Biol Fertil Soils (2013) 49:803–818
DOI 10.1007/s00374-013-0773-y

ORIGINAL PAPER

Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices

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Received: 6 August 2012 / Revised: 8 October 2012 / Accepted: 17 October 2012 / Published online: 18 January 2013

Abstract No-tillage systems contribute to physical, chemical and biological changes in the soil. The effects of different tillage practices and phosphorus (P) fertilization on soil microbial biomass, activity, and community structure were studied during the maize growing season in a maize–soybean rotation established for 18 years in eastern Canada. Soil samples were collected at two depths (0–10 and 10–20 cm) under mouldboard plow (MP) and no-till (NT) management and fertilized with 0, 17.5, and 35 kg P ha^{-1}. Results show that the duration of the growing season had a greater effect on soil microbiota properties than soil tillage or P fertilization at both soil depths. Seasonal fluctuations in soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N), in dehydrogenase and alkaline phosphomonoesterase activities, and in total phospholipids fatty acid (PLFA) level, were greater under NT than MP management. The PLFA biomarkers separated treatments primarily by sampling date and secondly by tillage management, but were not significantly affected by P fertilization. The abundance of arbuscular mycorrhizal fungi (AMF; C16:1ω5) and fungi (C18:2ω6,9) was lower under NT than MP at the 10–20-cm soil depth in July. Phosphorus fertilization increased soil microbial biomass phosphorus (SMB-P) and Mehlich-3 extractable P, but had a limited impact on the other soil properties. In conclusion, soil environmental factors and tillage had a greater effect on microorganisms (biomass and activity) and community structure than P fertilization.

Keywords Maize–soybean rotation · Microbial biomass · Microbial community structure · P fertilization · Soil enzymes · Soil tillage

Abbreviations

AMF	arbuscular mycorrhizal fungi
MP	moldboard plow
NT	no till
PCA	principal component analysis
PLFA	phospholipids fatty acid
SMB-C	soil microbial biomass carbon
SMB-N	soil microbial biomass nitrogen
SMB-P	soil microbial biomass phosphorus

Introduction

Soil microbiota is a major driver of soil formation, nutrient cycling, and organic matter turnover (Sotomayor-Ramírez et al. 2009), and is a key factor to consider when designing sustainable cropping systems (Yeates et al. 1999). Enrichment of soil quality, in the long-term, will affect the size, composition, and activity of soil microbiota because they are sensitive to environmental factors (Nazih et al. 2001) and agricultural practices, including tillage (Drijber et al. 2000) and fertilization (Beauregard et al. 2010; Bossio et al. 1998; Lalande et al. 2005).

The choice of agricultural practices may lend themselves to different processes or steps to achieve a more diverse and even microbiome (Chaparro et al. 2012). Several studies report that NT increased soil microbial biomass C (SMB-C), N (SMB-N),

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P (SMB-P), and enzymatic activity, which favors soil microbial richness and biodiversity in the surface layers (Aslam et al. 1999; Doran and Linn 1994; González-Chávez et al. 2010; Liu et al. 2008). Moreover, NT was shown to promote fungal growth and slow-growing bacteria (Gram positive (G^+)). Under MP, microbial communities were dominated mostly by aerobic microorganisms with a high metabolism rate (Gram negative (G^-) bacteria) (Feng et al. 2003; Frey et al. 1999; Pankhurst et al. 2002).

Reports on how P fertilization affects soil microbial properties are contradictory. Zhong and Cai (2007) reports that mineral P fertilizer increased microbial biomass and diversity while others found they had no significant effect on the composition of soil microbial communities (Bünemann et al. 2004; Hamel et al. 2006). Cruz et al. (2009) reports differences in microbial community structures for soils fertilized with or without P. The negative impact of P fertilization on the development of arbuscular mycorrhizal fungi (AMF) is well documented (Bilalis and Karamanos 2010; Hu et al. 2009; Shukla et al. 2012). Despite research on how soil microbial properties are affected by tillage and P fertilization, the interactive effect of tillage and P fertilization has not yet been evaluated for their effect on soil microbiota, particularly in terms of microbial biomass, activities and the composition of microbial communities.

Long-term studies are needed to fully understand how agricultural practices affect the evolution of soil ecosystem processes over time (Mitchell et al. 1991). In 1992, a long-term experiment was established at the experimental farm of Agriculture and Agri-Food Canada at L'Acadie, in southwestern Quebec, Canada, to evaluate the effects of tillage and mineral fertilization practices on the soil ecosystem processes and nutrient dynamics under a rotation of soybean (*Glycine max* (L.) Merrill) and maize (*Zea mays* L.).

Over the growing season, developing crops can affect soil microbial dynamics by altering the spatial distribution and quality of organic materials from rhizodeposition and decomposing residues (Franzluebbers et al. 1995). Concurrently, soil microorganisms respond to varying climatic conditions, soil moisture content, porosity, and particularly organic matter content, all of which are interrelated and dependent, in part, on soil management (Bardgett et al. 1999; Hamel et al. 2006; Spedding et al. 2004). By assessing specific soil microbial properties at several intervals over the growing season, soil microbial biomass, activities, and the composition of microbial communities can be monitored to determine their seasonal fluctuations, and the interaction of tillage with P fertilization.

This study sought to discern how soil microbial dynamics are affected by two different tillage management practices and three P fertilization treatments by determining seasonal changes in the soil surface layer of microbial biomass (C, N, and P), specific enzyme activity (dehydrogenase, alkaline phosphomonoesterase), and microbial community structure (phospholipids fatty acid [PLFA]) during the maize growing season of a long-term maize–soybean rotation.

Materials and methods

Description of the experimental site

The experiment was established in 1992 at the L'Acadie Experimental Farm of Agriculture and Agri-Food Canada in southwestern Quebec, Canada (45°18′ N, 73°21′ W). The soil was a clay to clay loam (Humic orthic gleysol, typic haplaquept) with 364 g clay kg^{-1} and 204 g sand kg^{-1} in the Ap horizon (Messiga et al. 2011).

This long-term experimental site consisted of a completely randomized split-plot design with two tillage practices: moldboard plow (MP) and no-till (NT) as main plots, and mineral N (0, 80, and 160 kgN ha^{-1}) and P (0, 17.5, and 35 kg P ha^{-1}) fertilizers set out in a factorial as subplots. From the onset of the experiment, crop residues were left on the soil surface after harvest. The treatments were replicated 4 times for a total of 72 subplots, each measuring 25×4.6 m. Details on treatment management practices are described in Shi et al. (2012). In 2010, maize (Pioneer 38M58; 2800 corn heat units) was sown at 74×10^3 plants ha^{-1} on May 12. Each plot comprised of six rows with 0.76 m between rows. Nitrogen treatments, as urea, were first band-applied (5 cm from the seeding row) at planting with 0, 48, and 48 kgN ha^{-1}, and completed at sidedress (June 20) with 0, 32, and 112 kgN ha^{-1}. The P treatments, as triple super-phosphate, were band-applied as a single application at planting. Glyphosphate (0.89 kg ae ha^{-1}) was used to control weeds. Air temperature and daily precipitation data were obtained from a weather station less than 1 km from the experimental site (Environment Canada 2012).

Soil sampling and chemical analysis

Soil samples (composite of five cores, 2.0 cm diameter) were collected at pre-planting (April 30), in mid-season (July 5), and prior to maize harvest (October 12) at 0–10 and 10–20 cm depths from the experimental plots that received 0, 17.5, and 35 kg P ha^{-1} and 160 kgN ha^{-1} for a total of 48 samples. The soil samples were stored at 4 °C until analysis finished; all analyses were completed within 4 weeks.

