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Soil Microbial Communities and Enzyme Activities under Various Poultry Litter Application Rates

V. Acosta-Martínez* and R. Daren Harmel

ABSTRACT

The potential excessive nutrient and/or microbial loading from mismanaged land application of organic fertilizers is forcing changes in animal waste management. Currently, it is not clear to what extent different rates of poultry litter impact soil microbial communities, which control nutrient availability, organic matter quality and quantity, and soil degradation potential. From 2002 to 2004, we investigated the microbial community and several enzyme activities in a Vertisol soil (fine, smectitic, thermic, Udic Haplustert) at 0 to 15 cm as affected by different rates of poultry litter application to pasture (0, 6.7, and 13.4 Mg ha^{-1}) and cultivated sites (0, 4.5, 6.7, 9.0, 11.2, and 13.4 Mg ha^{-1}) in Texas, USA. No differences in soil pH (average: 7.9), total N (pasture: 2.01–3.53, cultivated: 1.09–1.98 g kg^{-1} soil) or organic C (pasture average: 25–26.7, cultivated average: 13.9–16.1 g kg^{-1} soil) were observed following the first four years of litter application. Microbial biomass carbon (MBC) and nitrogen (MBN) increased at litter rates greater than 6.7 Mg ha^{-1} (pasture: MBC = >863, MBN = >88 mg kg^{-1} soil) compared to sites with no applied litter (MBC = 722, MBN = 69 mg kg^{-1} soil). Enzyme activities of C (β-glucosidase, α-galactosidase, β-glucosaminidase) or N cycling (β-glucosaminidase) were increased at litter rates greater than 6.7 Mg ha^{-1}. Enzyme activities of P (alkaline phosphatase) and S (arylsulfatase) mineralization showed the same response in pasture, but they were only increased at the highest (9.0, 11.2, and 13.4 Mg ha^{-1}) litter application rates in cultivated sites. According to fatty acid methyl ester (FAME) analysis, the pasture soils experienced shifts to higher bacterial populations at litter rates of 6.7 Mg ha^{-1}, and shifts to higher fungal populations at the highest litter application rates in cultivated sites. While rates greater than 6.7 Mg ha^{-1} provided rapid enhancement of the soil microbial populations and enzymatic activities, they result in P application in excess of crop needs. Thus, studies will continue to investigate whether litter application at rates below 6.7 Mg ha^{-1}, previously recommended to maintain water quality, will result in similar improved soil microbial and biochemical functioning with continued annual litter application.

Poultry litter has been used as a fertilizer and soil amendment for corn, small grain, fruit, forage grass, and vegetable production in the United States for decades. The application of animal manures and litters to agricultural soils can be an excellent alternative resource utilization that improves crop yields (Ginting et al., 1998) and produces potentially beneficial shifts toward a new equilibrium in soil physical (Sommerfeldt and Chang, 1985; Sommerfeldt et al., 1988; Haynes and Naidu, 1998), chemical (Whalen and Chang, 2002), and biological (Ritz et al., 1997; Peacock et al., 2001; Parham et al., 2002) properties. However, negative impacts to soil and water quality are also possible from mismanaged land application of animal manures. Long-term and/or high rate application of poultry litter or manure can produce elevated soil test phosphorus (P) levels, which increases the potential of P transport to surface water (James et al., 1996; Vadas and Sims, 1998; Harmel et al., 2004). In recent years, as a result of the shift to fewer and larger confined animal operations, environmental and economic issues associated with utilization or disposal of animal manures and litters have become a focal point of conservation efforts (USDA and USEPA, 1999; Ribaudo et al., 2003). The potential excessive nutrient and/or microbial loading from mismanaged land-applied organic fertilizers has drawn increased media, public, and regulatory attention forcing changes in the animal waste management, especially in the rates of application.

Although land application is the most common and usually most desirable method of utilizing manure because of nutrient and organic matter addition to soils (USDA and USEPA, 1999), it is not clear to what extent poultry litter application impacts soil microbial communities. The composition of the soil microbial communities strongly affects the potential of a soil for enzyme-mediated substrate catalysis (Kandeler et al., 1996). These communities, and enzymes as mediators, control nutrient availability, organic matter quality and quantity, and soil degradation potential, and thus, control soil quality and functioning. Thus, changes in soil microbial community size and composition and enzyme activities due to poultry litter application can impact environmental quality. Because of the economic and environmental implications of managing poultry by-products, the microbial effects of applying poultry litter to soils warrant further investigation.

The present investigation is a component of a broader study on the economical and environmental impacts of poultry litter application to pasture and cultivated agricultural sites in the Texas Blackland Prairies ecosystem. Our objective was to investigate selected soil microbiological, chemical, and biochemical effects of applying various rates of poultry litter to pasture and cultivated soils. Specifically, the microbial biomass C and N, microbial community structure as indicated by fatty acid methyl ester (FAME) profiles, and several enzymatic activities key to C, N, S, and P availability were determined during the first four years of poultry application to pasture and cultivated sites.

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Abbreviations: FAME, fatty acid methyl ester; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; PCA, principal component analysis.

MATERIALS AND METHODS

Site Description

This study was conducted at the Grassland Soil and Water Research Laboratory near Riesel, TX, USA. The laboratory is located in the Texas Blackland Prairies ecosystem, an important agricultural region encompassing 4.45 million ha (Fig. 1). The region is known for its Houston Black clay soils (fine, smectitic, thermic, Udic Haplustert), which exhibit a strong shrink swell potential. Slopes generally range from 1 to 4% and are classified as gently rolling. Land use in the region currently consists of improved pasture, rangeland, and corn, wheat, sorghum, and oat production under a wide range of tillage and management operations. The region also contains rapidly growing metropolitan populations in the Dallas–Fort Worth, Austin, and San Antonio areas (United States Census Bureau, 2001).

Land Management

A total of nine fields (six cultivated and three pasture) were used in this study (Fig. 1). The cultivated sites ranged in size from 4.0 to 8.4 ha, and the pasture sites ranged from 1.2 to 8.0 ha. Background soil and water data obtained before litter application indicated no significant inherent differences between sites before litter application (Harmel et al., 2004). For example in the pasture sites in 2000, soil organic C was about 25.1 g kg^{-1}, total nitrogen ranged only from 2.13 to 2.28 g kg^{-1}, and soil pH ranged from 7.5 to 7.7 (Table 1). For the six cultivated sites in 2000, soil organic C ranged from 12.4 to 15.3 g kg^{-1}, total N ranged from 1.09 to 1.28 g kg^{-1}, and soil pH was 7.7. The study sites were managed the same within each land use category (pasture and cultivated) beginning in 2000. In 2000–2001, no fertilizer was applied and no crops were planted in the cultivated sites. Beginning in 2001, the cultivated sites received one annual application of poultry litter at rates of 0, 4.5, 6.7, 9.0, 11.2, or 13.4 Mg ha^{-1}, and the three pasture sites received rates of 0, 6.7, or 13.4 Mg ha^{-1}. Litter application on pasture and cultivated sites occurred in July 2001, September 2002, September 2003, and September 2004.

