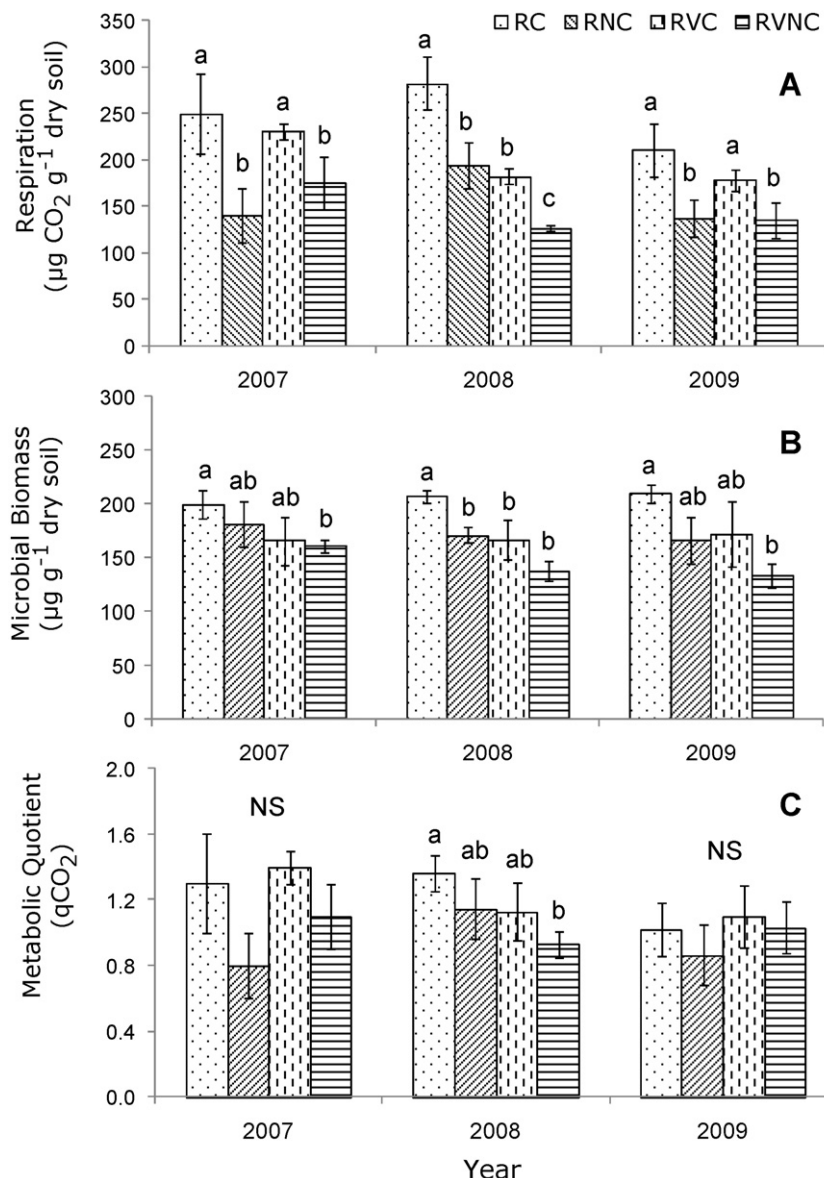


Fig. 2. Soil respiration, microbial biomass, and metabolic quotient in soils amended with rye-compost (RC), rye-no-compost (RNC), rye-vetch-compost (RVC), and rye-vetch-no-compost (RVNC) in 2007, 2008, and 2009. Bars with different letters within a year are significantly different ($P \leq 0.05$).

analysis, with the original variables drawn as vectors to summarize the correlation between the variable and both illustrated axes.

3. Results

3.1. Cover crop biomass and tomato yield

Amount of biomass produced by cover crops varied each year. Over all biomass produced in 2007 was lower than 2008 and 2009 (Fig. 1). Differences in biomass between cover crop treatments were largely due to crop type and compost treatments. Rye-vetch-no-compost treatment consistently performed below average in all the years. This is partly due to low seeding rate of rye in rye-vetch treatment, low fertility, and poor establishment of vetch as a result of late planting dates. Rye-compost treatment produced abundant biomass in 2008 and 2009. Unlike studies that reported higher biomass yield with rye-vetch mixture than their respective monocultures (Kuo and Jellum, 2002; Sainju et al., 2005), rye-vetch

biomass yields were statistically similar to rye alone biomass in 2007 and 2008, but were lower in 2009.