Soils for chemical analysis were air-dried and sieved to<2 mm. Soils were extracted with the Mehlich-3 solution (Mehlich 1984), a method recommended for eastern Canada (CRAAQ 2003), and P in the extracts was determined by colorimetry using the ascorbic acid–molybdate reaction (Murphy and Riley 1962). Soil pH was measured in distilled water with a 1:2 soil to water ratio (Hendershot et al. 2007).

Total C and N were determined by dry combustion on 0.20 mm ground soils with a LECO CNS-1000 analyzer (LECO Corp., St. Joseph, MI). Soil bulk density determinations were made in triplicate per plot as described by Lamontagne et al. (2001).

Soil microbial biomass C, N, and P

The SMB-C and SMB-N were determined using the chloroform fumigation-K_2SO_4 soil extraction method of Voroney et al. (1993) with minor modifications (Shi et al. 2012). The K_2SO_4-extracts were quantified for SMB-C by an automated combustion total organic C (TOC) analyzer (Model Formacs, Skalar Analytical, De Breda, The Netherlands). The difference in C content between fumigated and non-fumigated samples was corrected using a K_{EC} factor (the percentage of total microbial C extracted by K_2SO_4) of 0.45 to estimate SMB-C (Wu et al. 1990). The SMB-N was determined by autoclaving the K_2SO_4-extracts at 121 °C for 30 min to oxidize NH_4^+ to NO_3^- (Cabrera and Beare 1993), which was then quantified with an automated continuous-flow injection colorimeter using (Model QuickChem 8000 FIA+, Lachat Instruments, Loveland, CO) following the cadmium-copper reduction procedure (method 12-107-04-1B). The SMB-N was calculated as the difference in total N content between fumigated and non-fumigated samples using an extraction efficiency factor (K_{EN}) of 0.54 (Brookes et al. 1985).

The SMB-P was determined according to Brookes et al. (1982) as detailed in Shi et al. (2012). Three sets of soil including non-fumigated, fumigated, and P-spiked were extracted with $NaHCO_3$ and the inorganic P content in all extracts was determined by spectrophotometry at 882 nm wavelength on a U-1000 spectrophotometer (Hitachi Ltd., Tokyo, Japan) using the ascorbic acid–molybdate reaction (Murphy and Riley 1962). The SMB-P concentration (mg P kg^{-1} dry soil) was calculated as described in Shi et al. (2012).

Enzyme activity

To study the enzyme activity, we measured the dehydrogenase activity as an index of microbial activity and the alkaline phosphomonoesterase activity as an index of organic P mineralization (Dick 1997; Nannipieri et al. 2011). Dehydrogenase activity was determined by the reduction of 2,3,5 triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) using the method of Casida et al. (1964) as reported in Shi et al. (2012). Briefly, field-moist soil was incubated in the dark with TTC for 24 h at 25 °C without shaking. The TPF was extracted with methanol by filtration through a Whatman 934-AH glass microfiber filter and quantified by spectrophotometry (U-2010, Hitachi Ltd., Tokyo, Japan) at 485 nm, and expressed as μg TPF g^{-1} dry soil h^{-1} using the standard calibration curve of TPF (Sigma-Aldrich, Oakville, ON).

Alkaline phosphomonoesterase activity was determined as described by Shi et al. (2012). Briefly, soil samples were incubated with *p*-nitrophenol phosphate (Sigma 104) at 25 °C for 1 h, and the *p*-nitrophenol released by the enzyme was filtered through a Whatman #5 filter paper and then measured by spectrophotometry at 420 nm. Data were expressed as μg *p*-nitrophenol g^{-1} dry soil h^{-1} using the standard calibration curve of *p*-nitrophenol (Sigma-Aldrich, Oakville, ON).

Phospholipids fatty acid analysis

The PLFA profile analysis was carried out as described by Shi et al. (2012). Briefly, total lipids were extracted from fresh soil samples using methanol:chloroform (70:30 vol/vol), according to Bligh and Dyer (1956) and Kolarovic and Fournier (1986). Dried filtrate was fractionated into neutral, glyco, and polar fractions using chromatography on silicic acid (Zelles and Bai 1993). The fatty acid methyl esters were got from the polar lipid fraction (White et al. 1979). Each sample received methyl nonadecanoate fatty acid (Sigma Aldrich, Oakville, ON) as an internal standard and samples were dried and stored until analysis at −18 °C. Gas chromatography analysis was conducted using a Varian 3900 equipped with a flame ionization detector (FID) and a CP-8400 auto-sampler, according to Beauregard et al. (2010).

Peaks were identified and quantified as reported in Shi et al. (2012). Bacterial and fungal fatty acids were identified according to the Supelco standard based on the International Union of Pure and Applied Chemistry system as reported in Lalande et al. (2005), while the AMF (C16:1ω5) and fungal (C18:2ω6,9) markers were named as follows: total number of C followed by a colon, the number of double bonds, the symbol ω, and the position of the first double bond from the methyl end of the molecule.

Individual fatty acids have been used as signatures for various functional groups of microorganisms (Zelles et al. 1995). Biomarkers 2OH-C12:0, 2OH-C14:0, 3OH-C14:0, 2OH-C16:0, C14:0, a-C15:0, i-C15:0, C15:0, C16:0, i-C17:0, $C17:0^{\Delta}$, C17:0, C18:0, C18:1c, and C18:1t were chosen to represent bacteria (Bardgett et al. 1999; Bossio et al. 1998; Grayston et al. 2001). The biomarkers C18:2ω6,9 and C16:1ω5 were chosen to identify fungus (Frostegård and Bååth 1996) and AMF, respectively. The latter marker was successfully used in several studies (Balser et al. 2005; Beauregard et al. 2010; Helgason et al. 2010) in spite of its presence in some gram negative (G^-) bacteria (Olsson 1999). Total PLFA represents all the bacterial biomarkers plus the fungal and mycorrhizal markers.

Statistical analysis

All data were converted to area unit by bulk density except pH and moisture content. All the converted data were subjected to Bartlett's test for homogeneity of variance of residuals and were log transformed to achieve normality of distribution for the analysis of variance. The analysis of variance was performed with the PROC Mixed Procedure of SAS (SAS Institute 2004) to evaluate the effects of sampling date, tillage, P fertilization, and their interactions for the properties at each soil depth. When treatment effects were significant ($P \leq 0.05$), the LSD procedure was used to identify differences among treatment means. The PLFA (relative abundance) as affected by sampling date, tillage, and P fertilization in each soil depth, was analyzed further with SAS software using principal component analysis (PCA) after standardizing the data (SAS Institute 2004). Correlations between soil chemical (pH, Mehlich-3 P, total C and N, moisture content) and microbial (biomass, activity, and community) properties were determined in MP and NT by a redundancy analysis using the library vegan (Oksanen et al. 2010) in R (2012).

Results

Climatic conditions

The growing season of 2010 was warmer and generally wetter than the long-term 16-yr (1995–2010) average, although July was slightly drier with 80 mm of precipitation compared with 105 mm for the long-term average. Maximum air temperature at time of sampling was 19 °C on April 30, 34 °C on July 5, and 12 °C on October 12.

Effect of sampling date

Sampling date had a greater influence on soil properties than tillage and P fertilization at both soil depths (0–10 and 10–20 cm; Table 1). Overall, the July samples differed from the two others; in particular, warm and dry weather conditions combined with an actively growing crop to substantially decrease the soil moisture content (Fig. 1).