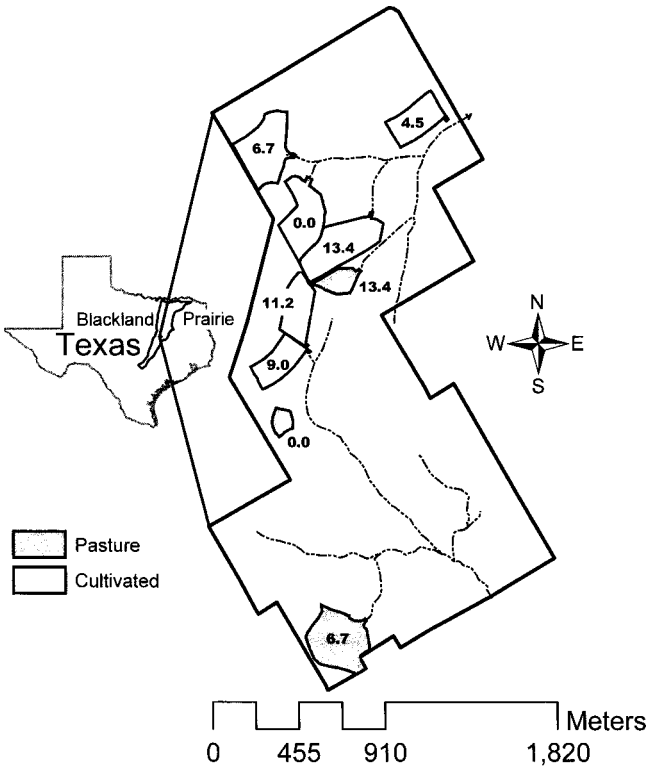

Fig. 1. This study investigated the soil microbial populations and enzyme activities in pasture and cultivated sites located in the Texas Blackland Prairie ecosystem, an important agricultural region encompassing 4.45 million ha. The region is known for its Houston Black Clay soil (fine, smectitic, thermic, Udic Haplustert), which is the representative soil for Texas, USA.

Management practices common to the region were employed as described in Harmel et al. (2004). In brief, the cultivated sites were under a 3-yr rotation of corn (*Zea mays* L.)–corn–wheat (*Triticum aestivum* L.) with conservation tillage. In the corn years, varying rates of supplemental N were applied to balance the available N rates across treatments. No supplemental N was applied in the wheat year. Corn was generally planted in March and harvested in August. After harvest, corn stalks were shredded. In the wheat year, wheat was planted in October and harvested in May and June. A combination of tillage and herbicides was used to control weeds. Poultry litter was surface applied and incorporated into the top 7 cm of the soil with a disc and/or field cultivator.

In the pasture sites, poultry litter was applied to the soil surface and not incorporated. These sites received no supplemental fertilizer, but herbicide was applied once per year in the spring to control weeds. These sites were managed as improved pasture with a monoculture of coastal Bermudagrass [*Cynodon dactylon* (L.) Pers.] or kleingrass (*Panicum coloratum* L.). Vegetative growth was removed either by grazing or hay production.

Poultry Litter Sampling and Analysis

Each year at the time of litter application, litter samples were collected in the field, transferred to a freezer, and stored at 0°C. The litter was then analyzed for moisture content by drying at 40°C for 24 h, total and extractable N and P, and organic C content (Table 2). An average from four years (2001–2004) showed that the poultry litter contained 39.9 g total N kg^{-1}, 33.3 g total P kg^{-1}, 29.2 g organic C kg^{-1}, 461 mg NO_3–N kg^{-1}, 3147 mg NH_4–N kg^{-1}, and 927 mg of soluble reactive phosphorus (SRP) kg^{-1}.

Soil Sampling and Analysis

Selected basic soil properties were determined each year from 2000 (before the initiation of the study) to 2004 in all the sites (Table 1). For microbiological analyses, soil samples were taken annually in November 2002, 2003, and 2004 following the second, third, and fourth annual litter application. From each site, three sampling locations were randomly selected but were adjusted to maintain at least a 50-m separation. At each sampling location (field replication), a composite sample comprised of ten 0- to 15-cm depth cores (2.54-cm diameter) was obtained. Samples were cooled with ice packs in the field immediately after collection. The samples were then sieved through a 5-mm mesh screen and stored at 4°C. Microbiological analyses were performed within the same month of sampling.

The microbial biomass carbon (MBC) and nitrogen (MBN) were determined on a 15-g oven-dry equivalent field-moist soil sample (<5 mm) by the chloroform-fumigation–extraction method (Vance et al., 1987). In brief, organic C and N from the fumigated (24 h) and non-fumigated (control) soil were quantified by a CN analyzer (Model TOC-V/CPH-TN; Shimadzu, Kyoto, Japan). The non-fumigated control values were subtracted from the fumigated values. The MBC and MBN were calculated using a kEC factor of 0.45 (Wu et al., 1990) and kEN

Table 1. Chemical properties of the soils studied under different rates of poultry litter amendments.

