MICROBIAL COMMUNITY AND ENZYME ACTIVITIES IN PRAIRIE SOIL ECOSYSTEMS UNDER DIFFERENT MANAGEMENT

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«Αυτός ο Κόσμος ο μικρός, ο Μέγας!»

Οδυσσέας Ελύτης

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TABLE OF CONTENTS

Chapter Page

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Natural phenomena together with management can affect soil productivity and sustainability. During the "Dust Bowl" years (1929 to 1941) in the Great Plains of the United States, drought combined with unsustainable farming practices on marginal land resulted in severe wind erosion and decline in soil productivity (Troeh et al., 2004). Although attempts have been made to protect soil ecosystems against degradation, and to ensure sustainable food production for future generations, regional and global assessments indicate that human induced degradation (soil erosion, salinization, soil organic matter decline) causes the loss of millions of hectares of agricultural land every year (Sanders, 1992).

Grasslands are among the most intensively managed ecosystems in the world, providing important land area for rangeland and cultivation (Burke et al., 1997). However, soil disturbances associated with management practices are likely to alter nutrient storage and cycling and may impact the biogeochemistry of grasslands. Understanding biochemical and microbiological responses of grasslands to management practices can help us make science-based decisions to enhance the sustainability of grassland ecosystems.

Management practices such as tillage, cropping patterns, fertilization, grazing, and land clearing, can affect the cycling of energy and matter on a global scale (Doran and Parkin, 1994). Soil influences atmospheric quality through its capacity to produce and consume atmospheric gases such as CO_2, N_2O, and CH_4 (Mosier et al., 1991). It is well established that soil organic matter content is an indicator of soil fertility and quality (Dick and Gregorich, 2004). Research suggests that mechanical cultivation, continuous and intensive growing of crops, and overgrazing decrease soil organic matter content (Burke et al., 1989; Davinson and Ackerman, 1993). Declining soil organic matter could negatively affect many soil physical (structure, infiltration rate, water holding capacity) and chemical properties (cation exchange capacity, fertility, pH), and lead to decline in soil productivity.

However, different management systems cause different degrees of soil disturbance and different effects on soil ecosystem functions. The combination of tillage, cropping, fertilization and manuring could either increase or decrease soil organic matter (Paustian et al., 1997). In addition, these effects vary with soil types, climate zones, and past management histories. One way to approach the problem is to separate it into components. That is, to try to evaluate the basic processes that determine the organic matter cycling, and then analyze how specific management practices affect these processes.

Organic C generally is considered healthy for soils because it improves most functions associated with productivity and sustainability. The level of organic C is determined by the difference between inputs of organic matter and outputs through mineralization (mostly as CO_2), erosion, and leaching (Fig. 1). The controls in