Tomato marketable yield was comparable among treatments, except in rye-compost treatment which had higher yield than rye-vetch-no-compost treatment. The rye-vetch-compost treatment significantly increased yield in 2008. Yield from the treatment was almost double than that of rye-compost treatment. Marketable yields were influenced to a greater extent by compost than cover crop in 2009. Rye-compost and rye-vetch-compost had significantly higher yields than rye-vetch no-compost treatment.

3.2. Soil respiration, microbial biomass, and metabolic quotient

There were significant differences in basal soil respiration (CO_2 evolution) between compost and no-compost treatments in all years, however, effect of cover crop on soil respiration was only significant in 2008 (Fig. 2A). Soil samples from compost amended plots showed higher respiration than non-amended plots. Soil respiration ranged from $126.5 \mu\text{g CO}_2 \text{ g dry soil}^{-1}$ (rye-vetch-no-compost

Table 1Nematode population distribution, affected by cover crop and compost treatment, in 100 cm³ soil samples collected at the end of growing season from 2007 to 2009.

Treatment ^x	Nematode counts ^y				
	Plant parasites	Omnivorous	Predatory	Bacterivore	Fungivore
2007					
RC	69	4	2	83c	18b
RNC	78	4	3	28c	23b
RVC	67	5	2	456a	105a
RVNC	67	4	2	211b	97a
Significance					
Cover crop	NS	NS	NS	***	*
Compost	NS	NS	NS	***	NS
Cover crop × Compost	NS	NS	NS	***	NS
2008					
RC	140b ^z	15	10	45	28
RNC	160b	10	8	35	47
RVC	222a	8	5	40	30
RVNC	255a	18	15	28	30
Significance					
Cover crop	*	NS	NS	NS	NS
Compost	NS	NS	NS	NS	NS
Cover crop × Compost	NS	NS	NS	NS	NS
2009					
RC	170	5	0	78	58
RNC	78	8	0	138	65
RVC	225	10	0	273	70
RVNC	43	13	0	172	30
Significance					
Cover crop	NS	NS	–	NS	NS
Compost	NS	NS	–	NS	NS
Cover crop × Compost	NS	NS	–	NS	NS

^x RC, Rye-compost; RNC, Rye-no-compost; RVC, Rye-vetch-compost; RVNC, Rye-vetch-no-compost.^y Data collected from three replications of 100 cm³ soil each.^z Mean separation within columns for individual years. Means followed by same letter(s) are not significantly different *, **, *** represent significance at $P \leq 0.05$, 0.01, 0.001, respectively.

treatment) to 282.3 $\mu\text{g CO}_2 \text{ g dry soil}^{-1}$ (rye-compost treatment). A difference in respiration due to cover crop treatment was not observed in 2007 and 2009, however, in 2008, soil respiration in rye treatment was higher than rye-vetch treatment.

Microbial biomass significantly differed among soils with cover crop and compost treatments (Fig. 2B). In 2007, the only statistically significant difference was found between rye-compost and rye-vetch-no-compost treatment. In 2008 highest microbial biomass was found in plots treated with rye-compost (195–210 $\mu\text{g g dry soil}^{-1}$) in 2008. In 2009 microbial biomass followed a trend similar to 2007. Metabolic quotient, which is the ratio of soil respiration to microbial biomass, did not differ among the cover crop/compost treatments, except in 2008, where rye-compost treatment had higher $q\text{CO}_2$ than rye-vetch-no-compost treatment (Fig. 2C). Rye-compost, rye-no-compost, and rye-vetch-compost treatments showed similar values. Soil microbial biomass constitutes the active pool of SOM. Soil microbes typically are C-limited (Smith and Paul, 1990) and lower microbial biomass in soils can be explained with low organic C in the soil (Fließbach and Mäder, 2000).

3.3. Nematode community distribution

In 2007, there were no differences in plant parasite, omnivore, or predatory nematode counts (Table 1). Populations of bacterivore nematodes were significantly higher in rye-vetch than rye alone treatments. Between rye-vetch-compost and rye-vetch-no-compost, bacterivore nematodes were higher in the former treatment. Omnivore, predatory, bacterivore, or fungivore nematode counts did not differ among treatments in 2008, however, higher abundance of plant parasitic nematodes was observed in

rye-vetch treatments. By the end of the final growing season (2009), there were no differences in nematode populations among treatments.