	Pasture			Cultivated					
	Poultry litter amendment rate (Mg ha^{-1})								
Soil property†	0	6.7	13.4	0	4.5	6.7	9.0	11.2	13.4
				December 2000 (background)					
TC, g kg^{-1}	45.70	58.20	51.40	39.10	33.30	27.00	45.10	39.80	44.70
OC, g kg^{-1}	25.10	25.10	25.20	15.30	13.20	14.50	12.40	13.60	14.60
TN, g kg^{-1}	2.28	2.13	2.15	1.28	1.24	1.28	1.09	1.14	1.19
TP, g kg^{-1}	0.57	0.59	0.57	0.57	0.66	0.52	0.54	0.49	0.57
pH (H_2O)	7.5	7.6	7.5	7.7	7.7	7.7	7.7	7.7	7.7
				January 2002 (first application)					
TC, g kg^{-1}	38.50	57.10	47.00	45.50	36.40	24.50	48.60	40.50	51.30
OC, g kg^{-1}	22.90	28.80	22.90	15.40	16.40	14.00	13.20	14.10	16.00
TN, g kg^{-1}	2.01	2.62	2.11	1.25	1.43	1.25	1.15	1.27	1.37
TP, g kg^{-1}	0.41	0.62	0.78	0.67	0.74	0.56	0.69	0.71	0.68
pH (H_2O)	7.8	7.8	7.8	8.0	8.0	7.8	8.0	7.9	7.9
				November 2002 (second application)					
TC, g kg^{-1}	44.00	61.80	47.30	41.90	34.90	25.90	48.90	40.60	48.30
OC, g kg^{-1}	23.80	29.60	23.80	14.60	15.20	14.70	13.90	14.60	16.50
TN, g kg^{-1}	3.48	3.16	3.53	1.21	1.06	1.11	1.96	1.98	1.91
TP, g kg^{-1}	0.59	1.27	1.28	1.35	0.91	1.02	1.03	0.89	1.06
pH (H_2O)	7.6	7.6	7.6	7.7	7.6	7.6	7.7	7.8	7.7
				November 2003 (third application)					
TC, g kg^{-1}	40.40	51.70	51.40	41.40	39.80	32.60	49.50	44.70	42.70
OC, g kg^{-1}	22.20	24.10	28.70	16.90	16.20	14.70	12.80	14.20	16.80
TN, g kg^{-1}	2.13	2.64	2.06	1.72	1.98	1.81	1.49	1.70	2.15
TP, g kg^{-1}	0.40	0.54	0.55	0.58	0.63	0.57	0.57	0.71	0.82
pH (H_2O)	8.1	7.9	8.0	8.0	8.0	8.0	8.0	8.0	7.9
				November 2004 (fourth application)					
TC, g kg^{-1}	40.40	44.70	46.60	40.30	33.30	33.00	50.30	39.30	44.70
OC, g kg^{-1}	25.90	25.20	28.90	16.20	17.30	16.70	14.10	15.20	17.00
TN, g kg^{-1}	2.25	2.74	2.50	0.93	1.01	0.86	1.09	1.03	1.03
TP, g kg^{-1}	0.20	0.41	0.56	0.32	0.43	0.36	0.53	0.49	0.46
pH (H_2O)	8.4	7.7	7.8	7.9	8.1	7.9	8.1	7.8	8.0

† TC, total carbon; OC, organic carbon; TN, total nitrogen; TP, total phosphorus.

factor of 0.54 (Jenkinson, 1988), respectively. Each sample had duplicate analyses and results were expressed on a moisture-free basis.

The assay procedures for the activities of β-glucosidase, α-galactosidase, arylsulfatase, and alkaline phosphatase are described in Tabatabai (1994), and the assay procedure for β-glucosaminidase activity is described in Parham and Deng (2000). The enzyme activities were assayed (<5 mm air-dried soil) at their optimal pH values in duplicates including one control.

Fatty acids were extracted from the soil samples following the MIDI (Microbial ID, Inc., Newark, DE) protocol as previously applied to soil analyses (Acosta-Martínez et al., 2004a, 2004b). Briefly, 3-g (<5 mm field moist) soil samples were treated according to the four steps of the MIDI protocol for biological samples: (i) saponification of fatty acids at 100°C with 3 mL 3.75 *M* NaOH in aqueous methanol (methanol to water ratio = 1:1) for 30 min, (ii) methylation (esterification) at 80°C in 6 mL of 6 *M* HCl in aqueous methanol (1:0.85) for 10 min, (iii) extraction of the FAMEs with 3 mL of 1:1 (v/v) methyl-*tert*-butyl ether/hexane by rotating the samples end-over-end for 10 min, and (iv) washing of the solvent extract with 1.2% (w/v) NaOH by rotating the tubes end-over-end for 5 min. The FAMEs were analyzed in a 6890 GC Series II (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and a fused silica capillary column (25 m × 0.2 mm) using ultra high purity hydrogen as the carrier gas. The temperature program was ramped from 170°C to 250°C at 5°C min^{-1}. The FAMEs were identified, and their relative peak areas (percentage) were determined with respect to the other FAMEs in a sample using the MIS Aerobe method of the MIDI system. The FAMEs are described by the number of C atoms, followed by a colon, the number of double bonds and then by the position of the first double bond from the methyl (ω) end of molecules, *cis* isomers are indicated by c, and branched fatty acids are indicated by the prefixes *i* and *a* for iso and anteiso, respectively.

Table 2. Characteristics of poultry litter applied to the soils.†

		Total N		Total P			Water extractable		
Date applied	Water	Moist	Dry basis	Moist	Dry basis	Organic C	NO_3–N	NH_4–N	SRP‡
	%	g kg^{-1}					mg kg^{-1}		
July 2001	49.5	23.2	46.0	21.4	42.3	284	211	1170	895
September 2002	9.8	30.5	33.8	34.7	38.6	312	857	3775	1234
September 2003	32.1	32.7	48.2	16.7	24.5	289	265	4726	778
September 2004	28.0	22.7	31.7	19.9	27.8	284	510	2917	799
Average	29.9	27.3	39.9	23.2	33.3	292	461	3147	927

† Values reported are average of n = 4 to 6 replications.
‡ Soluble reactive phosphorus.

Statistical Analyses

In this study, the treatments included land use (cultivated and pasture) and litter rates from 0 to 13.4 Mg ha^{-1}. Statistical analyses, including ANOVA and mean separation by least significant differences, were performed for the cultivated and pasture sites using the general linear model procedure of the SAS system (SAS Institute, 1999) to determine significant effects by the different poultry litter application rates. In the FAME analysis, indicators of fungal (18:1ω9c, 18:2ω6c and 18:3ω6c) and bacterial (Gm+: 15:0, *a*15:0, *i*15:0, *a*17:0, *i*17:0; Gm−: cy17:0, cy19:0; actinomycetes: 10Me16:0, 10Me17:0) groups were evaluated with the PC-ORD statistical software (Version 4) to determine differences in their relative abundance at various litter rates (McCune and Mefford, 1999). The data was examined by principal component analysis (PCA) using cross-products matrix with variance/covariance centered, and calculating scores for FAMEs by weighted averaging.

RESULTS

Microbial Biomass Carbon and Nitrogen

The ANOVA showed significant effects of poultry litter application on soil MBC ($P < 0.05$) (Fig. 2A). The soil MBC did not significantly increase in the pasture sites with applied litter in 2002 but did increase compared to the untreated control after the third and fourth poultry litter application in 2003 and 2004. For the cultivated sites in 2002, MBC exceeded the control for the three greatest poultry litter rates (9.0, 11.2, 13.4 Mg ha^{-1}). In 2003 and 2004, MBC was greatest for rates exceeding 4.5 Mg ha^{-1}. Unexpectedly, the addition of 13.4 Mg litter ha^{-1} to cultivated soils in 2003 increased MBC to comparable levels as observed in non-treated pasture soils.