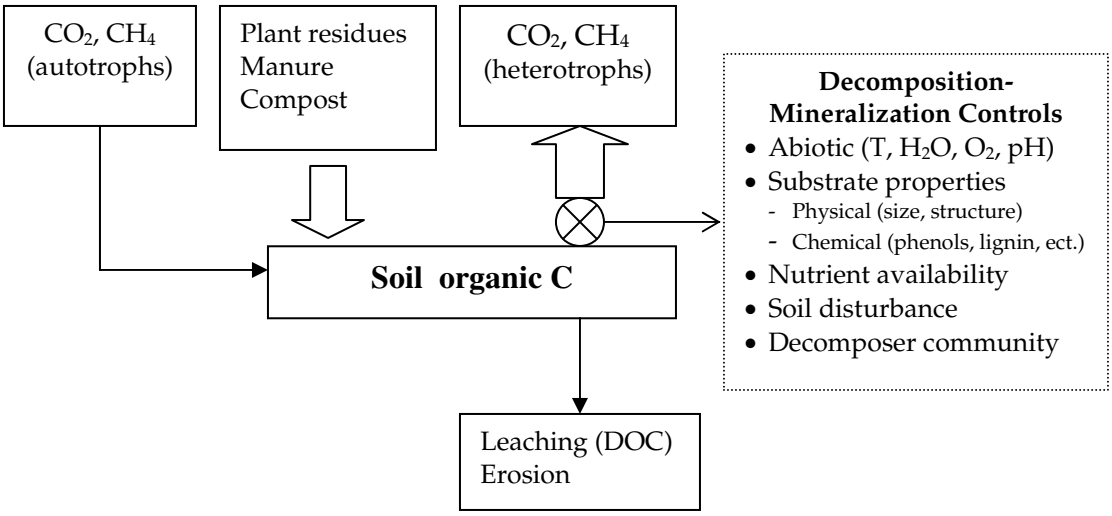

Fig. 1. Simplified inputs and outputs of C from organic matter in soils. The main controls on decomposition regulate CO_2 evolution by heterotrophs (DOC: dissolved organic C) (adapted from Paustian et al., 1997).

decomposition processes are complex and less easily manipulated than are inputs of organic materials. The inputs are above and below ground plant residues, manure, compost, and wastes. The controls include abiotic factors (soil temperature, water, aeration, pH), the physiological nature of organic matter (chemical composition, structure, particle size), the physical exposure of organic materials to decomposers, the availability of mineral nutrients for microbial growth and metabolism, and the nature and composition of the decomposer community, microbial biomass and activity. Soil management affects all of these process controls. A specific management practice could affect more than one control, and multiple controls could act synergistically or competitively (Paustian et al., 1997). Therefore, microbial biomass, activity, diversity,

and community structure are important controls of soil quality (Kennedy and Smith, 1995).

Microbial biomass and activity

Microbial biomass represents the living component of the soil. Microbially mediated processes affect ecosystem functions, including nutrient cycling, soil fertility, soil organic matter turnover, and global C changes. Although microbial biomass accounts for less than 5% of the soil organic matter (Dalal, 1998), it is considered the "eye of the needle" through which all organic matter must eventually pass (Jenkinson and Rayner, 1977).

Because of its multiple key roles in soil, microbial biomass has been used as an indicator to detect the effect of management practices on soil ecosystems (Dalal, 1998). Although the literature is inconclusive, microbial biomass usually declines when forest or grassland soils are cultivated, and increases with crop rotations, reduced tillage practices, and applications of plant residues or other organic amendments (Gil-Sotres et al., 2005; Saviozzi et al., 2001). Specifically, soils under permanent grass contained 50% more microbial biomass and supported approximately 150% more dehydrogenase activity than those under conventional tillage and no-till (Carpenter-Boggs et al., 2003). Soil microbial biomass C decreased with intensive grazing in semiarid grassland soils (Banergee et al., 2000; Sankaran and Augustine, 2004). On the other hand, year round grazing by herds of large herbivores did not deplete soil organic C or soil microbial biomass C (Tracy and Frank, 1998). Moreover, long-term exclusion (more than 50 years) of grazing from

grassland resulted in significant reductions in microbial biomass and activity in the surface soil (Bardgett et al., 1997). Tillage and cropping systems affect microbial biomass C. When a field under wheat-fallow rotation entered to a Conservation Reserve Program, changes in microbial biomass C were not detected seven years after the conversion (Staben et al., 1997). Continuous cotton resulted in reduction of microbial C compared to adjacent perennial pasture (Acosta-Martinez et al., 2004). Ladd et al. (1994) observed reduction in microbial biomass in continuous wheat in an Alfisol after eight years of N fertilizer application. However, continuous cropping resulted in increases in soil microbial biomass compared to a wheat-fallow rotation (Collins et al., 1992). In the Great Plains, 50 years of abandonment from cultivation did not significantly increase microbial C in a field compared to adjacent grasslands (Elliott et al., 1994). In summary, inconsistent results were reported in the literature about the effect of cultivation and grazing on microbial biomass C.