3.4. Community-level physiological profile

As expected average well color development increased with incubation period (Fig. 3). Rye-vetch-no-compost treatment consistently exhibited the lowest AWCD at all sampling periods in 2007. Cover crop treatments without compost had significantly lower AWCD in 2008. Rye-compost treatment had the highest AWCD after 72, 96, 120, and 144 h of incubation, followed by rye-vetch-compost treatment ($P \leq 0.05$). After 168 h of incubation, both rye and rye-vetch compost treatments, had similar AWCD which was higher than their no-compost counterparts. In 2009, rye-no-compost treatment constantly had the lowest AWCD throughout the sampling period. Readings from the fifth day of incubation were found most significant and are reported here. The richness index (number of substrates utilized) was lowest in the rye-vetch-no-compost treatment in 2007 (Fig. 4). That difference, however, was not observed in 2008 and 2009. Soil bacterial functional diversity index (Shannon–Wiener diversity index) was significantly affected by cover crop treatment in 2007. Within the two cover crop treatments, there was no difference between compost or no-compost application.

Principal component analysis showed distinct differences among treatments (Fig. 5). The proportion of variation explained by PC1 ranged from 33 to 55%. Principal component loadings, comprising of six categories of Biolog-EcoPlate™ C substrates, contributed towards the spread of variables along PC1 and PC2. During the 2007 season, microorganisms that utilize amides,

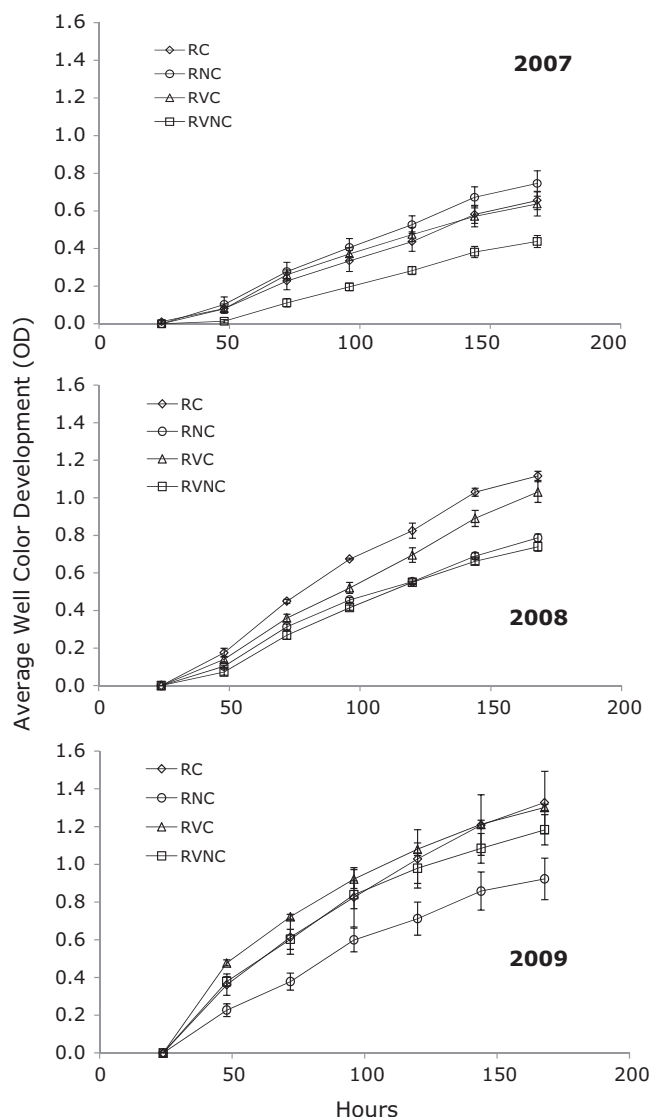


Fig. 3. Average well color development (AWCD) obtained by Biolog-EcoPlate™ incubation of all treatments. Treatments: RC, rye-compost; RNC, rye-no-compost; RVC, rye-vetch-compost; and RVNC, rye-vetch-no-compost. Error bars represent standard error.

carboxylic acids, and polymers influenced the spread of treatments along PC1 axis, while, carbohydrates and carboxylic acids influenced differences along PC2 axis (Table 2). Rye compost treatment increased utilization of carbohydrate substrates while rye-vetch compost treatment increased carboxylic acid utilization (Fig. 5A and B). For the most part similar treatments

clustered around each other in 2007 with exception to rye-vetch-no-compost treatment (Fig. 5A). Rye-vetch compost and rye-vetch no-compost treatments were widely separated from each other on PC1 axis; however, PC1 could not separate compost or no-compost treatments of rye cover crop treatment. In 2008 microorganisms that metabolize amino acids and carbohydrates influenced segregation of treatments on PC1 axis, whereas, those that breakdown polymers and miscellaneous compounds influenced the spread of treatments on PC2 (Table 2). Distinct patterns were visible in 2008, with PC1 separating compost and no-compost treatments, irrespective of cover crop treatment (Fig. 5C). Similar to 2007, majority of compost treatments positively correlated with the miscellaneous loading variable (Fig. 5D). Microbial substrate utilization of all substrates, except carbohydrates, was significant in separating treatments on PC1 axis in 2009; however, only amide and carbohydrate metabolizing bacteria significantly influenced treatment difference along PC2 axis. The spatial pattern of treatments was mixed in 2009, with rye-no-compost treatment placed far apart from all other treatments (Fig. 5E). All treatments except rye-no-compost, positively correlated with all loading variables on PC1 (Fig. 5F). Both PC1 and PC2 separated treatments in all years, but the degree of separation varied.