Poultry litter application also affected soil MBN significantly ($P < 0.001$) (Fig. 2B). Soil MBN content in pasture sites under the highest litter application rate (13.4 Mg ha^{-1}) exceeded the control in every year of this study, but results for the 6.7 Mg ha^{-1} rate were variable. For the cultivated sites, however, MBN results did not exhibit increasing trends with application rate due to balanced available N application rates across treatments. In 2002, only the highest application rate increased MBN compared to the untreated control and produced levels similar to the non-treated pasture site. All application rates increased MBN in 2003 compared to the control but only rates exceeding 4.5 Mg ha^{-1} increased MBN in 2004.

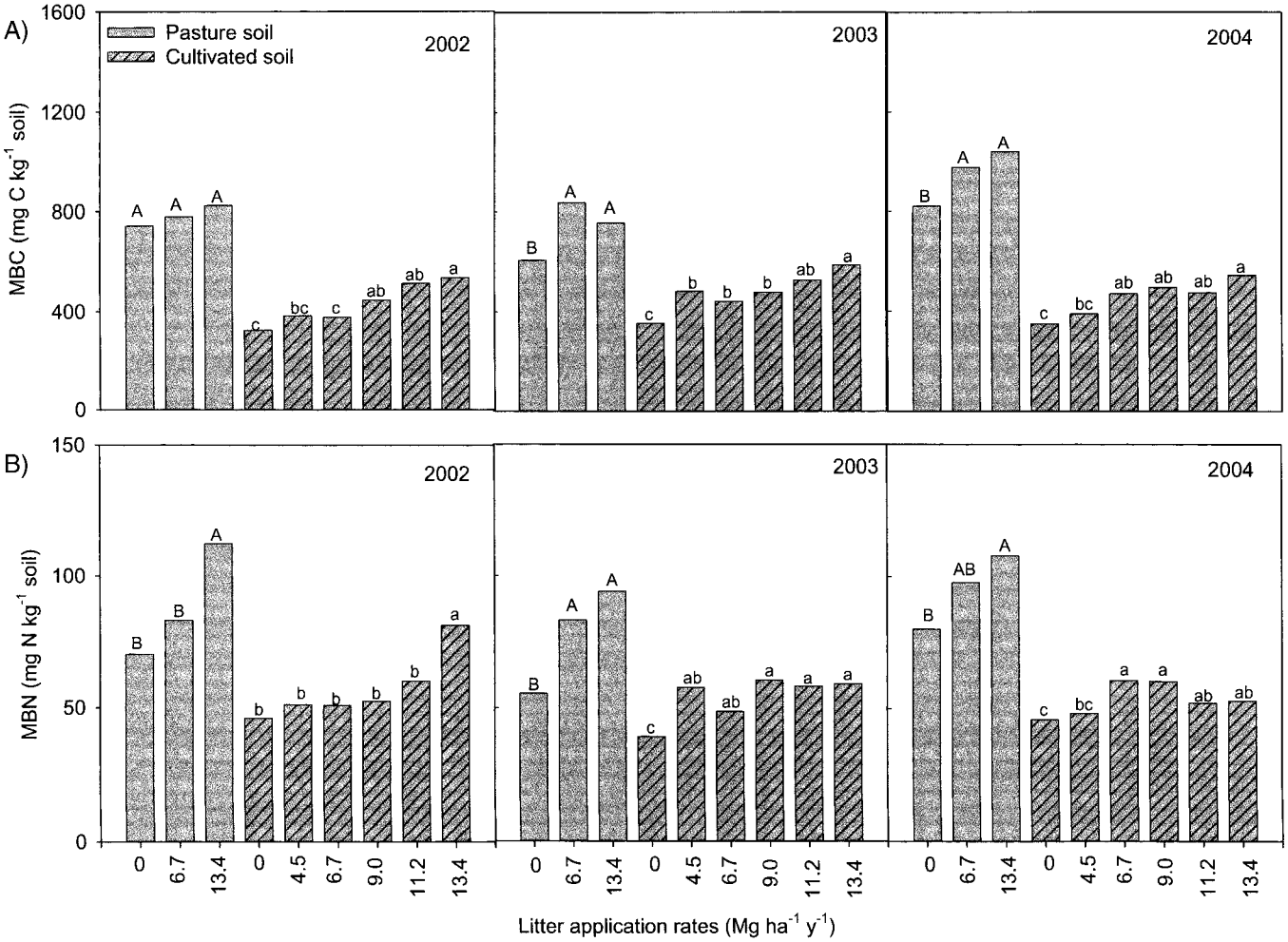

Fig. 2. Soil microbial biomass carbon (A) and microbial biomass nitrogen (B) in pasture and cultivated sites as affected by different rates of poultry litter applications in 2002, 2003, and 2004 following the second, third, and fourth annual litter application, respectively. Bars with different letters are significantly ($P < 0.05$) different.

Both MBC and MBN were significantly higher in the pasture soils compared to the cultivated soils ($P < 0.001$). Although we found significant increases in the microbial biomass due to poultry litter application to the cultivated soils compared to the control, the increases were not proportional to the increased poultry litter application rates.

Enzyme Activities

For the pasture sites, the activities of both glycosidases (β-glucosidase and α-galactosidase) were significantly greater under litter application as compared to the untreated control (Fig. 3A and 3B). For the cultivated sites, β-glucosidase activity significantly increased at rates greater than 6.7 Mg ha^{-1} after 2002 (Fig. 3A). The activity of α-galactosidase significantly increased due to litter application, except at the 4.5 Mg ha^{-1} rate in 2002 and 2004 (Fig. 3B). Regression analyses showed that the activities of the two glycosidases increased with increasing poultry litter application rates ($r > 0.55$, $P < 0.01$). The β-glucosidase activity was not as consistent across the study years as α-galactosidase activity in the untreated pasture or cultivated sites.