The effects of management on the soil ecosystem can also be reflected by changes in microbial activity. Activity of dehydrogenase, an intracellular enzyme that is active only in viable cells, is a measure of microbial activity. Dehydrogenase activity has been used as an index of soil biochemical activity in the A_p horizon (Bergstrom et al., 2000), and as a sensitive marker of soil degradation and soil microbial activity (Garcia et al., 1997b). Dehydrogenase activity decreased one year after cultivation of grassland or after seeding with native grasses (Dormaar and Willms, 2000). Bergstrom et al. (2000) found that plowing can either increase or decrease dehydrogenase activity. Although soil abandonment initially had a negative influence on biomass C and dehydrogenase activity, their values rose again after 15 years (Garcia et al., 1997a). In a de-intensification

experiment, a 10 to 15% increase in microbial biomass and dehydrogenase activity was detected following shifting from conventional to an integrated farming system for five years. However, the detected differences were not observed 10 years later (Emmerling et al., 2001).

Microbial diversity and community structure

Soil processes such as mineralization and nutrient cycling are carried out by a diverse community of microorganisms that employ many metabolic processes. Responses of microbial community to management practices are also reflected in changes of microbial diversity and community structure. Generally, a decrease in microbial diversity may decrease the microbial functionality in soils if an important group of microorganisms (e.g. nitrogen-fixing microorganisms) is negatively affected (Nannipieri et al., 2002). In other cases, a reduction in any group of species may have little effect on the overall processes because other microorganisms can fulfill the same function (Giller et al., 1998). Cultivation reduces microbial population and diversity (Øvreås and Torsvik, 1998). Buckley and Schmidt (2001) found that microbial community composition does not differ between fields managed conventionally and fields abandoned from cultivation for nine years. Microbial community composition in both of these cases differed from that of fields that had never been cultivated. Allison et al. (2005) found that bacterial communities are under stress in agricultural soil but not in prairie soils, probably due to low C inputs in the agricultural soils. It is not well understood how management practices alter microbial diversity and community structure and the related soil processes. It is

possible that the underlying basis for these changes varies for each specific soil ecosystem.

Soil enzymes

Soil enzymes are proteins, metabolites of microbial cells and plant roots. Certain enzymes can exist only in viable cells (intracellular) providing assessments of the activity of the biological component of the soil. Many other enzymes can exist in both viable cells and as extracellular enzymes in the soil solution or complexed with the soil matrix reflecting the cumulative effect of soil management on soil biology (Dick, 1997). Extracellular and intracellular enzymes play an important role on numerous catalytic reactions. Many soil functions that are related to microbial activity and diversity are also closely related to enzyme activities (Fig. 2). The microbial community of a soil ecosystem determines its potential for substrate catalysis because microbes produce most of the enzymes (Kandeler et al., 1996). Enzyme activities reveal information about the functional diversity and capacity of soil ecosystems, such as the ability to carry out organic C and nutrient cycling. As such, soil enzyme activities have been suggested as indicators of changes or disturbances of the soil ecosystem (Naseby and Lynch, 2002). Land management practices have significant influence on soil enzyme activities. Amidase, arysulfatase, deaminase, invertase, cellulase, and urease activities in grasslands were higher than in adjacent cultivated fields (Bandick and Dick, 1999). The activities of β-glucosidase, β-glucosaminidase, arylamidase, acid and alkaline phosphatase, phosphodiesterase and arysulfatase were lower in cotton fields than in uncultivated native

