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Spatial variation of soil enzyme activities and microbial functional diversity in temperate alley cropping systems

Abstract Spatially dependent patterns in microbial properties may exist in temperate alley cropping systems due to differences in litter quality and microclimate in areas under trees compared to those in the alleys. The effect of tree row location was evaluated for its impact on soil enzyme activities and Biolog substrate use patterns. Soils were sampled to a depth of 30 cm at the tree row and at the middle of the alley at two sites: a 21-year-old pecan (*Carya illinoinensis*)/bluegrass (*Poa trivials*) intercrop (Pecan site) and a 12-year-old silver maple (*Acer saccharinum*)/soybean (*Glycine max*)–maize (*Zea mays*) rotation (Maple site). Sampling was done in fall 2001 and summer 2002. β-Glucosidase activities, Biolog patterns expressed as average well color (AWC), substrate richness, and Shannon diversity index, and total Kjeldahl nitrogen (TKN) were significantly higher ($P<0.05$) in the tree row than at the middle of the alley for surface soils at the Pecan site. Fluorescein diacetate (FDA) hydrolytic activity was also higher at the tree row for soils sampled in the fall, but did not differ significantly for soils sampled in the summer. At the Maple site, AWC and substrate richness were significantly higher at the tree row for soils sampled in 2001. Soil volumetric water content and temperature were generally lower in the tree row at the Maple site. The results of this study suggest that functionally different microbial populations may be present under pecan trees compared to cropped alleys which may promote disparities in nutrient availability necessitating differential long-term nutrient management in such alley cropping systems.

Keywords β-Glucosidase · Fluorescein diacetate · Biolog · Temperate alley cropping · Microbial diversity

Introduction

In alley cropping, differences in litter quality between the tree and intercropped components litter can lead to differential enzyme activities and microbial functional diversity in relation to tree rows. Plant litter quality influences saprophytic microbes in soil that in turn regulate ecosystem functions such as decomposition and nitrogen (N) mineralization (Wardle and Lavelle 1997). Trees used in tropical regions where most research in alley cropping have been conducted are usually leguminous and produce litter rich in N relative to the intercrops. In contrast, trees used in temperate alley cropping are mainly hardwood species grown for nut and timber production (Jose et al. 2000) intercropped with food or forage crops. Litter from such trees is usually lower in N than that from the intercrops (Jose et al. 2000; Saggar et al. 2001).

Temperate alley cropping systems may contribute to soil organic matter (SOM) content through addition of tree leaf litter, fine roots, and crop residues. Changes in SOM usually occur over long periods, and alternative rapid, inexpensive, and more sensitive measurements may be needed to monitor SOM dynamics. Microbial parameters such as β-glucosidase activities can provide advance evidence of changes in soil organic carbon (SOC) long before it can be accurately measured by routine methodologies (Dick 1994). Fluorescein diacetate (FDA) hydrolysis is a broad-spectrum assay that depicts primary decomposers activities in soil (Dick et al. 1996), and their activities are correlated to SOC and total N (Gasper et al. 2001). Another

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microbial parameter is microbial functional diversity, which is defined as the numbers, types, activities, and rates at which a suite of substrates in Biolog microplates are utilized by bacterial communities (Zak et al. 1994). Enzyme activities (Bandick and Dick 1999) and Biolog microbial functional diversity (Gomez et al. 2000; Myers et al. 2001) have been shown to consistently differ among soil management practices and ecosystems. Myers et al. (2001) attributed differences in microbial functional diversity among forest ecosystems to variations in plant litter quality of substrate inputs to the soil.

Microclimate differences due to the presence of trees in alley cropping systems can cause variations in soil temperature and water content. Ko and Reich (1993) found that light intensity and soil temperature increased with increased distance from the tree's trunk. In general, soil temperature increases in clearcuts relative to adjacent forest, whereas soil moisture content is usually greater than in adjacent forest (Cadenasso et al. 1997).

Differences in tree litter characteristics compared to intercropped components, and the additional effect of trees on microclimate may cause spatially dependent soil enzyme activities and microbial functional diversity. We hypothesized that soil microbial properties would be different near the tree row compared to the middle of the alley because of the influence of trees on soil temperature, water content, and differences in litter quality. The objective of this study was to assess spatial and temporal variability of soil enzyme activities and microbial functional diversity in established temperate alley cropping systems.