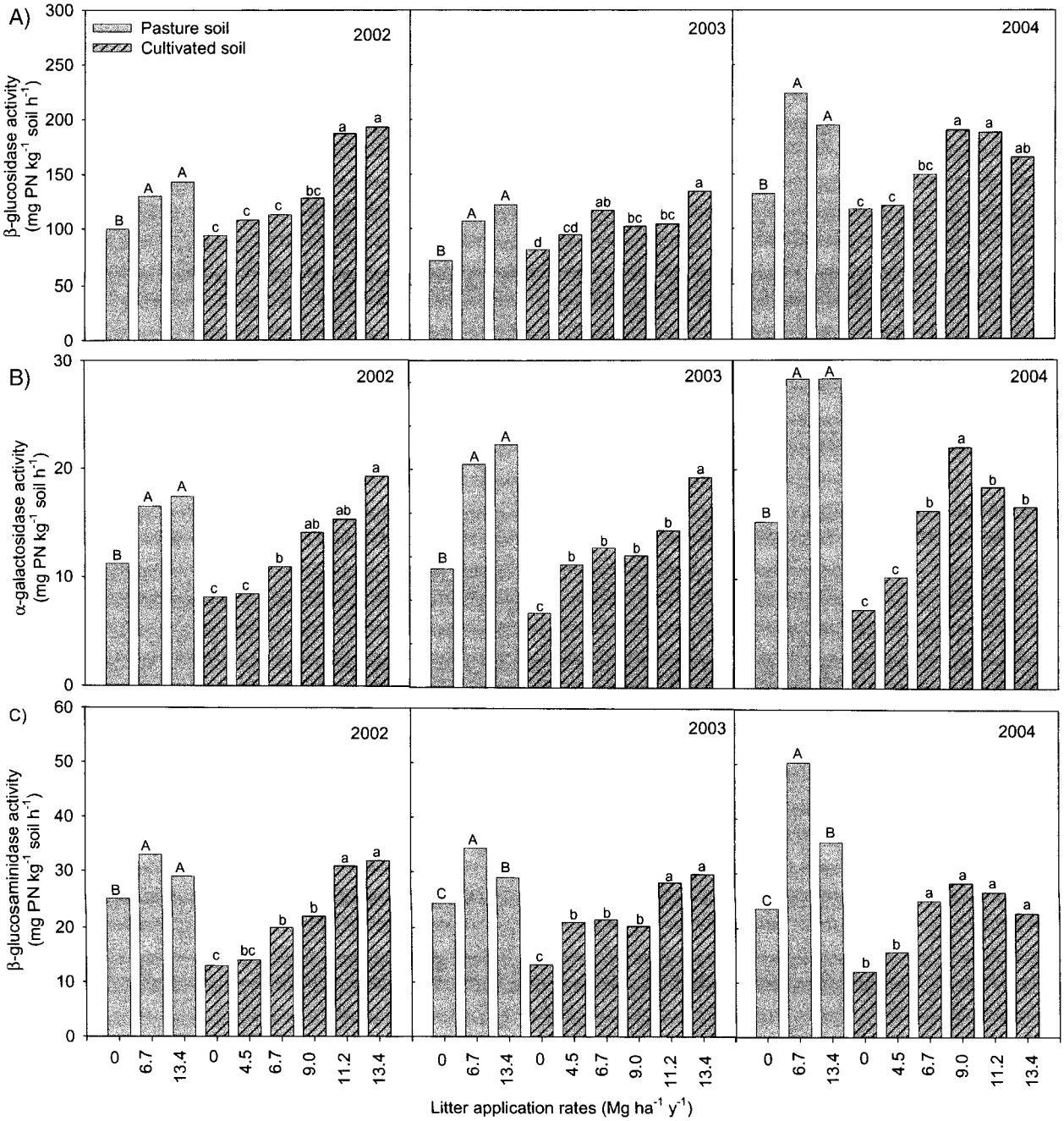

Fig. 3. The activities of soil β-glucosidase (A), α-galatosidase (B), and β-glucosaminidase (C) in pasture and cultivated sites as affected by different rates of poultry litter amendments in 2002, 2003, and 2004 following the second, third, and fourth annual litter application, respectively. Bars with different letters are significantly ($P < 0.05$) different.

Interestingly, β-glucosaminidase activity increased more in pasture sites with the 6.7 Mg ha^{-1} rate than at the highest rate of 13.4 Mg ha^{-1} (Fig. 3C). For cultivated soils, this enzyme activity typically exceeded that of the control at rates greater than 4.5 Mg ha^{-1}. Regression analyses demonstrated that β-glucosaminidase activity increased with increasing litter rates in 2002 ($r = 0.59$, $P < 0.01$) and 2003 ($r = 0.49$, $P < 0.01$) but not in 2004. The activity of β-glucosaminidase was constant in the control pasture and cultivated sites over the three study years.

A three-dimensional plot for the activities of β-glucosaminidase, β-glucosidase, and α-galactosidase as a group showed a separation of the cultivated sites with litter rates of 0 and 4.5 Mg ha^{-1} from the rest of cultivated sites with higher application rates and from all pasture sites (Fig. 4). Similar trends were observed in 2004 after four annual litter applications, but the enzyme activities at the 6.7 Mg ha^{-1} cultivated treatment were slightly greater than for the pasture control. These plots showed that four annual litter applications at 6.7 Mg ha^{-1} were required to increase these enzyme activities at cultivated sites to levels comparable to non-treated pasture.

The increases of arylsulfatase and alkaline phosphatase activities with increasing litter rate were not as consistent as observed for the other enzyme activities (Fig. 5A and 5B). For example, alkaline phosphatase activity under high litter rates (11.2 and 13.4 Mg ha^{-1}) was greater than the control in 2002 and 2004, but no differences were observed in 2003. Similar trends were observed for arylsulfatase activity. The ANOVA demonstrated that these enzyme activities involved in P (alkaline phosphatase) and S (arylsulfatase) soil cycling were greater in soils at pasture sites than at cultivated sites as observed for the other enzyme activities. Alkaline phosphatase activity was consistent in the untreated pasture and cultivated soils during this study, but arylsulfatase activity fluctuated annually.

Microbial Community Structure

Preliminary evaluation of the FAME data obtained with the MIDI protocol system revealed between 1.5 and 2 times higher total peak area of FAMEs named in pasture soils under poultry litter application compared to the untreated control soils (data not shown). Similarly, we observed between 1.5 and 2.2 times higher total peak area of FAMEs named in cultivated soils with applied litter compared to the control soils. We did not, however, observe increments proportional to increased litter application rates.