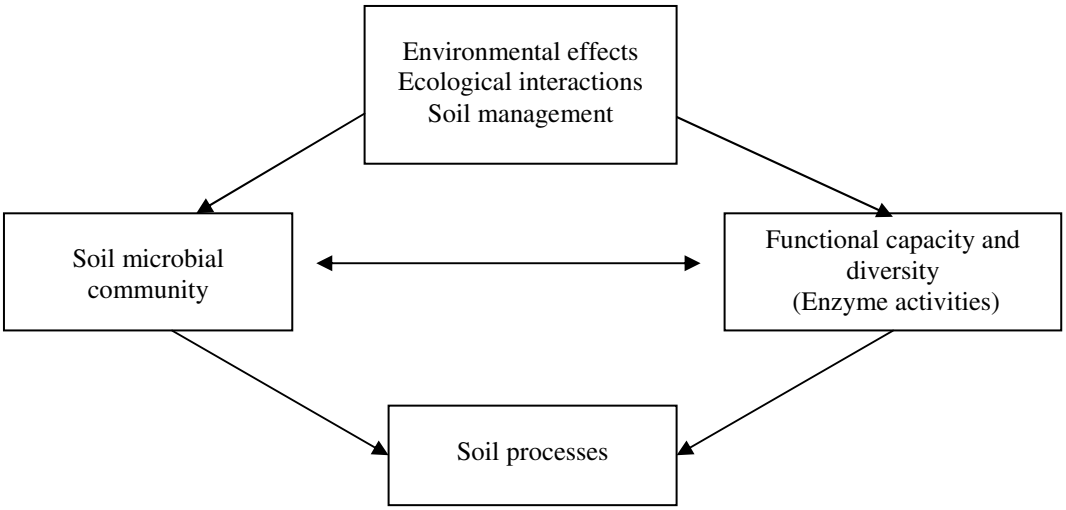

Fig. 2. Potential relations among management, microbial community, and enzyme activities in soil ecosystem (modified from Nannipieri et al., 2002).

grasslands (Acosta-Martinez et al., 2003). Acid phosphatase activity was 150% higher in soil under no-till continuous fertilized corn than under unfertilized conventional tillage; alkaline phosphatase activity was 50% higher in soil under continuous fertilized corn than without fertilizer (Jordan et al., 1995). In addition, enzyme activities decline in proportion to the loss of organic matter (Deng and Tabatabai, 1996a, 1996b, 1997). These findings indicate that enzyme activities can change with management or changes in land use and may be used as indicators of soil functions.

Soil heterogeneity

A soil system is heterogeneous. As an inherent property, heterogeneity of a soil reflects certain pedogenic processes, operating and interacting over a continuum of

spatial and temporal scales (Trangmar et al., 1985). It has been reported that soil properties vary noticeably even in uniformly managed ecosystems (Robertson et al., 1997). Changes in land use may alter the spatial distribution of soil properties at multiple scales. Land use may alter the local patchiness of soil nutrients by disconnecting interactions among microclimate, microtopography, vegetation, and soil biota (Fraterrigo et al., 2005). Soil spatial heterogeneity complicates monitoring and predicting terrestrial ecosystem functions and nutrient transformations. On the other hand, spatial heterogeneity may promote healthy functioning of ecosystems. Therefore, Fuhlendorf and Engle (2001) recommend a management approach to promote above ground heterogeneity for rangelands of the Great Plains. Spatial variability and structure of soil microbial properties may control ecosystem functions, and may be connected to the above ground heterogeneity. It is not surprising that spatial dependency of soil properties affects the estimate of biomass turnover rate (Harden and Joergensen, 2000), nutrient cycling processes (Yanai et al., 2003), and transport of organic pollutants (Søvik and Aagaard, 2003). However, most studies focus on revealing spatial structure of the above ground biota and biomass (Burke et al. 1999; Robertson et al., 1997), and of abiotic soil properties such as moisture, hydraulic conductivity, CEC, and texture (Bruckner et al., 1999; Feng et al., 2004; Søvik and Aagaard, 2003). Lately, several studies were about the heterogeneity of biological soil properties, mostly the microbial community structure (Nunan et al., 2002; Nunan et al., 2003; Ritz et al, 2004). Only a few studies included soil microbial and biochemical properties (Böhme et al., 2004; Cavigelli et al., 2005; Mummey et al., 2002). There is little information on the impact of management on spatial variability of soil microbial properties. Understanding heterogeneity of soil properties

that regulate microbial activity would be useful in evaluating the effects of management on the soil ecosystem.