Materials and methods

Experimental sites and soil sampling

Two sites in north central Missouri with mature trees were selected for this study. Table 1 shows a summary of site characteristics. At one site (Pecan site), pecan trees were planted in 1981 in rows spaced 12 m apart and 4.5 m between each tree in the row, intercropped with a maize–soybean–wheat rotation for the first 11 years, after which the trees were thinned to 12×9 m and bluegrass was planted in the alleys. In 1999, the Pecan site received broadcast application of lime at 6.2 t ha^{-1} and 35 kg N ha^{-1}. Weed control under trees was accomplished by spot spraying with atrazine, while the alleys were mowed and bluegrass removed for hay at least once a year. The soil is classified as a Gosport silty clay loam (fine, illitic, mesic Typic Dystrochrepts). For this site, mean annual temperature is 13°C and precipitation is 850 mm.

At the second site (Maple site), silver maples were planted in 1990 in rows spaced 18 m apart and with 1.5 m distance between each tree within the row. Tree rows were in an east/west orientation and intercropped with a maize–soybean rotation under no-till management. In 1996, silver maples were thinned based on stem form. Maize was planted at 70,000 seeds ha^{-1} as the intercrop in 2000 and 2002. Soybeans were planted at 500,000 seed ha^{-1} in 2001.

Table 1 Selected site characteristics

	Site	
	Pecan	Maple
Latitude/longitude	39°4′N/92°7′W	39°58′N/92°5′W
Age of trees (years)	21	12
Trees (ha^{-1})	289	370
Mean tree height (m)	7±1[a]	12±1
Mean DBH[b] (cm)	17±2	24±2
Tree leaf amounts (kg ha^{-1})	1323	2942
Other components, litter amounts (kg ha^{-1})	2000 (Bluegrass)	3,000 (Soybean) 5,000 (Maize)
Tree leaf litter, C/N ratio	32±3	45±7
Other components, C/N ratio	19±1 (Bluegrass)	20±4 (Soybean) 28±2 (Maize)

[a]Values show ±1 standard deviation
[b]Diameter of tree trunk measured at breast height

Weed control was applied by a spray application of glyphosate, ammonium sulfate, and 2,4-D. In fall 2001, 20, 39, and 93 kg ha^{-1} of N, P, and K fertilizer, respectively, were applied and another 130 kg N ha^{-1} in 2002. Mean annual temperature and precipitation at this site is 12°C and 760 mm, respectively. The soil is classified as a Mexico silt loam (fine, smectic, mesic Aeric Vertic Epiaqualfs).

Soils were sampled using a 2-cm diameter stainless steel push probe at 0 (tree row), 1.5, 3.0, 4.5, and 6.0 m (middle of the alley) from the tree row at the Pecan site and at 0, 3.0, 6.0, and 9.0 m from the tree row at the Maple site. Sampling was done at depths of 0–10, 10–20, and 20–30 cm at each position. At each sampling position, five to six cores were taken and composited to yield one sample. Five locations were sampled at each site in fall 2001 and summer 2002 to give at total of 150 bulk samples at the Pecan site (5 distances from the tree row ×5 different locations ×3 depths ×2 seasons) and 120 bulk samples at the Maple (4 distances from the tree row ×5 different locations ×3 depths ×2 seasons). One half of each soil sample was air-dried, ground, and passed through a 2-mm mesh sieve for chemical analysis and the other half was maintained field moist and stored at 4°C for soil enzyme and microbial functional diversity analysis. Stored soils were preincubated at room temperature (25–27°C) for 24 h before analysis. Results reported in this paper show comparisons of the tree row and middle of the alley positions because differences between the other positions were minimal.

Two HOBO soil temperature probes (Onset Computer Corp., Bourne, MA) were installed in and between the tree rows at 10-cm depths at each site in June 2002. Temperature probes were attached to a data logger accumulating data at 30-min intervals. Time domain reflectometry (TDR) waveguides were also installed at both locations, at two depths (10 and 20 cm) and at two positions in the alley at each site. Soil volumetric water content was measured at least once a month with a Trase TDR unit (Soilmoisture Equipment, Santa Barbara, CA).

Maize and soybeans were harvested at the Maple site using a Massey 10 small plot combine (Kincaid Equipment

Manufacturing, Haven, KS). Two rows of maize and a 1.5-m width of the narrow-row soybean were harvested and grain moisture was collected from a 15.2-m plot length. Maize and soybean grain yields were adjusted to 15.5 and 13.0% moisture, respectively. Yield measurements of bluegrass were not recorded at the Pecan site.

Laboratory analysis

β-Glucosidases (Enzyme commission # 3.2.1.21) catalyze the hydrolytic conversion of cellulose to glucose. Cellulose is quantitatively the most important organic compound and its mineralization in soil is an important process in the carbon cycle (Sinsabaugh et al. 1991). β-Glucosidase activities were analyzed using the method of Dick et al. (1996), which is based on colorimetric determination of ρ-nitrophenol released by β-glucosidase when soil is incubated with a buffered (pH 6.0) ρ-nitrophenol-β-D-glycopyranoside as the substrate.