All three microbial biomasses (SMB-C, SMB-N, and SMB-P) were significantly affected by date at both soil depths (Table 1, Fig. 2). The interaction of sampling date with tillage significantly affected SMB-C and SMB-N at the 0–10-cm soil depth, with values the greatest in April and least in July for NT; little variation was observed for MP. The content of SMB-P was generally greater in October and April than in July at the 0–10-cm soil depth. At the 10–20-cm soil depth, SMB-C significantly decreased from April to July and kept constant until October with no P or 35 kg P ha^{-1} and kept constant all the time with 17.5 kg P ha^{-1} under MP, but was least in July under NT with all P application rates; whereas the amounts of SMB-N were least in July in NT, and no significant seasonal fluctuation was observed in MP. At the same soil depth, there was a marked increase in the content of SMB-P at the end of the season for the 35 kg P ha^{-1} treatment under MP; lower values were observed for the other P treatments. Under NT, slight increases were obtained over time but only for the P0 and P17.5 treatments.

Dehydrogenase and alkaline phosphomonoesterase activity varied over the growing season at both soil depths, and increased without tillage (Table 1; Fig. 3). Both enzyme activities were more negatively affected by the low soil moisture condition in July under NT than MP, particularly at the 0–10-cm soil depth.

All phospholipids data (AMF, bacterial, fungal, and total PLFA) were significantly affected by sampling date at both soil depths (Tables 1 and 2). Bacterial, fungal (C18:2ω6,9), and total PLFA levels were least in July for both tillage systems (MP and NT) and depths. The level of AMF biomass (C16:1ω5) was also least in July for both tillage systems at the 0–10-cm depth, but it did not vary significantly during the season under MP at the 10–20-cm depth. No significant variation of the fungal to bacterial (F/B) ratio was observed at the 0–10-cm depth. The interaction of sampling date with P fertilization significantly affected the F/B ratio at the 10–20-cm depth, which was least in July with no P, kept constant with 17.5 kg P ha^{-1}, and was least in April with 35 kg P ha^{-1}.

Sampling date significantly affected soil pH, moisture content, and total C at both soil depths (Tables 1 and 2; Fig. 1). However, fluctuations of these properties were more apparent under NT than MP at the 0–10-cm depth. Seasonal variations in soil Mehlich-3 P were also observed under MP and NT at both soil depths. Sampling time in interaction with P fertilization showed that concentrations of Mehlich-3 P substantially increased in October with P treatments, compared with July, especially under NT at the 10–20-cm depth.

Effect of tillage

Tillage had a greater effect on soil microbial and chemical properties at the 0–10-cm soil depth than at the 10–20-cm depth (Table 1). Tillage significantly affected all properties, except for F/B ratio, pH and total N at the 0–10-cm depth, but only significantly affected SMB-C, F/B ratio and Mehlich-3 P at the 10–20-cm depth.

At the 0–10-cm depth, when compared with MP, NT generally increased soil pH, moisture content, Mehlich-3 P, SMB-C, SMB-N, SMB-P, both enzyme activities, AMF, and bacterial, fungal, and total PLFA in April and October, but not significantly in July (Figs. 1, 2, and 3; Table 2). For

Table 1 Analysis of variance for the effects of tillage, P fertilization, and sampling date on soil microbial biomass C, N, and P, dehydrogenase, alkaline phosphomonoesterase, phospholipid fatty acids (bacterial, fungal, AMF, and total PLFA) and selected chemical properties at 0–10 and 10–20 cm soil depths

Source of variation	SMB-C[a]	SMB-N	SMB-P	Dehydrogenase	Alkaline phosphomonoesterase[b]	AMF PLFA	Bacterial PLFA	Fungal PLFA	Total PLFA	F/B ratio	pH	Moisture content	Total C	Total N	Mehlich-3 P
0–10 cm															
Tillage (T)	0.001	0.020	<0.001	<0.001	0.018	0.002	0.007	ns	0.006	ns	ns	<0.001	0.029	ns	<0.001
P fertilization (F)	ns	ns	<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.028	ns	<0.001
T×F	ns	ns	0.003	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	<0.001
Sampling dates (D)	0.012	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	ns	<0.001	<0.001	0.004	0.002	<0.001
T×D	0.001	<0.001	ns	<0.001	<0.001	0.013	0.020	0.013	0.037	ns	0.029	<0.001	<0.001	ns	<0.001
F×D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.037	ns	ns
T×F×D	ns	ns	0.043	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.011
10–20 cm															
T	<0.001	ns	ns	ns	ns	ns	ns	ns	ns	<0.001	ns	ns	ns	ns	0.024
F	ns	ns	<0.001	0.015	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	<0.001
T×F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.016
D	<0.001	<0.001	<0.001	0.013	<0.001	<0.001	<0.001	<0.001	<0.001	0.016	<0.001	<0.001	0.011	ns	<0.001
T×D	ns	<0.001	ns	0.023	0.032	0.011	ns	ns	ns	ns	ns	ns	ns	ns	<0.001
F×D	ns	ns	0.004	ns	ns	ns	ns	ns	ns	0.032	ns	ns	ns	ns	<0.001
T×F×D	0.020	0.016	<0.001	0.009	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.012

ns not significant at $P=0.05$

[a] SMB-C, SMB-N, SMB-P=soil microbial biomass C, N, and P; AMF=arbuscular mycorrhizal fungi; PLFA, phospholipids fatty acids; F/B ratio=ratio of fungal to bacterial PLFA