The higher total peak area of FAMEs named in the litter treated soils was attributed to an increase in the relative abundance of most FAMEs compared to the control. The PCA obtained for pasture sites revealed that the control site contained similar soil microbial community structure every year with generally high fungal to

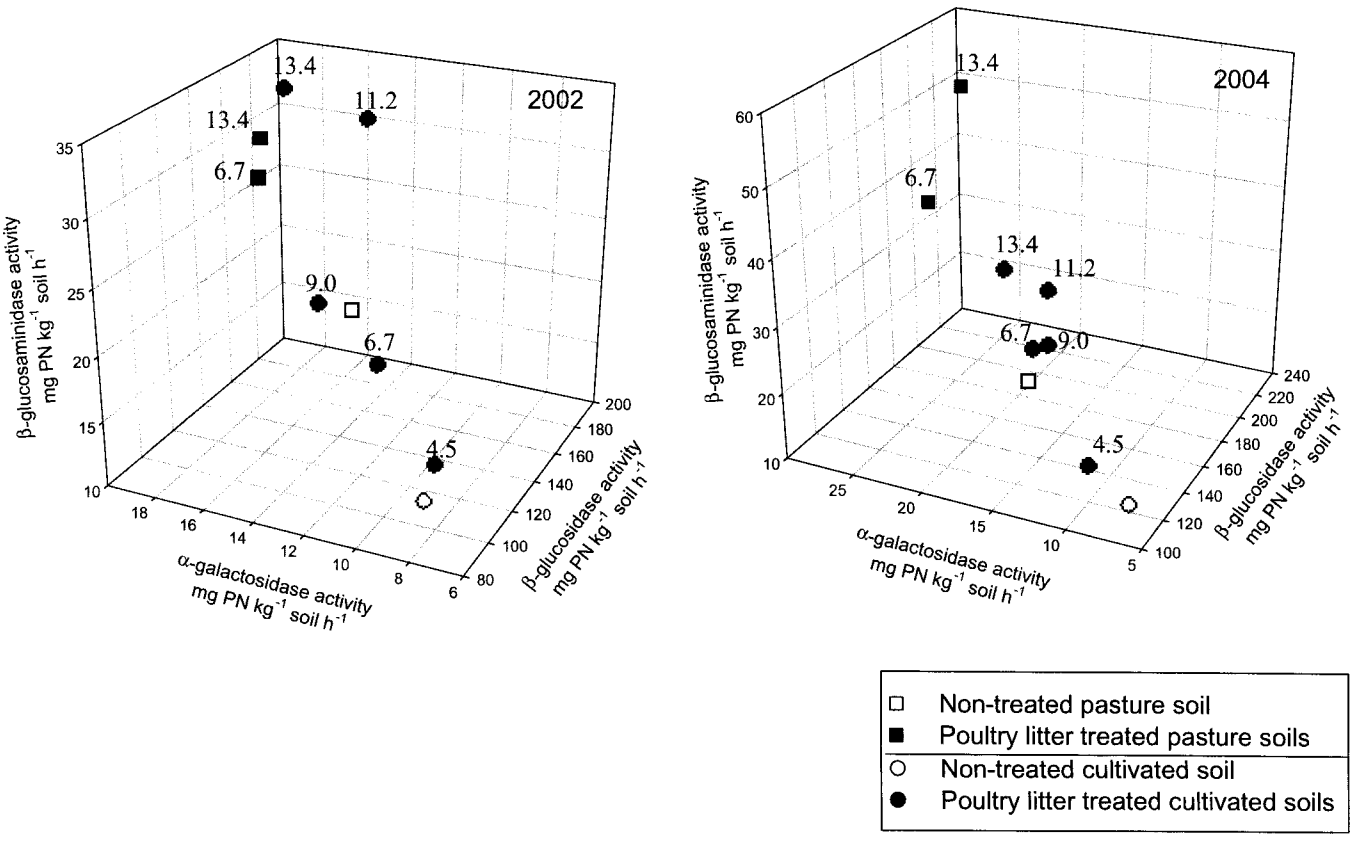

Fig. 4. Three-dimensional plots for the group of soil β-glucosidase (*y* axis), α-galactosidase (*x* axis), and β-glucosaminidase (*z* axis) activities as affected by different rates of poultry litter amendments. Plots show the results in 2002 and 2004 following the second and fourth annual litter application, respectively.