Distribution along PC2 did not reveal distinct patterns in any of the years. Percent of total variance in data set attributed to PC2 was low and ranged from 23 to 28%. Along the PC2 axis, in 2007, soil microorganisms that utilize carbohydrates and amino acids were positively correlated with rye-compost and negatively correlated with rye-vetch-compost treatment (Fig. 5). On the contrary such an association could not be established in 2009. It is difficult to draw any logical inference from PC2 in 2009 as the distribution of treatments (cover crops/compost) does not follow any set pattern or trend.

4. Discussion

The objective of this study was to understand changes in below ground biology brought out by two very commonly used organic amendments, cover crops and compost, under an organic production system. We focused on rye and arye-vetch mixture, as they are suitable to temperate climatic regions and a widely accepted cover crop system. During the course of our study the amount of cover crop biomass added to the treatments varied between treatments. Although organic growers generally mix rye with a legume cover crop to meet crop N demand, we used a rye-only treatment to simulate grower practice in our region. In general, it produced higher biomass than rye-vetch mixture. This is primarily due to higher seeding rate of rye in rye alone cover crop treatment and sub-optimal establishment of vetch due to severe fall and spring weather conditions. Under Michigan conditions rye can be established even when seeded in October or November. Vetch, on

Table 2
Principal component loadings (correlation between original loading variable and principal component) as a measure of influence of a loading variable on overall treatment differences.

Loading variable	2007		2008		2009	
	PC1 (33%) ^a	PC2 (26%)	PC1 (45%)	PC2 (23%)	PC1 (55%)	PC2 (28%)
Amides	0.85 ^b	−0.38	−0.29	−0.15	0.67 ^b	−0.57 ^b
Amino acids	0.15	0.22	−0.50 ^b	−0.12	0.89 ^b	−0.32
Carbohydrate	0.02	0.93 ^b	0.54 ^b	−0.32	0.32	0.94 ^b
Carboxylic acids	−0.67 ^b	−0.53 ^b	−0.45	0.15	0.88 ^b	0.41
Polymers	0.85 ^b	−0.23	−0.10	0.74 ^b	0.93 ^b	0.26
Miscellaneous	−0.28	−0.41	0.40	0.53 ^b	0.58 ^b	−0.39

^a Percent of total variance in data set, including all cover crop/compost treatments, attributed to principal component 1 (PC1) and 2 (PC2).

^b Factor loadings statistically significant ($P \leq 0.05$). A high positive or negative correlation indicates higher degree of influence of loading variable on differences among treatments determined for a principal component.