FDA hydrolysis provides a broad-spectrum indicator of soil biological activity, and was determined following the method of Bandick and Dick (1999). The FDA procedure is based on hydrolysis of the FDA substrate by microbial cells releasing fluorescein, which can be quantified by spectrophotometry.

Biolog Eco-plates (Biolog Inc., Hayward, CA) were used to assess the microbial functional diversity of whole soil from the two sites. Eco-plates have 31-carbon substrates that are dominant in ecological samples, each replicated three times. Eco-plates were inoculated with 125 μl of soil suspension (10^{-3} dilution) made by diluting 5-g soil samples with sterile 0.85% NaCl. Inoculated plates were incubated at 25°C and color development monitored every 12 h up to 84 h by measuring optical density values with an automated plate reader (Dynatech MR5000, Dynatech Laboratories, VA, USA) at 570 nm. A heated potassium dichromate oxidation was used to analyze soil organic carbon (SOC) (Nelson and Sommers 1986). Soil total nitrogen was determined by Kjeldahl digestion and NH_3 in the digest was measured using a Lachat QuikChem Automated Ion Analyzer (Zellweger Analytics Inc. 1996).

Statistical analysis

All statistical procedures were performed using the SAS statistical program (SAS Institute 2001). The *t*-test was used to test for differences between sites and positions in the alley using input data sets of summary statistics. Principal component analysis (PCA) was performed on Eco-plate data to characterize microbial communities from different sites and sampling times. PCA was computed on samples after 72 h of incubation. Color developed in the control well was subtracted from optical densities in all other wells. Average well color (AWC), substrate richness, and Shannon Index were computed following Gomez et al. (2000) and an analysis of variance was performed on the processed data. Pearson linear correlation analysis was performed to determine associations among soil enzyme activities and Biolog parameters in relation to each other and to SOC and TKN.

Results

Microclimate modifications

Soil temperature differences between the tree row and the middle of the alley at the Pecan site were less than 2.5°C during the hottest week of summer in 2002 (Fig. 1a). Temperature differences between the middle of the alley and the tree row varied from −1.93 to 2.31°C. Additionally, soil water content at 10- and 20-cm depths did not differ with positions in the alleys at the Pecan site (Fig. 2a). At the Maple site, soil temperature differences of greater than 6°C were observed (Fig. 1b). Differences between the middle of the alley and the tree row varied from 0.46 to 8.74°C. Soil volumetric water content was higher at the middle of the alley than at the tree row at 10- and 20-cm depths (Fig. 2b). No differences in soil water content were observed between the two soil depths.
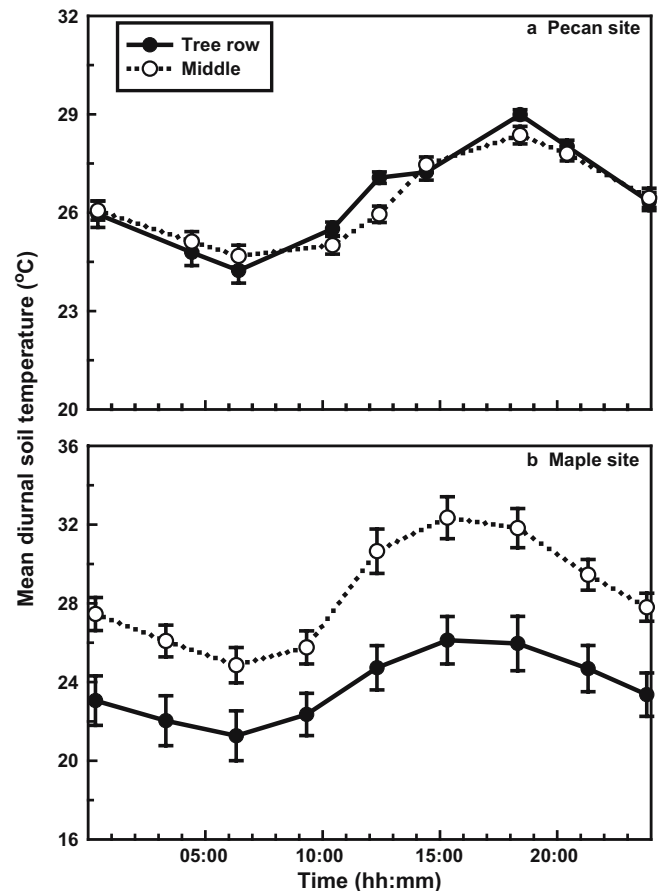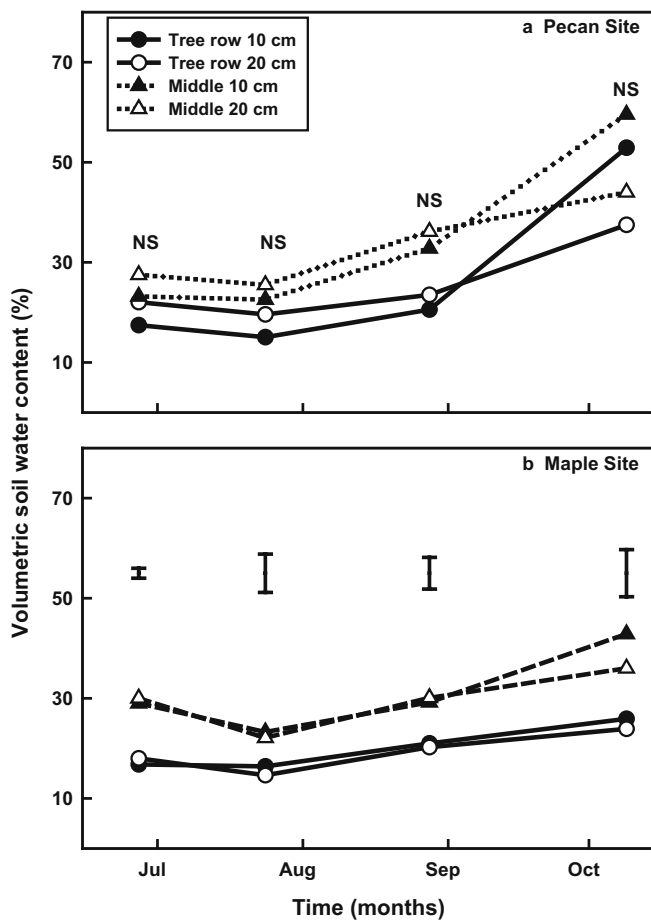