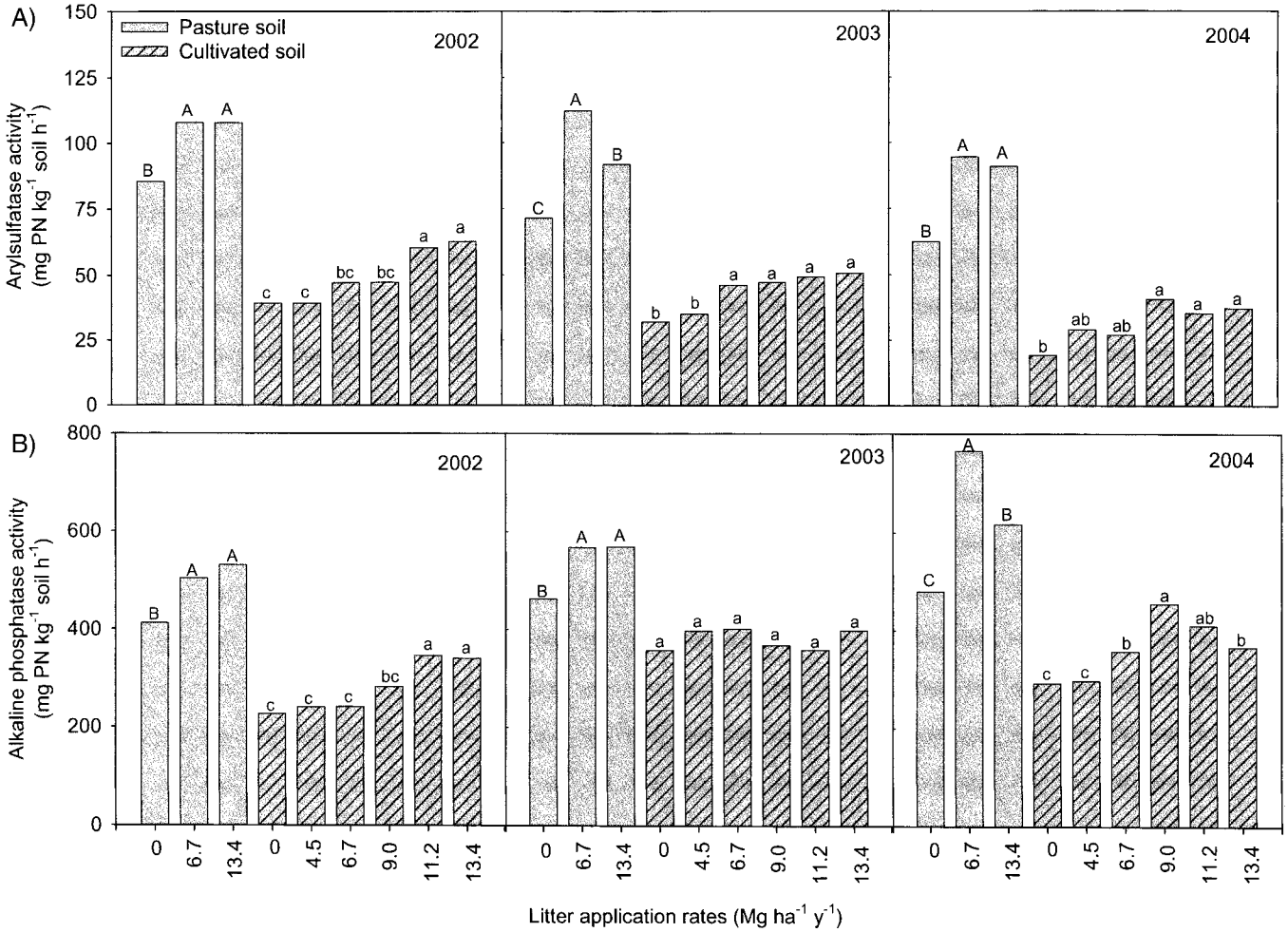

Fig. 5. Soil arylsulfatase activity (A) and alkaline phosphatase activity (B) in pasture and cultivated sites as affected by different rates of poultry litter amendments in 2002, 2003, and 2004 following the second, third, and fourth annual litter application, respectively. Bars with different letters are significantly ($P < 0.05$) different.

bacterial (F:B) ratios (Fig. 6). In 2002 and 2003, highest soil bacterial populations were shown at the 6.7 Mg ha^{-1} rate, but higher fungal populations occurred at the 0 (control) and the 13.4 Mg ha^{-1} rate sites. However, higher bacterial populations were found at the two treated sites studied (6.7 and 13.4 Mg ha^{-1}) compared to the control in 2004. The PCA obtained for cultivated soils showed microbial community shifts to higher fungal populations at the 9.0, 11.2, and 13.4 Mg ha^{-1} litter rates (Fig. 7).

DISCUSSION

Nutrient contents of poultry litter are among the highest of all animal manures, and the use of poultry litter as a soil amendment for cultivated soils can provide appreciable quantities of all important plant nutrients (Sims and Wolf, 1994). This may explain the observed higher soil microbial biomass C and N in the pasture and cultivated sites after only four poultry litter applications. Because soil organic C was generally similar between sites within the same land use category, changes in the microbial biomass are expected to foreshadow changes in organic matter quality and quantity in soils with continued litter application. Other studies have reported a significant correlation between the annual rate of manure applied and the increase in organic matter content at the 0- to 15-cm depth in the long term (e.g., Sommerfeldt and Chang, 1985). Information on soil microbial biomass responses to poultry litter application is limited, but previous studies have found increases of MBC in cultivated soils with the application of municipal solid wastes (Pascual et al., 1999) and cattle manures (Ritz et al., 1997; Parham et al., 2002). A recent study by Bittman et al. (2005) also found increases in MBN in pasture soils with manure application compared to the untreated and inorganic fertilizer-treated counterparts. In addition, Forge et al. (2005) reported that manure applications increased microbial turnover and flux of nutrients through the soil food web in the same soils as those studied by Bittman et al. (2005).

Our findings were consistent with the increases in enzyme activities observed due to the application of other organic amendments such as cattle manures (Diaz-Marcote et al., 1995; Pascual et al., 1999) and municipal solid wastes (Pascual et al., 1999) in cultivated soils. The significant increases in β-glucosaminidase, β-glucosidase, and α-galactosidase activities due to poultry litter application represent increases in limiting steps of

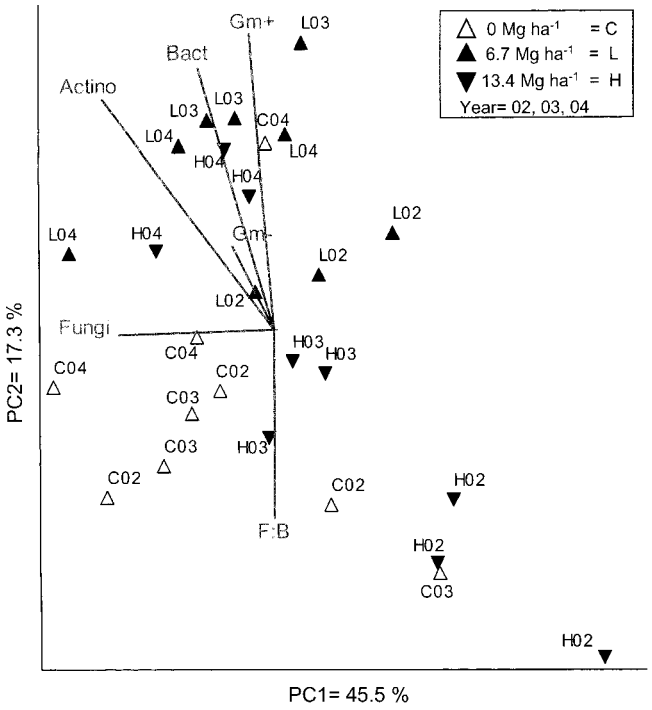

Fig. 6. Principal component analysis (PCA) of the soil microbial community shifts in pasture sites as affected by different poultry litter application rates according to the abundance of fatty acid methyl ester (FAME) indicators of fungal, bacterial, and actinomycetes groups. Each symbol specifies the year of sampling (02 = 2002, 03 = 2003, 04 = 2004), and the litter rates (C = control, L = 6.7 Mg ha^{-1}, H = 13.4 Mg ha^{-1}). The PCA provides a comparison of the fungal to bacterial (F:B) populations ratio among the soils. The soils located near the microbial group specified indicate they have higher relative abundance of such microbial group.

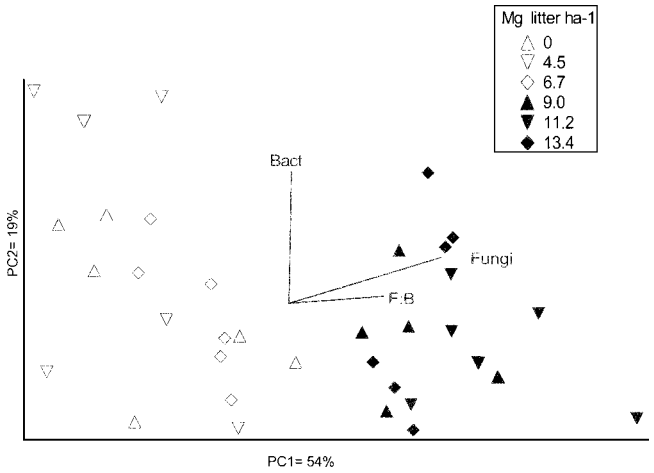

Fig. 7. Principal component analysis (PCA) of the soil microbial community shifts in cultivated sites as affected by different poultry litter application rates for 2003 and 2004 samplings according to the abundance of fatty acid methyl ester (FAME) indicators of fungal, bacterial, and actinomycetes groups. The symbols indicate the different poultry litter application rates applied to the soils (open symbols = 0, 4.5, and 6.7; closed symbols = 9.0, 11.2, and 13.4 Mg ha^{-1}). The PCA provides a comparison of the fungal to bacterial (F:B) populations ratio among the soils. The soils located near the microbial group specified indicate they have higher relative abundance of such microbial group.