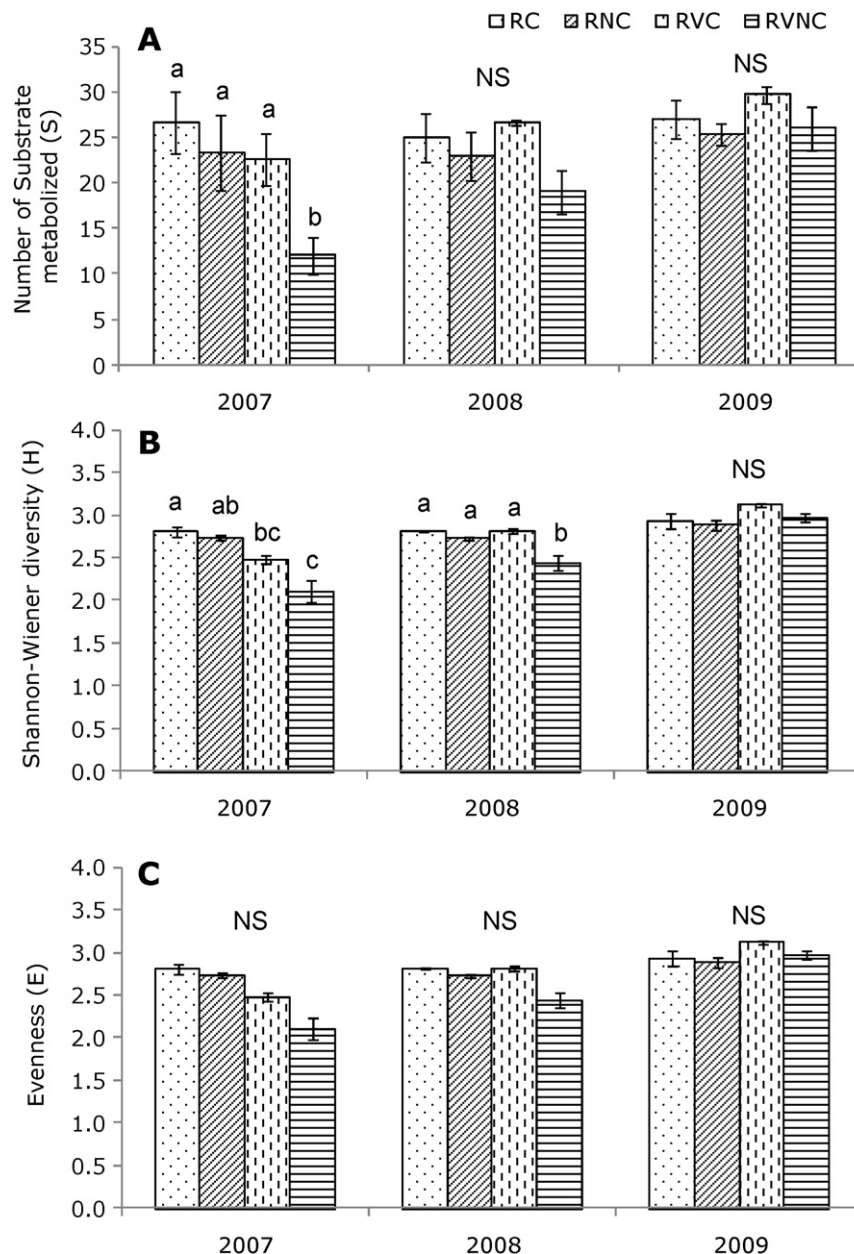


Fig. 4. Bacterial richness (A), functional diversity (B) and evenness (C) indices of soils under cover crop and compost treatments based on community level physiological profile. Treatments: RC, rye-compost; RNC, rye-no-compost; RVC, rye-vetch-compost; and RVNC, rye-vetch-no-compost. Bars with different letters within a year are significantly different ($P \leq 0.05$). NS = means are not significantly different.

the contrary, needs to be seeded in late August or early September for adequate establishment. Therefore, vetch does not establish well when it follows tomato in the rotation since tomato harvest is sometimes not completed until the end of September. Tomato yields were significantly higher in compost amended treatments due to higher soil fertility status. Tomato yields have been shown to respond positively to compost applications (Carrera et al., 2007). Unlike, Teasdale and Abdul-Baki (1998), rye-vetch mixture did not increase yields when compared to rye alone treatment, likely due to the poor vetch establishment and low rye seeding rate in the cover crop mixture. One of the major objectives of integrating vetch in a cropping system is its ability to fix atmospheric N; however, use of vetch with rye without any additional nutrient source, as was the case in this study, was not enough to increase yields. Biculture of vetch and rye cover crop may increase N supply, crop yields, and N-uptake compared with rye (Sainju et al., 2005),

however, cover crop performance and its effect on successive crop can greatly vary depending upon climate, geography and length of growing season (Abdul-Baki et al., 1996).

4.1. Soil respiration, microbial biomass, and metabolic quotient

There was no difference in soil respiration between cover crop treatments (rye or rye-vetch) in 2007; however, within those treatments compost had a significant impact. Soil respiration was high in compost incorporated soils indicating high microbial activity. Respiration was highest in rye-compost treatment in 2008 which could be attributed to higher cover crop biomass added to that system that year (Fig. 1). Other studies have also shown high soil respiration following manure or compost application (Gunapala and Scow, 1998; Treonis et al., 2010; Tu et al., 2006). Soil microbes typically are C-limited (Smith and Paul, 1990), thus, with the addition