[b] Data transformed by log prior to statistical analysis

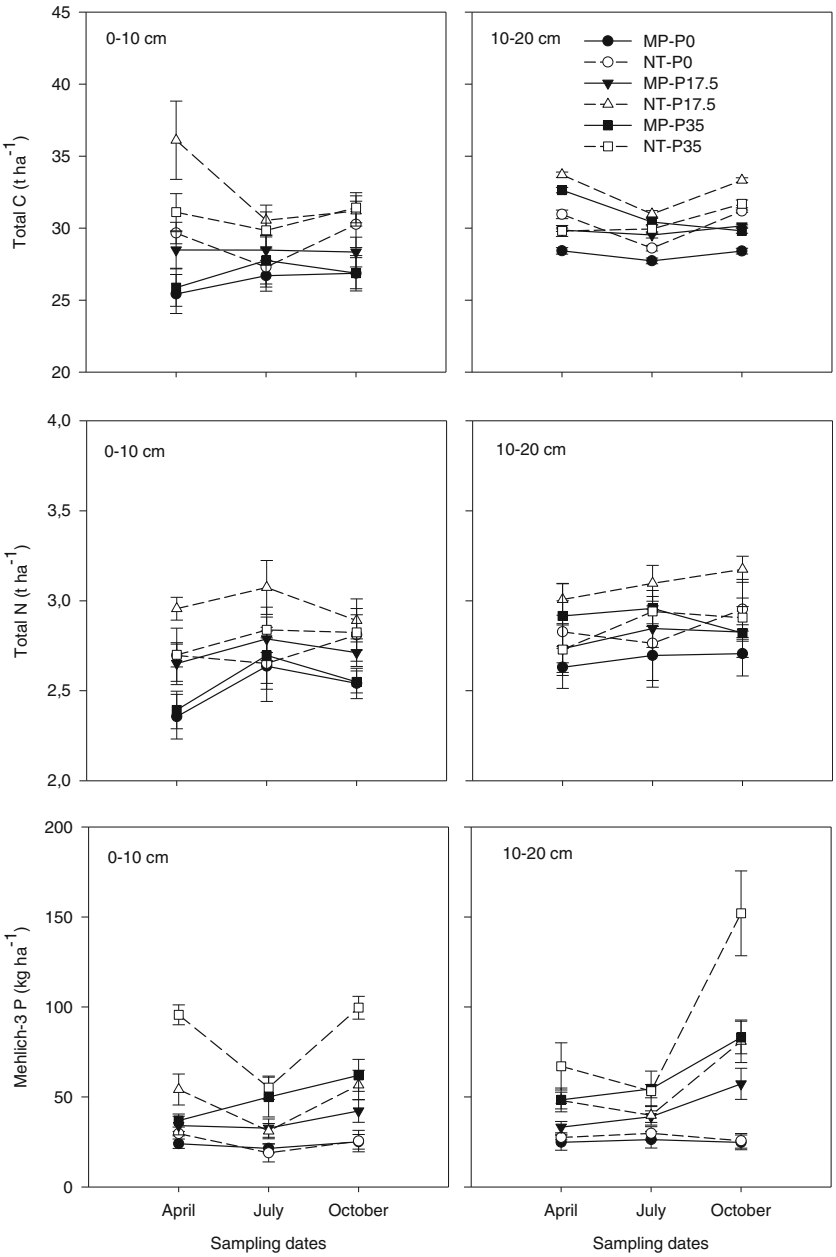

Fig. 1 Seasonal variation in soil pH, moisture content, and Mehlich-3 extractable P as affected by tillage and P fertilization at 0–10 and 10–20 cm soil depths. Soils sampled on April 30, July 5, and October 12, 2010. *MP* moldboard plow, *NT* no-till, *P0* 0 kg P ha^{-1}, *P17.5* 17.5 kg P ha^{-1}, *P35* 35 kg P ha^{-1}. *Bars* represent the standard errors of the mean for each treatment (n=4)

Mehlich-3 P and SMB-P, the increase with NT was only in combination with P applications of 17.5 and 35 kg P ha^{-1}.

At the 10–20-cm soil depth, NT increased SMB-C, SMB-N, and enzyme activities in April and October, and Mehlich-3 P in October, relative to MP (Figs. 1, 2, and 3). Tillage had a complex relationship with SMB-P, which depended on date and P application. In April, NT tended to produce a higher SMB-P with 35 kg P ha^{-1} than MP, whereas in October the same P application under MP increased the concentration of SMB-P (Fig. 2). On the other hand, AMF and fungal PFLA were significantly greater with MP than with NT in July (Table 2). The F/B ratio was significantly greater under MP than NT for all sampling dates at the 10–20-cm depth.

Effect of P fertilization

Phosphorus fertilization had a limited impact on the measured soil properties (Table 1). Soil Mehlich-3 P increased with increasing P additions at both the 0–10- and 10–20-cm soil depths, especially under NT in April and October (Fig. 1). At the 0–10-cm soil depth, P fertilization increased SMB-P from 18 to 21 mg kg^{-1} without P to an average of 26 and 39 mg kg^{-1} with 35 kg P ha^{-1} under MP and NT, which represents an increase of 41 % and 79 %, respectively (Fig. 2). At the 10–20-cm soil depth, P fertilization increased SMB-P from 20 to 21 mg kg^{-1} without P to an average of 31 and 29 mg kg^{-1} with 35 kg P ha^{-1} under MP and NT, which represents an increase of 57 % and 41 %,

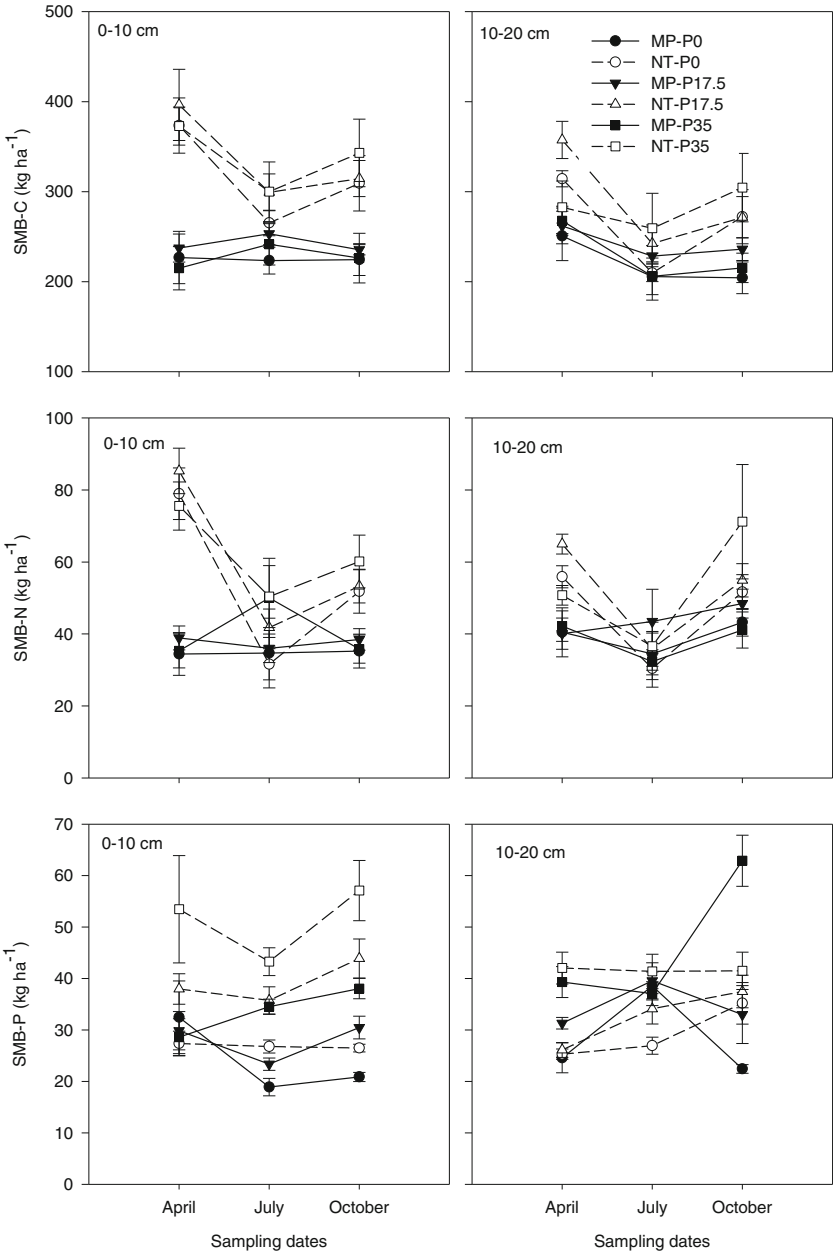

Fig. 2 Seasonal variation in soil microbial biomass C, N, and P (SMB-C, SMB-N, SMB-P) as affected by tillage and P fertilization at 0–10 and 10–20 cm soil depths. Soils sampled on April 30, July 5, and October 12, 2010. *MP* moldboard plow, *NT* no-till, *P0* 0 kg P ha^{-1}, *P17.5* 17.5 kg P ha^{-1}, *P35* 35 kg P ha^{-1}. *Bars* represent the standard errors of the mean for each treatment (n=4)

respectively. Phosphorus fertilization combined with tillage and sampling date influenced SMB-N and dehydrogenase activity at the 10–20-cm soil depth. Under NT, SMB-C, SMB-N, and dehydrogenase activity were significantly greater for the 17.5 kg P ha^{-1} application than for 35 kg P ha^{-1} in April; the opposite effect was found in October (Figs. 2 and 3). Alkaline phosphomonoesterase activity was not significantly affected by P fertilization at both soil depths.