Objectives and significance

The overall objective of this study was to evaluate the impacts of different management systems on microbial properties and functional capacity related to nutrient cycling in prairie soils. The specific objectives were:

1. To reveal spatial variability and dependence of chemical and microbiological properties in these soils;

2. To evaluate impacts of different management systems on soil properties and activities of enzymes involved in C-, N-, and P-cycling; and

3. To assess impacts of different management systems on microbial community structure and diversity.

Although considerable research effort has been made to elucidate effects of management practices on soil ecosystems, the results are inconsistent because of methodological, and soil and environmental differences. Many of the reported studies were conducted to compare different ecosystems under different environmental and climatic conditions. Little work has been done to evaluate effects of management practices on soil biochemical and microbiological properties in prairie ecosystems. As the most active fractions of soil properties, biochemical and microbiological properties hold the key in understanding nutrient flux and ecosystem function in a global context.

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CHAPTER II

EFFECTS OF MANAGEMENT ON SPATIAL VARIABILITY OF MICROBIAL BIOMASS AND ACTIVITY

Abstract

Microbial activity is critical to soil ecosystem function. Changes in microbial activity and biomass induced by changes in management practices may indicate alteration in ecosystem function and health. Understanding the spatial variability and spatial structure is essential in developing sampling strategies, and for data validation and application. The objectives were: (1) to determine microbial responses to grazing and cultivation in prairie soils; and (2) to reveal spatial variability and structure of chemical, biochemical and microbiological parameters in soils under different management systems. Treatments included: (1) undisturbed, (2) abandoned from cultivation, (3) moderately grazed, (4) heavily grazed, and (5) cultivated with continuous winter wheat (*Triticum aestivum*), no-till cotton (*Gossypium hirsutum*), or conventional cotton. The treatments have been conducted for a minimum of 30 years. The experimental site is located in a rolling upland mixed prairie in the south central United States. Samples were taken along a transect at 0.2 m intervals for each treatment. Results showed that 33 to 96% of overall variation in measured soil parameters was accounted for by management

practices. When compared with grazed or undisturbed systems, cultivated soils exhibited greater heterogeneity in microbial biomass C and N. Cultivation reduced contents of soil organic C, microbial biomass C, and N, and dehydrogenase activity, while grazing activities enhanced these soil and microbial parameters. Contents of organic C, dissolved organic C, soluble N, microbial biomass, and dehydrogenase activity in the abandoned from cultivation soils were similar to or higher than those in the undisturbed soils, indicating partial restoration of the soil parameters. Grazed systems showed higher soil organic C and supported greater microbial biomass and activity than the cultivated system, indicating that grazing can be a more sustainable management option. In the cultivated treatments, soil parameters such as soluble C and N that are more sensitive to perturbations showed higher spatial dependence than less active soil properties such as organic C.

1. Introduction

The impact of human activities, such as cultivation, on the soil ecosystem has been amply demonstrated (Lal, 2004). Such impact could lead to loss of ecosystem function, climax alteration, and soil capacity to sustain life (Foley et al., 2005). In the United States, some cultivated land has been returned to grassland for commodity reduction, soil conservation and biodiversity. However, we have limited understanding on the effectiveness of soil management practices on restoring, maintaining, and improving ecosystem functions. Elliott et al. (1994) reported that in the Great Plains of

the United States, cultivation led to significant reduction in microbial biomass C which took 50 yr of succession to restore to the level of adjacent grasslands.

Soil ecosystems are inherently heterogeneous and harbor a variety of microhabitats in which microbial life can evolve. In evaluating the complex soil ecosystem, heterogeneity can complicate detection of changes in soil properties. Average values of homogenized soil samples often lead to estimations that do not accurately reflect the natural soil ecosystems (Trangmar et al., 1985). Spatial distribution of soil properties may affect water movement, solute transport, and the fate of environmental contaminants in the soil ecosystem (Wagenet and Rao, 1990). Soil heterogeneity reflects certain pedogenic processes, operating and interacting over a continuum of spatial and temporal scales (Trangmar et al., 1985). Moreover, cultivation and grazing may alter the local patchiness of soil nutrients by disconnecting interactions between microclimate, microtopography, vegetation and soil biota, contributing to additional heterogeneity of soil properties at multiple scales (Fraterrigo et al., 2005). Therefore, spatial variability and spatial dependence of soil and microbial parameters may reflect perturbations by changes in land use in these soil ecosystems. Understanding spatial variability and structure would reveal factors controlling ecosystem function, improve data interpretation to illustrate the underlying changes, and maintain environmental quality (Wardle, 2002).