chitin, cellulose, and melibiose degradation of soil, respectively, and thus, it is further evidence of the recycling of nutrients from the poultry litter. These soil enzyme activities in the litter treated sites were in some cases twice that of the untreated control. It was evident that enzyme activities were more responsive to litter application than microbial biomass. In our study, disk tillage practices used to incorporate litter at the cultivated sites could have affected soil fungal populations, a major component of the microbial biomass, and thus diminished differences in microbial biomass.

The determination of soil β-glucosidase, α-galactosidase, and β-glucosaminidase activities may have provided an indirect comparison of the effect of litter on the rate of compound degradation. The fact that only β-glucosaminidase activity was greater under poultry litter applications of 6.7 Mg ha^{-1} than under 13.4 Mg ha^{-1} in pasture soils may indicate that the rate of 13.4 Mg ha^{-1} was already too high to be degraded as fast as the rate of 6.7 Mg ha^{-1}; given the circumstances that organic amendments are generally surface applied for pastures and because soil organic C was generally similar in the treated and untreated sites. Our findings could also be due to the fact that β-glucosaminidase activity is involved in degradation of chitin, which is a more complex substrate than the substrates of β-glucosidase (celubiose) and α-galactosidase (melibiose).

In contrast to other enzyme activities, alkaline phosphatase activity (involved in P cycling) and arylsulfatase activity (involved in S mineralization) did not consistently increase with increasing application rates in the cultivated soils, and they were generally highest at the 9.0, 11.2, and 13.4 Mg ha^{-1} rates in the cultivated soils. These findings demonstrate the importance of evaluating several enzyme activities in soils to represent different biochemical reactions, as their response may vary with amendment chemical compositions and application rates. In addition, phosphatases are significantly affected by soil pH, which controls phosphorus availability in soil, and this could occur despite the level of organic matter content or disturbance. Eivazi and Tabatabai (1977) reported that acid phosphatases are more predominant in acid soils and alkaline phosphatases are more predominant in alkaline soils. In an acidic soil with a pH increase from 4.2 to 5.7 due to cattle manure application, Parham et al. (2002) found increases in alkaline phosphatase and phosphodiesterase activities but no response of acid phosphatase activity. Thus, alkaline phosphatase activity did not consistently increase with increasing litter application rates in the cultivated soils studied, in part, due to the fact that the different rates of poultry litter did not affect the soil pH.

The higher microbial biomass and total peak area of FAMEs named in the litter treated soils suggest higher microbial diversity or richness compared to the nontreated counterparts (Parham et al., 2002; Sun et al., 2004). Similar to three-dimensional plots for the group of β-glucosaminidase, β-glucosidase, and α-galactosidase activities, the PCAs revealed that the extent of microbial community shifts were dependent on the litter application rates. In addition, the PCAs demonstrated

that the soil microbial community shifts influenced by poultry litter were dependent on the land use. For example, the microbial community structure under pasture experienced shifts to higher soil bacterial populations at litter rates of 6.7 Mg ha^{-1} and shifts to higher soil fungal populations at litter rates of 9.0, 11.2, and 13.4 Mg ha^{-1} in cultivated sites. Other studies have reported differences in microbial community shifts based on the organic amendment applied (Peacock et al., 2001; Larkin et al., 2005). In the present study, which included only poultry litter application, the differing microbial community shifts can be attributed to inherent differences in organic C, enzyme activities, microbial biomass, and community structure between the pasture and cultivated soils. Previous studies have reported a higher microbial biomass, enzyme activities, and abundance of indicator FAMEs of bacteria, fungi, and protozoa under pasture soils compared to cultivated soils (i.e., Acosta-Martínez et al., 2004b). In addition, the surface application of poultry litter in the pasture sites compared to incorporation in the cultivated sites may have some influence, particularly in the lack of response of the soil microbial community in pasture sites treated with the highest amendment of 13.4 Mg ha^{-1}.

The fact that the microbial community shifts were accompanied by changes in microbial biomass and enzyme activities (Ndiaye et al., 2000; Sun et al., 2004) provided evidence that the microbial community composition strongly influences the potential for enzyme-mediated substrate catalysis in soil (Kandeler et al., 1996). This theory is also supported by significant correlations previously found between fungal populations or microbial biomass and β-glucosaminidase activity (Miller et al., 1998; Parham and Deng, 2000; Acosta-Martínez et al., 2004b), and arylsulfatase activity (Bandick and Dick, 1999).

Other studies have showed that the changes in surface soils following organic fertilizer addition not only depended on amendment characteristics and rates of application but also on the soil types (Larkin et al., 2005; Pérez-Piqueres et al., 2006). The soil used in this study had relatively high clay and organic C contents, which could have led to an early response of the microbial and biochemical parameters during the first four years of poultry litter application. Although continued evaluations are needed to be conclusive, our study demonstrated that the effects of poultry litter amendments on enzyme activities under pasture or cultivated soils increase with increasing poultry litter application rates within a year, specially for the enzyme activities of C and N cycling, but were generally not additive with continued litter application. In terms of long-term trends, Parham et al. (2002) reported that application of cattle manure once every four years for more than 100 years did not cause excessive accumulation of P in the surface 0 to 30 cm but promoted microbiological activities and P cycling.

Several studies have shown that properly managed, land-applied manures can provide valuable nutrients and organic matter for agricultural production systems without harming the environment and public health (Janzen et al., 1999; Harmel et al., 2004). Our results indicated that litter rates of approximately 6.7 Mg ha^{-1} and greater have potential to increase soil microbial properties and enzymatic activities after only four consecutive annual applications compared to untreated soils. While rates greater than 6.7 Mg ha^{-1} provide rapid enhancement of soil microbial populations and enzymatic activities, they result in P levels exceeding crop needs, which leaves the potential for excess P loss in runoff (Harmel et al., 2004). It is expected that litter application at rates below 6.7 Mg ha^{-1}, such as the 2.2 to 4.5 Mg ha^{-1} recommended by Harmel et al. (2004) to maintain water quality, will result in similar improved soil microbial function with continued annual litter application.

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