Soil PLFA profiles

Principal component analysis of all PLFA biomarkers indicates that the composition of soil microbial community structure was affected by sampling date and tillage, while P fertilization had no significant effect at either soil depth (Table 3). Results indicate that the first and second principal components (PC1 and PC2) explain 67 % and 61 % of total variation at the 0–10-cm soil depth and 10–20-cm soil depth, respectively (Figs. 4 and 5). For PC1, all treatments were separated by sampling date at both soil depths, and for PC2 only at the first depth; PC2 was separated only at the 10–20-cm depth by tillage system. The overall loadings for PC1 show that most bacterial biomarkers (i-C15:0, a-C15:0, C15:0, 2OH-C14:0, 3OH-C14:0, i-C17:0+C17:0$^{\Delta}$, C17:0, 2OH-C16:0, and C18:1c) and AMF biomarker (C16:1ω5) primarily drove the separation of groups at the 0–10-cm depth. The bacterial biomarkers (3OH-C14:0, C16:0, i17:0

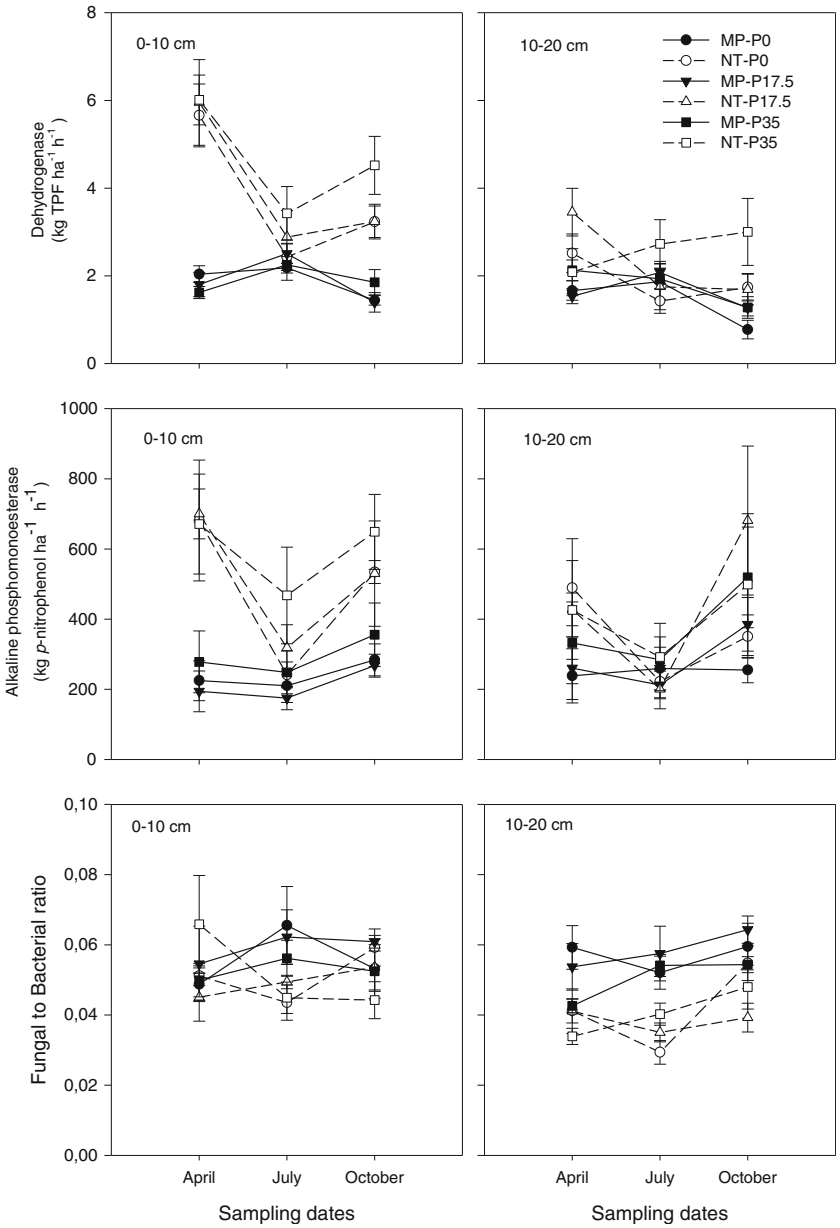

Fig. 3 Seasonal variation in soil dehydrogenase and alkaline phosphomonoesterase activity as affected by tillage and P fertilization at 0–10 and 10–20 cm soil depths. Soils sampled on April 30, July 5, and October 12, 2010. *MP* moldboard plow, *NT* no-till, *P0* 0 kg P ha^{-1}, *P17.5* 17.5 kg P ha^{-1}, *P35* 35 kg P ha^{-1}. *Bars* represent the standard errors of the mean for each treatment (n=4)

+C17:0$^{\Delta}$, and C18:0) and the AMF biomarker (C16:1ω5) were important contributors to PC2 at the first soil depth (Table 3; Fig. 4b). A similar trend was observed at the second soil depth, except for AMF and fungal (C18:2ω 6,9) markers (Table 3; Fig. 4b). At the 10–20-cm soil depth, the AMF marker was not the significant driving factor for PC1 and PC2, but the fungal marker was a major contributor to PC2.

Relationship between soil microbial and chemical properties

Redundancy analysis using microbial (biomass, activity, and community) and chemical properties indicates a different relational pattern that depended on tillage system (Fig. 6a, b). Under MP, SMB-C and -N were positively correlated to total C and N contents, and SMB-P and alkaline phosphomonoesterase activities were positively correlated to pH and Mehlich-3 (Fig. 6a). Soil microbial community components were positively correlated with each other and to C/N ratio, moisture content, and total C. Under NT, all microbial properties were positively correlated to soil chemical properties, especially pH, total C, and moisture content (Fig. 6b).

Discussion

Seasonal variation

Our results confirm that sampling date was the dominant factor since all measured soil microbial and physicochemical

Table 2 The interaction effect of tillage and sampling date on soil total C and N, and phospholipid fatty acids (bacterial, fungal, AMF, and total PLFA) at 0–10 and 10–20 cm soil depths

Source of variation		pH	Moisture content %	Bacterial PLFA g ha^{-1}	Fungal PLFA g ha^{-1}	AMF PLFA g ha^{-1}	Total PLFA g ha^{-1}
0–10 cm							
April							
	MP	6.1b[a]	21.1c	2394b	135b	76bc	2604b
	NT	6.6a	24.1a	4402a	238a	155a	4795a
July							
	MP	5.7c	15.7e	986d	60c	25e	1071d
	NT	5.7c	16.3e	1326cd	57c	36de	1419cd
Oct							
	MP	5.9bc	21.8d	1555c	86b	50cd	1691c
	NT	6.1b	22.6b	2465b	131b	83b	2679b
SEM (n=6)		0.14	1.4	504	27.7	19.1	550
10–20 cm							
April							
	MP	6.1a	22.3a	1788a	92a	58ab	1938a
	NT	6.3a	22.1a	2016a	75a	73a	2164a
July							
	MP	5.7bc	17.5b	734b	40b	48b	822b
	NT	5.6c	16.7b	794b	28c	23c	845b
Oct							
	MP	5.9b	21.9a	1714a	102a	58ab	1874a
	NT	5.8b	22.2a	2050a	96a	68a	2214a
SEM (n=6)		0.27	2.6	244	12.8	7.3	261

MP moldboard plow, *NT* no till, *AMF* arbuscular mycorrhizal fungi, *PLFA* phospholipids fatty acids, *F/B ratio* ratio of fungal to bacterial PLFA, *SEM* standard error of mean

[a]Means followed by the same letter in each column within a depth category are not significantly different at P=0.05

properties showed significant variability at both soil depths over the growing season. These seasonal variations are characterized by the low values for most microbial properties in July.