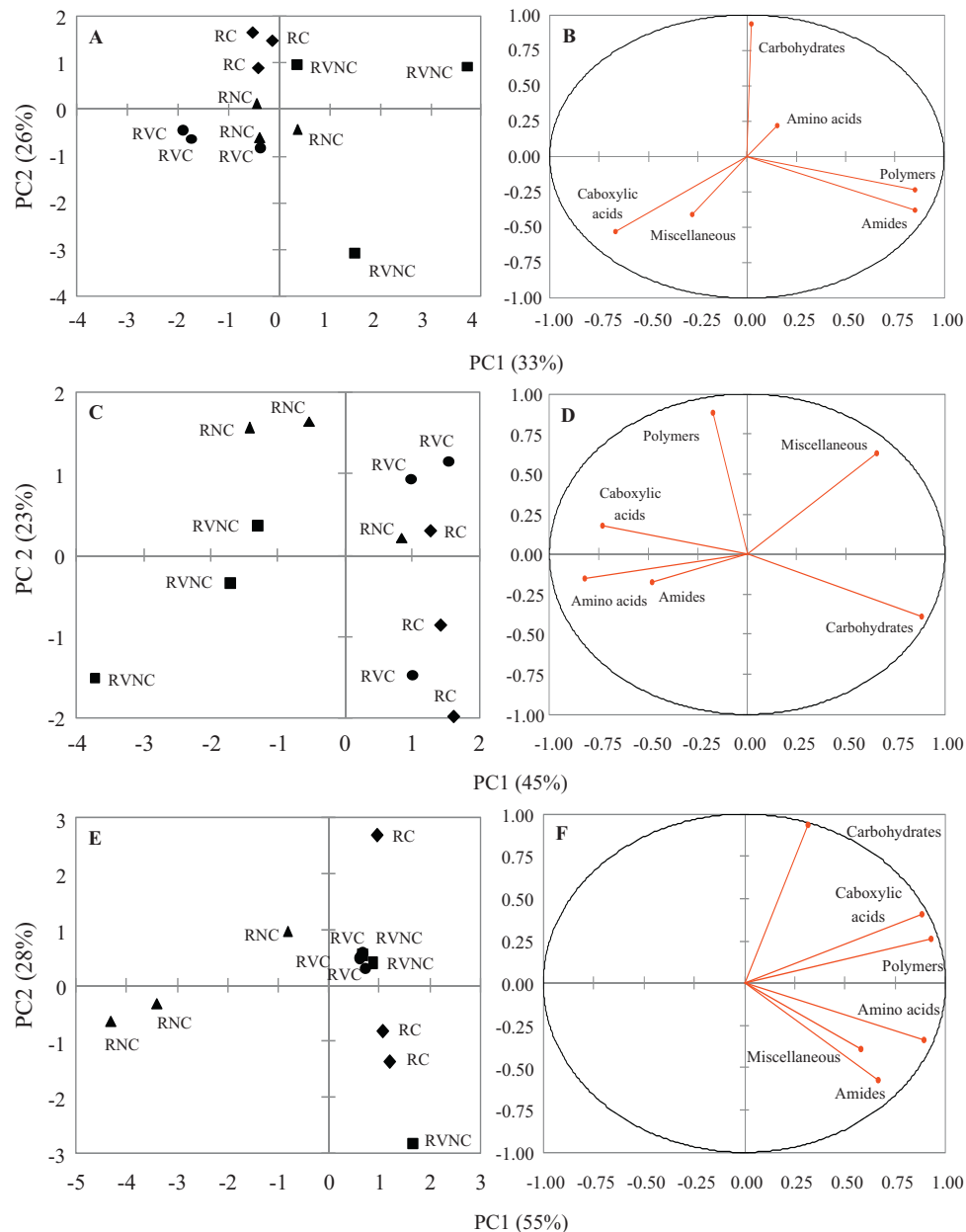


Fig. 5. Principal component analysis of absorbance data from Biolog-EcoPlate™ in 2007 (A and B), 2008 (C and D), and 2009 (E and F). Treatments included rye-compost (RC ♦), rye-no-compost (RNC ▲), rye-vetch compost (RVC ●), and rye-vetch-no-compost (RVNC ■). Loading variables explaining variation based on cover crop and compost treatments. A, C, and E = treatment scatter plot on PC1 and PC2 axis; B, D, and F = loading variables on PC1 and PC2 axis.

of compost a rapid increase in microbial activity and soil respiration occurs due to increased carbon reserves. Fließbach et al. (2007) also reported higher basal respiration in organically managed production systems when compared to unfertilized control plots.

Stable and sustainable soils are defined as those with high level of biological activity, high microbial diversity, and capability to release nutrients from SOM (Friedel et al., 2001; Smith and Paul, 1990). Differences in SMB and activity can directly affect crop nutrient availability. Thus SMB is an effective indicator to predict overall fertility and productivity of a cropping system (Bending et al., 2004). A number of studies have compared soil respiration, microbial biomass, and other microbial properties among contrasting management systems (organic vs. non-organic) and reported significant differences (Bending et al., 2004; Bulluck et al., 2002; Lundquist et al., 1999). In this study, only minor differences, especially for microbial biomass, were found in 2007 and 2009, primarily due