Most studies on soil spatial variability and dependence focus on the spatial structure of the above ground biota and biomass (Burke et al. 1999; Robertson et al., 1997), and of abiotic soil properties such as moisture, CEC, and texture (Feng et al., 2004; Robertson et al., 1993; Søvik and Aagaard, 2003). As a crucial component in ecosystem function, microbial activity and biomass are sensitive indicators of ecosystem

perturbations (Anderson and Domsch, 1989). Soil processes related to microbial activity and biomass often have high spatial variability (Cambardella et al., 1994; Robertson et al., 1997). Spatial patterns of a specific soil property may induce a similar spatial pattern for another property, affecting biomass turnover rate (Harden and Joergensen, 2000), nutrient cycling processes (Corre et al., 2002; Yanai et al., 2003), and transport of organic pollutants (Søvik and Aagaard, 2003). Although several recent studies were directed to evaluate heterogeneity of microbial community structure (Nunan et al., 2002; Ritz et al, 2004), and of microbial and biochemical properties (Böhme et al., 2004; Cavigelli et al., 2005), few studies have focused on the impact of soil management practices on spatial variability and structure of soil microbial properties. The objectives of the study were: (1) to determine responses of microbial properties to management practices in prairie soils; and (2) to reveal spatial variability and spatial structure of soil variables, focusing on biochemical and microbiological parameters, in different management systems.

2. Materials and methods

2.1. Site and soil description

The soil samples were taken from two sites located within 20 km of each other in the Southern Great Plains of the United States: The Marvin Klemme Research Range Station (MKRRS) in a rolling upland mixed prairie, and a nearby cotton farm. Detailed description of the MKRRS site can be found in Fuhlendorf et al. (2002).

Soils in the MKRRS are classified as Cordell silty clay loam. The dominant species are perennial grasses with variable statures. The dominant midgrasses are *Bouteloua cultipendula* (sideoats grama), *Aristida purpurea* (purple treeawn) and *Borthiochloa laguroides* (silver bluestem) (Fuhlendorf et al., 2002). The dominant shortgrasses are *Bouteloua gracilis* (blue grama), *Buchloe dactyloides* (buffalograss), and *Buteloua hirsuta* (hairy grama). Tallgrass species are less abundant: *Schizachyrium scoparium* (little bluestem), *Sorghastrum nutans* (yellow indiangrass), and *Andropogon gerardii* (big bluestem). Herbaceous dicots are also present. They are highly diverse and fluctuate with annual precipitation. A few woody species are found on site with the most dominant being subshrub *Gutierrezia sarothrae* (broom snakeweed), *Rhus glabra* (smooth sumac), and *Prunus angustifolia* (chickasaw plum) in isolated areas.

Pastures were divided into long-term grazing treatments of different grazing intensities. For this study, samples were taken from the undisturbed (UD, no grazing or cultivation for more than 50 yr), abandoned (AB, cultivated but returned to grassland for at least 30 yr and grazed at moderate intensity since 1996), heavily grazed (HG), moderately grazed (MG), and cultivated (CW, conventional tillage with continuous winter wheat for over 30 yr). The grazing intensities were from previous stocking rate studies and were based on the recommended rate of 25 animal unit days per hectare (AUD ha^{-1}) for moderately and 50 AUD ha^{-1} for heavily grazed treatments. The grazing season was from April to September each year (Gillen et al., 2000). No fertilizers or pesticides have been applied to the UD AB, HG, and MG treatments. At the time of sampling (February 2005), the vegetation was dead or dormant. The CW area was covered with wheat approximately 7 cm tall. The wheat crop was receiving 46 kg N ha^{-1}

and 16 kg P ha^{-1} (in the forms of urea and mono-ammonium phosphate) in early September of each year.

Soil at the cotton farm is classified as Dill fine sandy loam. Two tillage systems were conducted for at least 10 yr: No-till (NT) and conventional tillage (CT). In the NT field, ryegrass was seeded as a cover crop, whereas in the CT field the soil surface was bare.