In the present study, soil moisture, microbial biomass (C, N, and P), and enzyme activities were lowest in July. In 2010, July was particularly dry, below the long-term average, and usually drier than spring and fall. This result shows how microbial properties can be affected by fluctuations in their microenvironment. It is well documented that moisture and temperature are major environmental factors controlling microbial abundance and distribution (Díaz-Ravoña et al. 1995; McGill et al. 1986). Our results show a positive, and more clearly defined relationship between soil moisture content and SMB-C and -N under NT than MP (Fig. 6). This result indicates that environmental conditions, such as moisture, played a major role in the seasonal variation of SMB-C and SMB-N under NT. Soils under NT are known to have a higher organic matter and moisture content in the surface layer than those under MP (Feng et al. 2003). In addition, Jin et al. (2011) report that soil porosity was greater under NT than MP after 11 years of no-tillage farming, largely due to an increase in macro- and meso-soil porosity. This increase in porosity coupled with a higher organic matter content could be partly responsible for the greater moisture under NT.

Analysis of PLFA profiles reveals a higher variability due to sampling date than to tillage but without significant correlation with P fertilization. This result agrees with other studies reporting changes in community structure over the growing season, and which show a greater variability than that associated with crop management, such as fertilization, rotation, drainage, tillage, or residues left on soil (Bardgett et al. 1999; Spedding et al. 2004). Regardless of tillage and P fertilization, total PLFA and bacterial, fungal, and AMF biomass were greatest in spring or fall than in summer at both soil depths. Moreover, this enriched microbial community composition was closely related to soil moisture content under NT and MP (Fig. 6a, b). From our analysis of the PCA loadings, we observe that the fungal biomarker (C18:2ω6,9) was enriched in the April sampling (Figs. 4 and 5). It has been previously reported that fungi are important contributors to the decomposition of plant residues, particularly within the spring subsurface layer (Durrieu 1993; Schutter and Dick 2001). On the other hand, at both soil depths, the October sampling was characterized by markers of G^+ bacteria, predominantly composed of branched fatty acids and with G^- bacteria associated with hydroxyl fatty acids (Wilkinson 1988).

Table 3 Soil microbial community structure as affected by sampling date, tillage, and P fertilization, and PLFAs scores on the first two principal components at 0–10 and 10–20 cm soil depths

	0–10 cm		10–20 cm	
	PC1	PC2	PC1	PC2
Sampling date	<0.001	0.007	<0.001	ns
Tillage	ns	ns	ns	0.022
P fertilization	ns	ns	ns	ns

PLFAs ($P<0.05$) receiving scores on the first two principal components

Fatty acid	Scores - *PC1*		Fatty acid	Scores - *PC2*	
	0–10 cm	10–20 cm		0–10 cm	10–20 cm
i-C15:0	0.34	0.34	C15:0	–	0.27
a-C15:0	0.39	0.32	3OH-C14:0	0.41	0.28
C15:0	0.30	0.26	C16:0	0.48	0.18
2OH-C14:0	0.38	0.33	C16:1ω5	−0.40	–
3OH-C14:0	0.20	0.30	i-C17:0+C17:0$^{\Delta}$	−0.26	−0.20
C16:0	–[a]	0.31	C17:0	–	0.28
C16:1ω5	0.23	–	2OH-C16:0	–	−0.44
i-C17:0+C17:0$^{\Delta}$	−0.33	−0.34	C18:0	0.51	0.42
C17:0	−0.33	−0.32	C18:2ω6,9	–	−0.38
2OH-C16:0	0.27	–			
C18:0	–	0.22			
C18:1c	0.31	0.31			

ns not significant at $P=0.05$

[a]Means of biomarker on the principal components was not significant at $P=0.05$ and the scores are not shown in the table

Effect of tillage

Variation in SMB-C, SMB-N, SMB-P, dehydrogenase and alkaline phosphomonoesterase activities, and AMF, bacterial, fungal, and total PLFA biomass confirm the positive effect of NT, especially at the soil surface (0–10-cm depth), as reported previously (Aslam et al. 1999; Doran and Linn 1994; González-Chávez et al. 2010; Liu et al. 2008). This result also corroborates Needelman et al. (1999) who found that tillage largely impacted the topsoil constituents of agricultural systems. Drijber et al. (2000) detected a significant decline in the mycorrhizal marker from no-till to plowed soil, while Pankhurst et al. (2002) observed a higher F/B ratio at the 0–5-cm soil layer with direct drilling (NT) versus conventional cultivation (MP). In our study, we observed higher values in total PLFA under NT at both soil depths, indicating that many bacteria, either G^+ or G^- in association with fungi, were able to degrade complex substrates, such as lignin and humic acid, that are often found in the surface layer of no-till soils (Burke et al. 2003; Durrieu 1993; Schutter and Dick 2001).

In our study, the soil at the 0–10-cm depth of NT contained more microbial biomass and microbial activities (dehydrogenase, alkaline phosphomonoesterase activities) than the soil under MP, which agrees with other reports (Balota et al. 2004; Doran 1980; Doran et al. 1998; González-Chávez et al. 2010; Liu et al. 2008; Spedding et al. 2004). Paul and van Veen (1978) report that microbial biomass C, N, and P decreased with an increase in tillage disturbance because of lower organic matter inputs to the soil. Soils under NT have more crop residues on the surface, and therefore a greater organic matter content (Dick and Durkalski 1998; Feng et al. 2003), which in turn increases the soil microbial biomass due to higher substrates for microbial growth (Kandeler et al. 1999). Regardless of tillage, our study shows a strong correlation between SMB-C and -N and total C, which indicates that soil organic matter was an important factor affecting microbial biomass and its activities (Aciego Pietri and Brookes 2008). Other factors, such as soil temperature, pH, moisture, and clay content, also reportedly affect microbial biomass in soil (Carter 1986; Gestel et al. 1993; Nicojardot et al. 1994). Our results from the redundancy analysis show that with the higher organic matter content under NT than MP, all microbial properties were positively affected.