Fig. 1 Mean diurnal soil temperature at a depth of 10 cm at **a** the Pecan site (July 27–August 3, 2002) and **b** the Maple site (June 26–July 3, 2002). *Error bars* represent ±1 standard deviation. The *Y*-axis scale is different for **a** and **b**

Fig. 2 Soil volumetric water content at **a** the Pecan site and **b** the Maple site during summer 2002

Soil organic carbon and nitrogen

At the Pecan site, no differences were observed in soil organic carbon (SOC) between positions in the alley for all three depths (Table 2). Total Kjeldahl nitrogen (TKN) was, however, higher at the tree row for surface soils (0–10 cm). At the Maple site, both SOC and TKN were significantly higher at the tree row at 30 cm depth (Table 2). Observed differences between positions in the alley were not maintained in the second sampling (Table 2). Generally, SOC and TKN were higher in surface soils than in subsurface soils for both sites.

Enzyme activities

At the Pecan site, FDA activity was higher at the tree row compared to the alley for surface soils. No differences were observed at 20- and 30-cm depths among positions in the alley (Table 2). At the Maple site, FDA was significantly higher at the middle of the alley than at the tree row for surface soils (Table 2). No differences were observed at lower depths. FDA decreased only slightly with depth at both sites (Table 2). Additionally, FDA was significantly correlated to SOC and TKN at the Maple site (Table 3).

β-Glucosidase activities were significantly higher at the tree row than at the middle of the alley at all depths for soil sampled in fall 2001 at the Pecan site (Fig. 3a). In 2002, β-glucosidase activities maintained the same significant trends, except at the 20 cm depth (Fig. 3a). At the Maple site, β-glucosidase activities were higher at the tree row than at the middle of the alley at 30 cm depth in 2001 and at

Table 2 Soil organic carbon, total nitrogen and fluorescein diacetate activities at the Pecan and Maple sites by position in the alley in 2001

Site and position	Soil organic carbon (mg kg^{-1})			Total Kjeldahl nitrogen (mg kg^{-1})			Fluorescein DA (mg g^{-1})		
	10 cm	20 cm	30 cm	10 cm	20 cm	30 cm	10 cm	20 cm	30 cm
Fall 2001									
Pecan tree[a]	24.6	18.1	18.0	2.09	1.21	1.07	7.1	5.1	5.2
Pecan mid	21.1	18.8	18.0	1.80	1.28	1.10	6.2	5.6	6.1
$P>|t|$	NS	NS	NS	*	NS	NS	*	NS	NS
Maple tree	18.5	18.6	15.4	2.10	1.86	1.67	4.7	3.6	3.1
Maple mid	21.2	17.1	11.3	2.41	1.63	1.43	6.9	3.5	3.2
$P>|t|$	NS	NS	*	NS	NS	*	***	NS	NS
Summer 2002									
Pecan tree	26.8	17.8	16.0	2.32	1.55	1.34	5.5	4.9	4.5
Pecan mid	25.8	16.9	15.7	2.09	1.47	1.34	5.0	5.4	6.0
$P>|t|$	NS	NS	NS	NS	NS	NS	NS	NS	**
Maple tree	25.0	19.6	12.2	2.41	1.94	1.34	4.7	4.9	4.1
Maple mid	24.0	15.4	11.6	2.36	1.59	1.27	4.8	4.4	4.6
$P>|t|$	NS	NS	NS	NS	NS	NS	NS	NS	NS