2.2. Sampling and analysis

Surface soil samples to 0.05 m depth were taken along transects at 0.20 m intervals, with 10 samples taken from each transect in each management system. All transects were oriented east to west with the first point of each transect randomly chosen. The field-moist soil samples were sieved (2-mm sieve), mixed thoroughly, and stored in sealed plastic bags at 4^{o}C. A portion of each sample was air-dried for texture and chemical analysis. A small portion of air-dried samples was ground to pass an 80-mesh (180 μm) sieve for organic C determination. Microbial biomass C (C_{mic}) and N (N_{mic}), and dehydrogenase activity (DH) were determined in field-moist soils within seven days following sampling.

Soil texture was determined by the hydrometer method (Gee and Or, 2002). Soil organic C content (C_{org}) was determined by the Walkley-Black method (Nelson and Sommers, 1982). Soil pH was determined using a combination glass electrode (soil to 0.01 M $CaCl_2$ ratio = 1:2.5). Soil microbial C (C_{mic}) and N (N_{mic}) were both determined with fumigation-extraction method (Brookes et al., 1985a and b; Vance et al., 1987).

Contents of C and N extracted with 0.5 M K_2SO_4 in the unfumigated soils were used to indicate dissolved organic C (DOC) and inorganic N (N_{inorg}) in these soils (Haynes, 2005). Dehydrogenase activity (DH) was determined according to Casida et al. (1964). All analyses were conducted in duplicate with the exception of DH which was measured in triplicate. Results were expressed on a moisture free basis. Soil water content was determined gravimetrically after drying at 105°C for 48 h. The DOC to C_{org}, and C_{mic} to C_{org} ratios were calculated to indicate the contribution of the more dynamic fractions of C_{org} to the total C_{org}. The ratio of DH to C_{mic} was calculated to indicate the metabolic activity of the microbial community and the C_{mic} to N_{mic} ratio to indicate differences in the microbial community structure.

2.3. Statistical methods

Analysis of variance was performed and comparisons were made with least significant difference test (*LSD*, $P \leq 0.05$) (SAS Institute, Inc., 1999). The spatial variability was evaluated using the coefficient of variation (CV), and the spatial dependence using semivariance $\gamma(h)$:

$$\gamma(h) = (1/2)(1/N(h))\sum_{i=1}^{N(h)}[z(x_i) - z(x_i + h)]^2$$

where z is the measured parameter, x_i is the coordinate of the sample, $N(h)$ is the number of pairs of samples $z(x_i)$ and $z(x_i + h)$, separated by the separation distance (lag) h. Theoretically, semivariance increases with increasing separation distance to a more or less constant value (sill) (Trangmar et al., 1985; Webster and Oliver, 1990). The separation distance at the sill defines the limit of spatial dependence (range). The

semivariance at a lag of zero (nugget variance) represents random variability that is not detectable at the scale of sampling. Structural variance is the part of the sample variance that is spatially autocorrelated. Structural variance plus the nugget variance make the sill. Semivariance has units of the property squared. In this study, semivariance was divided by the sample variance to obtain a "normalized semivariance" for comparison among properties. The normalized semivariance was calculated for lags 0.20 to 1.60 m.

Because of observed significant impacts by cultivation, variograms were constructed using data that were pooled to achieve normal distribution and enable comparisons. The uncultivated group of soils had data pooled from UD, AB, HG, and MG treatments. The cultivated group had data pooled from the CW, NT and CT treatments. Variogram model parameters were calculated after normalizing distributions and removing local trends. C_{org} data were normalized by dividing each value with the treatment mean (C'_{org}), N_{inorg} by the square root transformation, and ratios of variables by natural logarithms. Based on the residual sum of squares, the best fitted model was chosen to calculate the nugget, sill, and range (Gamma Design Software, 1995). Spherical models were chosen for all the variables tested, except for the ratio C_{mic} to N_{mic} where the linear model was chosen.