Soil depth is an important factor in microbial community distribution (Acosta-Martinez et al. 2007; Bausenwein et al. 2008; Hansel et al. 2008), which explains some of the effects from management practices (Sun et al. 2011). Typically, NT promotes C storage in the surface soil layer. When soil is mouldboard plowed, the layer near the bottom of the plow, usually at a 20–30-cm depth, becomes richer in organic matter over cropping years (Angers et al. 1997; Poirier et al. 2009), which we also observed. This accumulation of total C beyond the 20-cm depth is due to long-term MP tillage; however, in our study the 0–10-cm depth had a higher total C content under NT than MP. This accumulation of total C near the soil surface in NT explains how a high level of soil microbial biomass (SMB-C and SMB-N) and

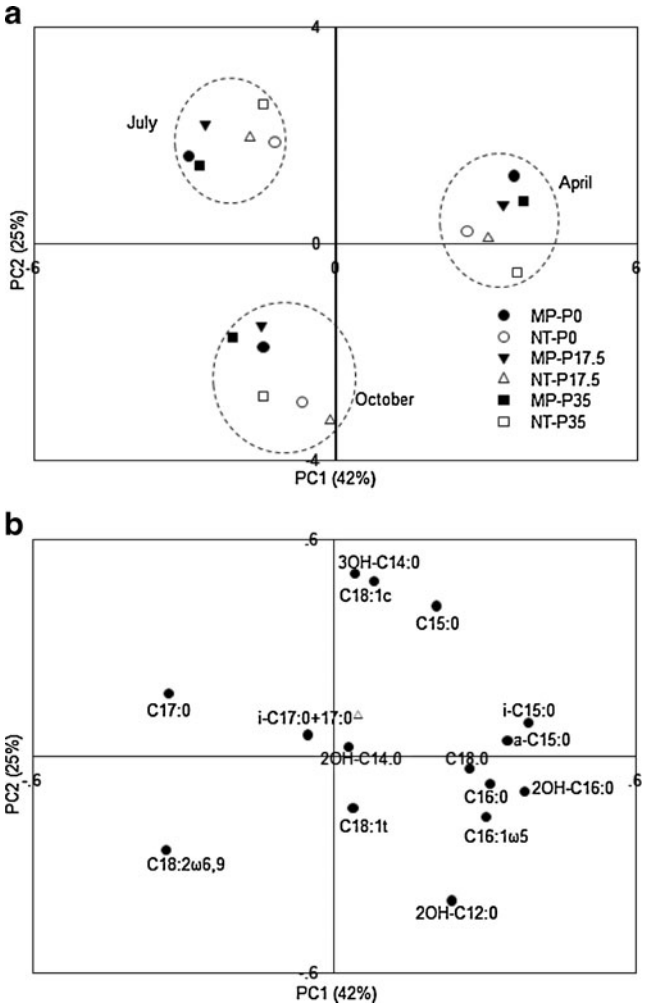

Fig. 4 Ordination plots of the microbial community composition (based on phospholipids fatty acids) as determined by principal component analysis (PCA) at a 0–10-cm soil depth under long-term management. PCA shows 67 % of the variation explained by two main axes. **a** Tillage and P fertilization treatments for three sampling dates. **b** Position of the signature fatty acids in the axes referring to the loading values of the principal component analysis. *MP* moldboard plow, *NT* no-till, *P0* 0 kg P ha^{-1}, *P17.5* 17.5 kg P ha^{-1}, *P35* 35 kg P ha^{-1}

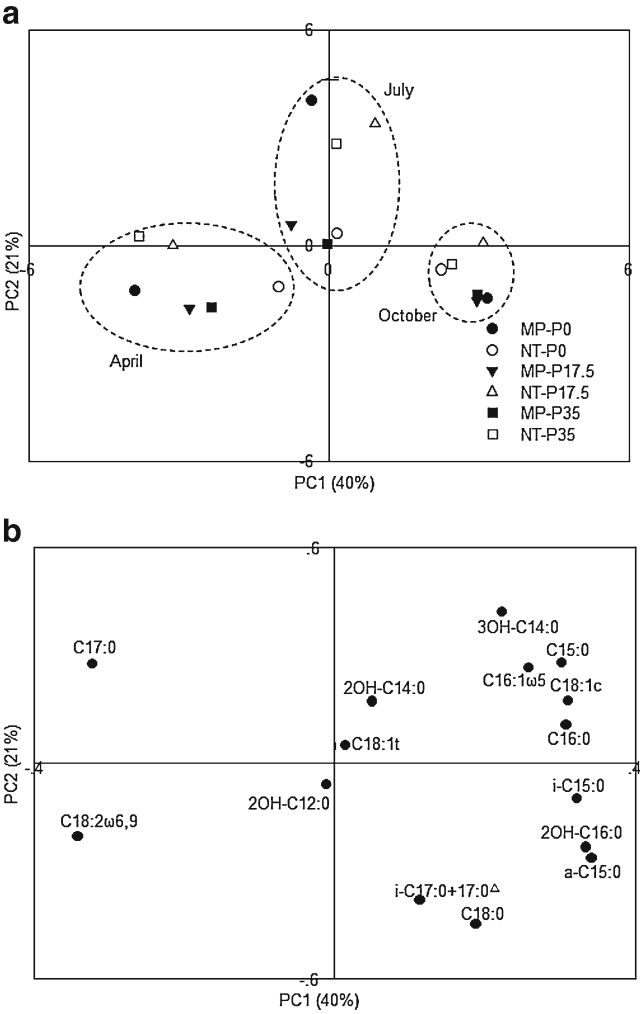

Fig. 5 Ordination plots of the microbial community composition (based on phospholipids fatty acids) as determined by principal component analysis (PCA) at a 10–20-cm soil depth under long-term management. PCA shows 61 % of the variation explained by two main axes. **a** Tillage and P fertilization treatments in three sampling dates. **b** Position of the signature fatty acids in the axes referring to the loading values of the principal component analysis. *MP* moldboard plow, *NT* no-till, *P0* 0 kg P ha^{-1}, *P17.5* 17.5 kg P ha^{-1}, *P35* 35 kg P ha^{-1}

enzyme activities can be obtained. More fungal and AMF biomass was observed at the 10–20-cm soil depth under MP, than NT, particularly in July. This predominance of fungi is also evident in the F/B ratio (Fig. 3). This observation confirms that the total C accumulation in the bottom of the plow layer might have set conditions for the establishment of some microorganisms, in particular fungi that are known to be fundamentally different in terms of decomposer groups (Mille-Lindblom and Tranvik 2003; Rousk and Bååth 2007; Six et al. 2006).

Effect of P fertilization

The effect of P fertilization on soil microbial properties was less than that attributed to sampling date and tillage. In the present study, P fertilization affected SMB-P at both depths, similar to the effect from Mehlich-3 P. The SMB-P can play an important role in P cycling, and its availability to plants, by acting as a source (mineralization), or as a sink (immobilization), of phosphate ions (Achat et al. 2009a, b; Oberson and Joner 2005). Regardless of tillage system, we found a positive correlation between SMB-P and soil Mehlich-3 P, in agreement with Gosai et al. (2010). In fact, P fertilizer additions increased soil available P and also enhanced SMB-P, which indicates that SMB-P was primarily acting as a source for recovering available P in the mineralization process. However, P fertilization had no significant effect on alkaline phosphomonoesterase activity. This enzyme activity is mainly of microbial origin (Juma and Tabatabai