to the overall organic framework of the study with every treatment having a cover crop component. Undoubtedly, differences would still exist, largely due to quantity and quality of plant residue incorporated into the soil. Noticeably, rye-compost treatment had significantly higher microbial biomass in 2008 which can be traced back to high amount of biomass entering that system. Increase in the quantity of organic inputs often result in high microbial biomass (Fließbach and Mäder, 2000). Organic farming systems with compost applications had 34% higher microbial biomass than treatments which did not get any manure application (Fließbach et al., 2007). Soil microbes typically are C-limited (Smith and Paul, 1990) and lower microbial biomass in soils can be explained with low organic C in the soil (Fließbach and Mäder, 2000). Our results support those findings, as we also, in general, observed lower soil respiration and microbial biomass in rye-vetch-no-compost treatment which corresponded with low cover crop biomass production

in that system, partly due to low fertility status (no-compost). Our results indicate a positive correlation between microbial biomass and SOM for all years (data not shown), except 2007. With the addition of cover crops and compost more organic matter is added which facilitates rapid microbial population growth.

An understanding of microbial processes is important for the management of farming systems, particularly those that rely on organic inputs (Smith and Paul, 1990). One of the key variables in microbial processes is qCO_2 . Metabolic quotient is an indicator of microbial C utilization efficiency and increase in its value indicates reduced microbial efficiency. There were clear differences in qCO_2 between rye-compost and rye-vetch-no-compost treatment. Higher organic matter input in rye-compost resulted in increased qCO_2 . Lundquist et al. (1999) also reported increase in qCO_2 with organic matter additions. Effects of fertilizer and manure applications on qCO_2 depend on soil nutrient status, with applications reducing qCO_2 under nutrient stress conditions, and vice versa (Wardle and Ghani, 1995). High metabolic quotient is often associated with stress; however, the validity of such a relationship is still questionable and needs further understanding (Wardle and Ghani, 1995).

4.2. Nematode community distribution

Organic amendments, including cover crops and compost, contribute to improved soil health by enhancing the activity and abundance of decomposer organisms in the soil. Such amendments have a positive impact on increasing SMB and abundance of bacterivore and fungivore nematodes (Bulluck et al., 2002; Ferris et al., 1999; Gunapala and Scow, 1998). We did observe a positive effect of rye-vetch cover crop mixture on population of free living nematodes (bacterivore and fungivore) in 2007. In the rye-vetch cover crop treatment, addition of compost significantly increased the count of bacterivore nematodes likely due to the increase in bacterial density due to compost application (Ferris et al., 1996). The population of free-living nematodes is affected by plant residue types (McSorley and Frederick, 1999). Incorporation of sunhemp (*Crotalaria juncea*), a plant belonging to the same family as vetch, has been shown to increase bacterivore and fungivore nematode populations in soil (Wang et al., 2001). Fungal feeding nematodes have been shown to be in higher numbers when the residue incorporated had high C:N ratio (Villenave et al., 2010). In contrast, we found higher fungivore nematode counts in rye-vetch treatment, which has a reduced C:N ratio, than rye alone in 2007. This difference was not observed in later years.

In 2008 rye alone cover crop treatment (both with and without compost) critically affected plant parasitic nematode counts. Rye has been shown to be a poor host to a number of key nematode pests (McSorley and Gallaher, 1992). In a study conducted on testing the effect of winter cover crops on nematode population levels, Wang et al. (2004), found that cereal cover crops, including rye, decreased numbers of a key nematode pest, *Meloidogyne incognita*, better than the leguminous cover crops during the winter season. Short-chain fatty acids along with other organic acids, found in decomposing rye residues, are toxic to plant parasitic nematodes (Patrick et al., 1965). Cyclic hydroxamic acids found in the family *Poaceae* have also been implicated in the nematicidal properties of rye. In our study, despite the presence of rye, rye-vetch mixture could not reduce the plant parasitic nematode population to the levels obtained by using rye alone as a cover crop. The reason could be the low seeding rate of rye in rye-vetch mixture, which was half of what was used for rye alone treatment. Surprisingly, a positive effect of compost in suppressing plant parasitic nematodes was not seen. Incorporation of animal manure or compost is generally considered to increase the number of nematode antagonistic microorganisms thereby providing nematode suppression, however, a clear relationship between

animal manure, antagonistic microorganisms, and nematode suppression has not been demonstrated (Oka, 2010). The abundance of omnivore and predator nematodes was fairly low in all years. Omnivore and predator nematodes are generally larger than other nematode types. Our research plots comprised of soils with higher clay content, thereby lesser pore space, which may limit the growth of omnivore and predator nematode population.