Fluorescein DA fluorescein diacetate, *NS* not significant
[a]Tree and mid represent tree row and middle of alley, respectively

Table 3 Correlation coefficients (*r*) among soil organic carbon and nitrogen, soil enzyme activities, and Shannon diversity index for surface soil (0–10 cm) in two alley cropping systems for fall 2001

Site soil property	Pecan		Maple		
	SOC	TKN	SOC	TKN	Yield[a]
β-Glucosidase	0.21	0.49*	0.46*	0.65**	0.59**
Fluorescein DA	−0.04	0.09	0.63**	0.81***	0.71***
Shannon index 48 h[b]	0.52*	0.65**	0.05	−0.17	−0.45*
Shannon index 60 h	0.55*	0.70***	0.11	−0.14	−0.48**
Shannon index 72 h	0.50*	0.69***	0.12	−0.10	−0.47**
Shannon index 84 h	0.51*	0.71***	0.13	−0.04	−0.40*

SOC Soil organic carbon, *TKN* total Kjeldahl nitrogen, *Fluorescein DA* fluorescein diacetate
[a]Soybean yields
[b]Shannon index at 48, 60, 72 and 84 h of incubation
*, **, ***Significance at $P<0.05$, $P<0.01$, and $P<0.001$, respectively

20 cm depth in 2002 (Fig. 3b). Lower soil depths had highly reduced β-glucosidase at both sites, which was more than 50% less than for surface soils (0–10 cm) (Fig. 3). Generally, β-glucosidase activities were positively correlated to SOC at the Maple site and to TKN at both sites (Table 3).

Fig. 3 Seasonal and spatial patterns of β-glucosidase activities at **a** the Pecan site and **b** the Maple site. *, **, and ***Significance at $P<0.05$, $P<0.01$, and $P<0.001$, respectively, using *t*-test. *NS* not significant

Substrate use: biolog

AWC, SR, and SI were significantly higher at the tree row for surface soils (0–10 cm) at the Pecan site for both years (Fig. 4a, b, and c). At the Maple site, AWC and SR were similarly higher at the tree row in 2001 but not in 2002 (Fig. 4d and e). SI was higher at the middle of the alley in 2002 (Fig. 4f). Additionally, SI was significantly higher at the middle than at the tree row at lower depths in 2002 and only at 30-cm depth in 2001 (data not shown). No differences were observed at lower depths for AWC and substrate richness at both sites. Shannon index values followed the same trend as AWC and substrate richness for surface soils at the Pecan site, for both years, while no differences between positions in the alley were observed at lower depths (data not shown). In both seasons, the middle of the alley at the Maple site had the lowest AWC and substrate richness (Fig. 4).

Principal component analysis (PCA) separated time of sampling but not positions in the alley at the Pecan site (Fig. 5a). At the Maple site, separation between positions in the alley was minimal but there was a seasonal separation for the soils sampled at the middle of the alley (Fig. 5b). The first two principal components (PC) accounted for 54 and 41% at the Pecan and the Maple sites, respectively. PCA only minimally separated the two sites.

AWC and substrate richness were highly correlated at both sites ($r>0.92$, ***), and both of these parameters were negatively correlated to Shannon index ($r=−0.37$ to $−0.53$, *) at the Maple site and but not at the Pecan site. Shannon index was also highly correlated to SOC and TKN at the Pecan site (Table 3). Shannon diversity index was also negatively correlated to soybean yields (Table 3).