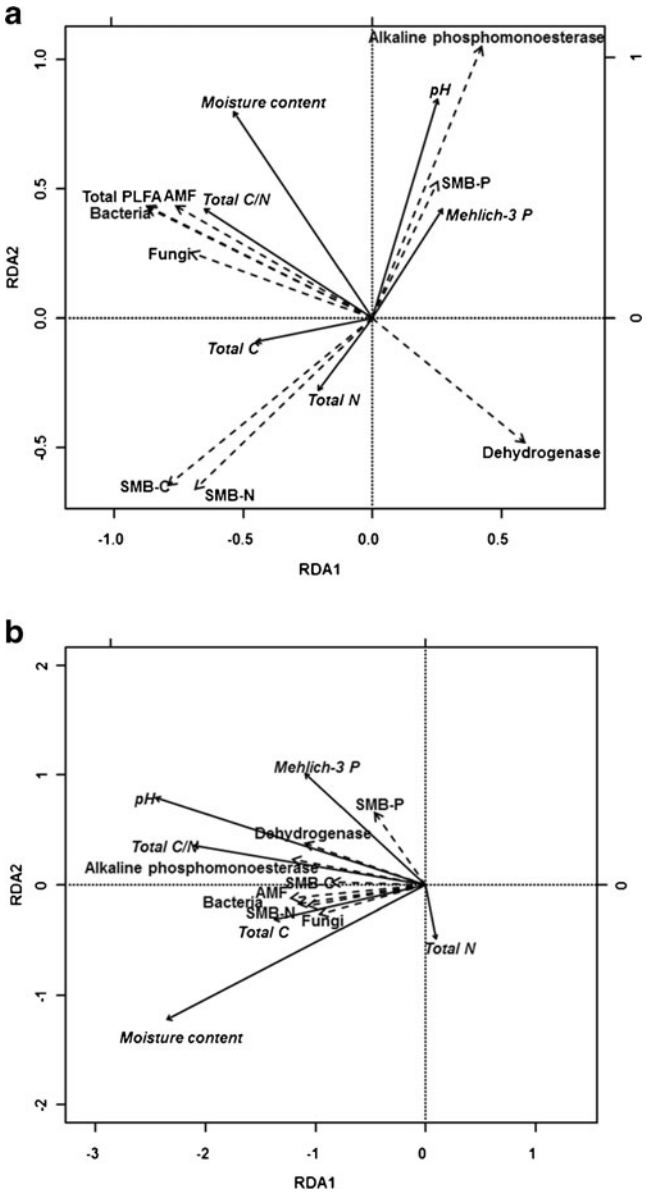

Fig. 6 Significant correlations between soil microbial (biomass, activity, and community) and chemical properties as tested by redundancy analysis at $P<0.05$. **a** *MP* moldboard plow. **b** *NT* no-till

1978), unlike acid phosphomonoesterase which originates from plants and microbes (Tabatabai 1994), and is strongly correlated to labile organic P but not to labile inorganic P (Turner and Haygarth 2005). This result could explain the lack of response in our study.

Results of the present study show that P fertilization had no significant effect on soil microbial community structure and its specific PLFA biomarkers. This result contrasts with Cruz et al. (2009) who report that soil microbial community structure was influenced by 37 years of P fertilization in a Brown Chernozem. However, Hamel et al. (2006) found that variations in P did not influence the composition of microbial communities in a wheat-based rotation, while Bünemann et al. (2004) observed that P fertilization did not significantly affect total amount of PLFAs, bacterial and fungal biomasses under a maize–crotalaria fallow rotation. Redundancy analysis shows that soil microbial community structure components were more closely related to total C and C/N ratio of soil than Mehlich-3 P, for both tillage managements. This result indicates that soil organic matter is the key factor affecting the biomass and composition of soil microbial community. Even in soils with low plant-available P, the growth of soil microorganisms are more limited by C and N than by P (Bünemann et al. 2004; Ehlers et al. 2010). According to resource-ratio theory, the microbial community structure composition and total hydrocarbon-degrader biomass vary in response to changes in the supply and the ratios of potentially growth-limiting resources within a system (Smith et al. 1998). Because P fertilization did not affect total C and N, even though Mehlich-3 P was increased, the composition of soil microbial communities could not adapt to changes in soil-available P. In other words, with unchanged values in total C, SMB-C and SMB-N, P could not be the limiting factor for soil microbial communities.

Interactive effects of sampling date, tillage, and P fertilization

We found a large interactive effect between tillage and sampling date. The NT system usually had a positive effect on soil microbial properties in April and October, but not in July. This result indicates that environmental factors, such as dry summer conditions, can change the effect of tillage on soil microorganisms; also, effects of the tillage system may differ during the growing season. Under NT, both enzyme activities and SMB-C and -N followed a similar seasonal pattern that could be attributed to soil moisture content. In MP, a different trend was observed with limited fluctuation during the growing season. According to the redundancy analysis, correlations between soil microbial and chemical properties differed with tillage system. This result shows that changes in soil microbial biomass and enzyme activities are influenced by a combination of factors including soil environmental conditions and organic matter content, which are important in controlling overall nutrient cycling and availability (Chen et al. 2003; He et al. 1997). However, the contribution of individual factors affecting soil enzyme activities and SMB-C and -N depended on tillage system. Usually these response variables were mainly regulated by organic matter between tillage systems, but when soil environmental factors changed, the growth of soil microorganisms were limited more under NT than MP.

The trends in SMB-P are difficult to be interpreted. Seasonal variation in SMB-P was already observed for tillage, P fertilization, and also with soil depth; the lowest

values were not always in July. This result contrasts with Liu et al. (2008) who found that regardless of tillage or P fertilization, SMB-P increased from seeding to the middle of the growing season, peaking either in June or July and then decreasing in October. The difference between the two studies indicates that SMB-P is not mainly constrained by environmental factors, which differs from the other microbial aspects. With a similar pattern as Mehlich-3 P, SMB-P was also affected by tillage × P fertilization; the increase under NT was only found with P applications of 17.5 and 35 kg P ha^{-1} in April and October. High correlations were obtained between SMB-P and Mehlich-3 P and pH both under NT and MP based on RDA analysis. This result indicates that the temporal variation of SMB-P could be affected by a combination of P availability, pH, and environmental conditions. Moreover, results also show that Mehlich-3 P was positively correlated with all microbial properties under NT but only with SMB-P and alkaline phosphomonoesterase under MP. In the present study, the absence of a response of alkaline phosphomonoesterase to P addition was observed, which disagrees with the point that the synthesis of phosphomonoesterases in soil is depressed by the application of inorganic P (Nannipieri et al. 2011). The fact that Mehlich-3 P was correlated with alkaline phosphomonoesterase both under NT and MP may mean that there were other factors affecting enzyme activity. Indeed, soil texture (clay to clay loam) particularly clay particles may stabilise extracellular phosphomonoesterase, which will become active and mask the real effect of inorganic P on enzyme synthesis. Thus, the observed relationship between Mehlich-3 P and microbial properties under our two tillage systems indicates that there was a greater microbial role in P availability through SMB-P turnover under NT than MP. Therefore, for maize grown in a long-term rotation, NT combined with P fertilization could help to sustain soil-available P through the soil P biological cycle during the growing season.

Conclusion

This study found that in addition to seasonal conditions affecting the variability of microbial and soil physicochemical properties throughout the growing season for maize, soil microbial biomass, activity, and composition of microbial communities responded to changes in soil conditions, with tillage having a greater effect than P fertilization. Phosphorus fertilization increased SMB-P and soil Mehlich-3 P, with soil microbial biomass and activity being more predominant under NT than MP; these effects were mostly related to soil moisture conditions and organic matter content. The PLFA analysis emphasizes that soil microbial community structure was primarily affected by environmental factors, minimally affected by tillage practice, and not significantly affected by P fertilization. Soil microbial properties varied less over time under MP than NT. This result indicates that soil microbial biomass, activities, and microbial community composition depend more on soil organic matter for their growth and were not limited by soil P status. However, we also found that NT combined with P fertilization could help to sustain a supply of soil available P by improving the soil P biological cycle. Further studies should be conducted to determine how environmental variations (i.e., winter conditions) will affect soil P dynamic associated to microbial change.

Acknowledgments This work was supported by Agriculture and Agri-Food Canada (AAFC) and the MOE (Ministry of Education of China)-AAFC Ph.D. research program. We thank Sylvie Côté, Keith Hanson, Claude Lévesque, Sylvie Michaud, and Jeannot Pelletier for their help in sampling soils and technical assistance. Special thanks to the people at the L'Acadie Experimental Farm of Agriculture and Agri-Food Canada who have supervised and maintained the trial since 1992.

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