4.3. Community-level physiological profile

Residue quantity, quality, soil type, environmental conditions, and their complex interactions significantly influence soil microbial functional diversity (Garbeva et al., 2004). Our data show that the functional diversity, indicated by C substrate utilization, was influenced by cover crops in one of the years (2007). Lower level of substrate utilization pattern by rye-vetch-no-compost treatment correlates with low microbial biomass in that system. Shannon–Wiener diversity index varied from year to year. Compost applications did not increase within cover crop treatments in 2007, however in 2008 compost application produced higher functional diversity in rye-vetch-compost than rye-vetch-no-compost treatment. Soil management practices have been shown to be affecting structure and metabolic diversity of soil microorganisms (Bending et al., 2002). Gomez et al. (2006) reported higher microbial diversity in soils amended with two types of vermicomposts or chicken manure than a control soil (without amendment). Although, our treatments differ in residue quality (rye vs. rye-vetch) and presence or absence of compost, in general, after four years of cover crop/compost treatments, CLPP, based on substrate utilization, the Shannon–Wiener diversity index, and Evenness index, had a converging trend. By the end of fourth year, there was no difference in either of those indices. Similarly, Bending et al. (2002) also found significant convergence in microbial community functioning during decomposition of residues which widely differed in quality. In soils with high SOM (in our case ranging from 3 to 4.5%), differences in CLPP due to crop residue tend to converge as residue decomposition progresses (Bending et al., 2002).

Average well color development is an index which reflects microbial density on Biolog-EcoPlate™. In 2008, the application of compost in both cover crop treatments significantly increased AWCD. Biolog substrate utilization assay detects the copiotrophic, or the “r” selected bacteria which would be rapidly affected by high level of organic carbon and nitrogen due to the addition of compost. Compost applications have been previously reported to increase microbial diversity indices possibly as a result of growth and development of diverse microorganisms (Fließbach and Mäder, 1997). The AWCD response in 2009 showed signs of treatment convergence, except for rye-vetch-no-compost, which had significantly lower AWCD. This could be attributed to the low amount of C entering that system due to reduced amount of cover crop biomass incorporated and lack of compost.

Principal component analysis in 2007 was not able to provide a clear interpretation of how microorganisms were affected by our treatments, however in 2008, the analysis clearly separated compost and no-compost treatment regardless of cover crop treatment (rye or rye-vetch). The PCA separation suggests significant impact of compost application on soil microbial activity and functional diversity. This is primarily due to differences in microbial substrate utilization in those treatments. Bucher and Lanyon (2005) had found similar results with annual or biennial applications of dairy manure resulting in soil microbial communities distinguishable from soil that received no manure or occasional applications. In our case, we found that compost treatments on PC1 were positively correlated with two loading variables (or class of substrates on Biolog-EcoPlate™), carbohydrates and miscellaneous. Miscellaneous group of substrates on Biolog-EcoPlate™

comprise of compounds that contain esters and phosphates. It has been established that application of dairy compost, can significantly contribute to higher levels of phosphorus in the soils which in compost treatments, could have favored the growth of microorganisms that actively metabolize phosphorus rich compounds.

Although crop diversity can impact microbial communities due to differences in the quality and quantity of C compounds incorporated entering the soil, influence of cover crop (rye or rye-vetch) on soil microbial diversity was less pronounced than compost treatment. This difference could be due to nature of cover crops which decompose with difficulty at short-term when compared to an organic amendment source like compost (Perez-Piqueres et al., 2006). Buyer et al. (2010), also reported similar effects with minimal differences in soil microbial communities between rye or rye-vetch cover crop treatment. In the current study, compost application was found to be most important in increasing soil microbial biomass and enhancing microbial diversity. The differences in microbial biomass and diversity between compost and no-compost treatment may have implications for nutrient availability to crops. High microbial biomass and diversity often lead to high nutrient availability (Zaman et al., 1999), through enhancing microbial biomass turnover. Compost amendments therefore maintain and enhance the fertility and productivity of agricultural soils, allowing a sustainable land use.

5. Conclusion

Overall our results demonstrate that soil management practices, such as the practice of cover cropping and compost application can enhance soil biological activity. Soil biological properties such as respiration, microbial biomass, nematode population, and microbial functional diversity can be used an indicator of management induced, changes to soil quality. For most soil biological properties evaluated, use of rye or rye-vetch mixture did not lead to major differences; however, the use of compost significantly altered various biological parameters within cover crop treatments. Compost application increased microbial biomass and had a positive impact on soil microbial functional diversity. The results highlight higher microbial activity in soils receiving yearly compost applications. Soil microbial functional diversity, based on CLPP patterns derived from Biolog-EcoPlate™, was not consistent in separating compost and no-compost treatments, however, it is possible that it may take more time to observe changes in community dynamics as soil microbial communities can be relatively robust towards short-term effects (Fließbach et al., 2007).

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