Crop yields for Maple site

Maize and soybean yields were generally higher at the middle of the alley than for rows next to silver maple trees. Average maize and soybean grain yields were 6,211 (±648) and 3,031 (±143) kg ha^{-1} for middle rows compared to 1,039 (±1,296) and 787 (±143) kg ha^{-1}, respectively, for
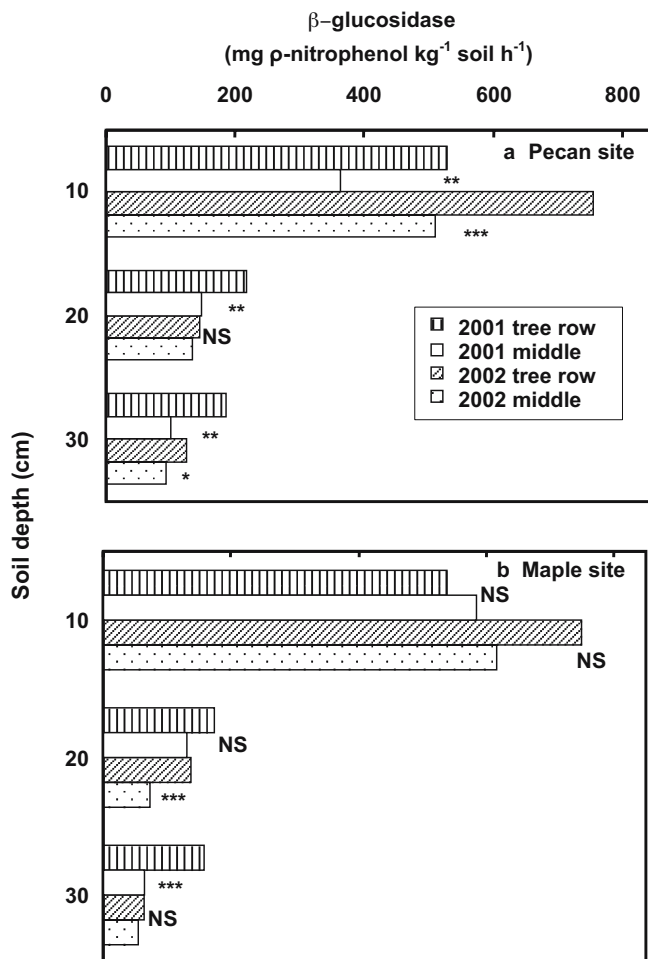
Fig. 4 Average well color (**a**, **d**), substrate richness (**b**, **e**), and Shannon diversity index (**c**, **f**) at the Pecan and Maple sites for surface soils (0–10 cm). *Error bars* represent the least significant differences (*LSD*) at *P*<0.05
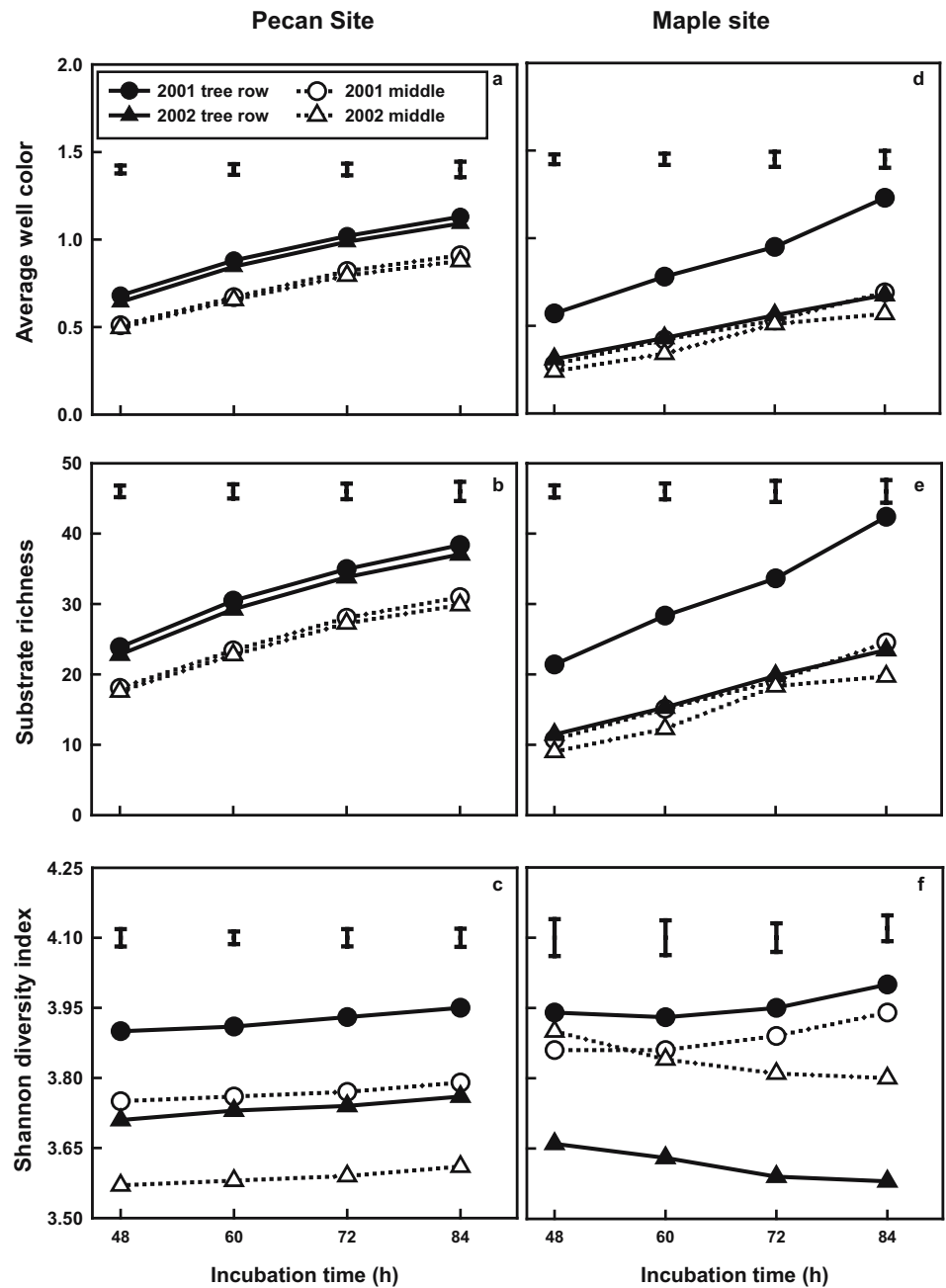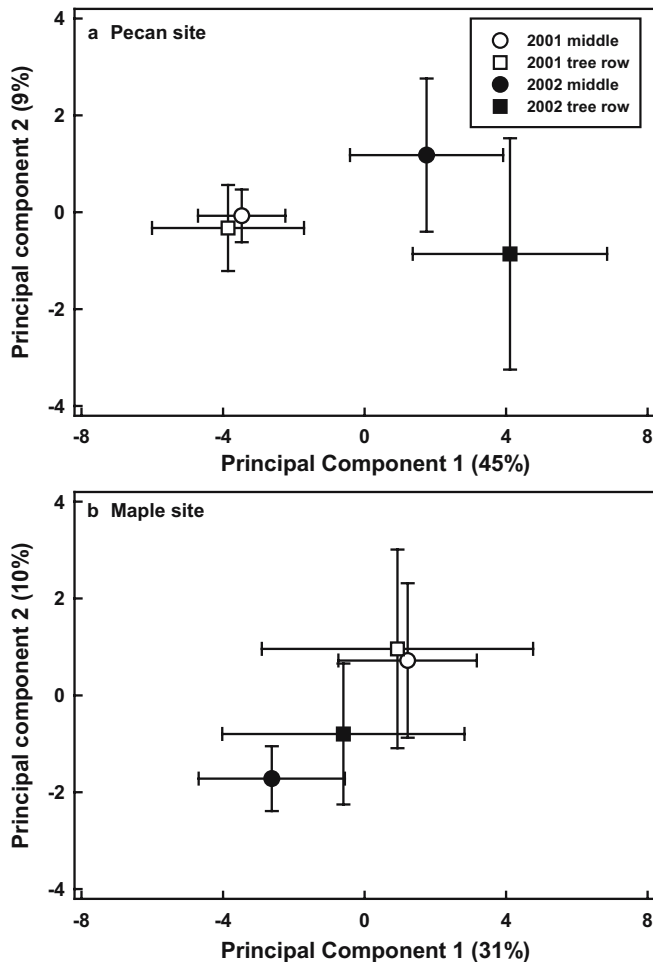

rows next to silver maple trees. The trend observed for yields is the same as that for soil water content (Fig. 2b), possibly implying greater water availability to the crops in the middle compared to those closer to the trees. Soybean yields were positively correlated to enzyme activities (Table 3).

Discussions

Soil enzyme activities and parameters used to describe microbial functional diversity were generally higher at the tree row than at the middle of the alley at the Pecan site. The observed differences may indicate the existence of metabolically different microbial populations with positions in the alley. Microbial functional diversity and activities in soil may be influenced by factors such as the availability and quality of organic substances, soil temperature, and soil water content. Because soil temperature and water content at the Pecan site were comparable between the tree row and the middle of the alley, the availability and quality of litter may have played a more pronounced role in influencing microbial dynamics.

Pecan trees produced lower amounts of litter with a higher C:N ratio than bluegrass (Table 1). Additionally, grasses have a more continuous supply of organic substrates because of the extensive root system. But as perennial grasses may inhibit nitrifying bacteria leading to low nitrate content (Alexander 1977), it is plausible that the area under bluegrass was more N-limited than under pecan trees,

Fig. 5 Principal component analysis of substrate use patterns for surface soils (0–10 cm) at **a** the Pecan site and **b** the Maple site

Results observed at the Maple site were temporally more variable. Soil temperature and water content were higher at the middle of the alley, and within the range that would favor optimum microbial growth (Zak et al. 1999), as reflected by FDA activities (Table 2) and Shannon index (Fig. 4). However, AWC and SR were higher at the tree row than at the middle of the alley. The lack of consistent results may be attributed to the annual variation in soil microbial communities associated with the crops in rotation. Soils sampled in 2001 were sampled after maize harvest, while those in 2002 were sampled during early soybean growth. Differences in litter quality and amounts (Table 1) of maize and soybean crops may influence microbial populations. Additionally, the age of the alley cropping system may influence microbial activities. Lee and Jose (2003) reported lower soil microbial biomass in a 3-year-old pecan–cotton alley cropping system compared to a similar system that was 47 years old. Pecan trees were 21 years old and both components (pecan trees and bluegrass) are perennial in comparison to the maple–maize–soybean system, which was 12 years old.

Organic matter decreases with depth and is usually correlated to microbial activities (Dick et al. 1996) as shown for β-glucosidase activities (Fig. 3) and Biolog parameters. Wick et al. (1998) observed similar reductions in β-glucosidase activities with depth in three agroforestry fields in Nigeria, where—after 4, 10, and 14 years of continuous alley cropping under minimum tillage—no differences were observed among sites in β-glucosidase activities but activities in the 0- to 5-cm depth were significantly higher than at the 5- to 10-cm depth.

Shannon index was highly correlated to TKN at the Pecan site (Table 3). Nitrogen is usually the limiting nutrient in soil for plant growth and for heterotrophic microorganisms. Anthropogenic activities have led to increased N in atmosphere and soils in some parts of Europe and in the northeastern USA. The negative effects of nitrogen saturation include shifts in plant species composition, decreases in species diversity, and leaching of nitrate to surface and ground waters (Vitousek et al. 1997). Studies on the relationship among different forms of N and Biolog Shannon index may provide important insights into how soil bacterial diversity is related to soil N transformations. Davidson et al. (1996) reported that repeated N fertilizer application resulted in development of larger and more active populations of nitrifying bacteria.

Lower crop yields near trees have been associated with reduced solar radiation (Miller and Pallardy 2001) and water availability (Jose et al. 2000) due to shading and competition with trees. Jose et al. (2000) reported a 31% reduction in water uptake by maize during the growing season when maize was intercropped with black walnut trees, and concluded that competition for water is a major concern for temperate alley cropping systems. In this study, however, we did not measure water or nutrient uptake and cannot rule out the role of soil water in affecting the observed differences in yield. Correlations between soybean yields and enzyme activities and Shannon index are interesting. Verstraete and Voets (1977) observed that win-

leading to overall lower microbial activities and functional diversity. Furthermore, different plant species may support functionally distinct microorganisms based on differences in root exudates and decomposition of sloughed off root tissue. Since both bluegrass and pecan are perennial crops, under no-till management, it is possible that the microbial populations associated with each plant species was accustomed to particular levels and cycles of organic substrate, and each microbial community has attained a functional equilibrium, with that of pecan flora utilizing more of the substrates in Biolog ecoplates. Myers et al. (2001) reported that three forest ecosystems which differed in leaf litter quality and quantities supported functionally different microbial communities based on Biolog substrate use patterns.

Seasonal shift in microbial functional diversity observed at the Pecan site (Fig. 5a) may be related to seasonal cycles of nutrient availability (Myers et al. 2001) due to temporal patterns in leaf- and root-derived substrates. In 2001, soils were sampled in the fall, when sloughed-off root tissue and leaf litter are the main substrates, while in 2002 sampling was done in early summer when root exudation may be highest due to rapid plant growth.

ter wheat grain yields were positively correlated to phosphatase activities while sugar beet yields were negatively correlated to urease activities. Correlations between enzyme activities and crop yields may be important in developing a soil biochemical index for assessing soil condition for crop production.

Conclusions

Soil enzyme activities and microbial functional diversity were higher near pecan trees compared to 6 m into the alley cropped to bluegrass. Microbial activities also varied with time at the Pecan site. In contrast, no consistent differences were observed between silver maple trees and alleys cropped to maize in rotation with soybean, despite consistently higher soil temperature and water content in the alley. Results at the Maple site underscore the role of litter quality and quantity in influencing microbial function in contrast to small variations in microclimate, while the results observed at the Pecan site confirm that alley cropping systems may have distinct microflora associated with trees that differ from those of the intercrop. This may have implications for long-term nutrient cycling in alley cropping systems that may require differential nutrient management to maximize productivity of such systems.

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