3. Results

3.1. Soil properties, and microbial biomass and activity

Soil samples from the two locations had the same clay content (19% clay, 49% silt, and 32% sand for MKRRS; 19% clay, 26% silt and 55% sand for the cotton farm), and similar soil pH (about 7.2) (Table 1). The contents of C_{org}, N_{inorg}, C_{mic}, and N_{mic} varied most, with CV around 60% (Table 1). Seventy-five to 96% of the detected variation was accounted for by management practices, except for N_{inorg} (45%) and DOC (33%) (Table 1). The highest N_{inorg} was found in the AB soils (Table 2). Cultivation of the soil ecosystem led to significant reduction in C_{org}, DOC, C_{mic}, N_{mic}, and DH (Table 2, Figs. 1, 2, and 3). These five parameters were highest in the HG soils. The increase in DH by grazing was significant compared to the other treatments (Fig. 3). Contents of C_{org} and DOC, N_{inorg}, microbial biomass, and DH activity in the AB soils were similar to or higher than those in the UD soils.

Overall, less than 2% of C_{org} was present as DOC and about 2% was present as C_{mic} (Table 3). The percentages of DOC in C_{org} were significantly higher in the cultivated soils when compared with the uncultivated ones (UD, AB, HG and MG). The increases in the C_{mic} portion of C_{org} by cultivation were, however, not significant. Despite the variations of C_{mic} to N_{mic} ratios in samples along a transect, cultivation generally increased these ratios (Table 3). Cultivation also enhanced metabolic activities of the microbial community, evidenced by the higher DH to C_{mic} ratios (Table 3). When

Table 1. Treatment effect based on one-way analysis of variance of measured soil properties.

Soil parameter	Mean	CV (%)	Treatment[1]		Treatment Effect[2]
			SSQ	EV (%)	
pH	7.2	6	10	93	***
C_{org} (g C kg^{-1} soil)	15	58	4871	96	***
DOC (mg C kg^{-1} soil)	91	39	29187	33	***
N_{inorg} (mg N kg^{-1} soil)	5.7	63	402	45	***
C_{mic} (mg C kg^{-1} soil)	319	56	1599967	75	***
N_{mic} (mg N kg^{-1} soil)	33	61	23955	89	***
DH (mg TPF produced kg^{-1} soil 24 h^{-1})	169	45	345343	87	***

C_{org}: Organic C, DOC and N_{inorg}: C and N extracted with 0.5M K_2SO_4, C_{mic} and N_{mic}: Microbial biomass C and N, DH: Dehydrogenase activity; CV: Coefficient of variation.

[1]Total $df = 69$, treatment $df = 6$; SSQ: Sum of squares; EV: Explained variation;

[2]F-test; *** $P \leq 0.001$.

Table 2. Summary statistics of pH, organic C (C_{org}), dissolved organic C (DOC), and inorganic N (N_{inorg}).

Soil property	Treatment[1]	Mean[2]	CV (%)	Min	Max
pH	UD	7.4 [a]	0.9	7.3	7.5
	AB	7.1 [b]	1.4	7.0	7.2
	HG	7.5 [a]	0.7	7.4	7.5
	MG	7.3 [c]	1.6	7.1	7.5
	CW	7.4 [a]	1.9	7.2	7.6
	NT	7.1 [b]	2.5	6.7	7.2
	CT	6.3 [d]	0.8	6.2	6.4
C_{org} (g C kg^{-1} soil)	UD	21.0 [b]	14	17.0	25.3
	AB	22.6 [b]	11	19.4	26.7
	HG	24.6 [a]	8	21.5	27.3
	MG	18.9 [c]	14	15.2	23.5
	CW	8.3 [d]	5	7.6	8.9
	NT	4.1 [e]	13	3.1	5.1
	CT	3.9 [e]	8	3.4	4.3
DOC (mg C kg^{-1} soil)	UD	104 [ab]	36	50	190
	AB	105 [ab]	18	68	134
	HG	115 [a]	49	27	224
	MG	84 [bc]	34	49	137
	CW	102 [ab]	19	72	141
	NT	74 [cd]	24	46	101
	CT	52 [d]	25	31	77
N_{inorg} (mg N kg^{-1} soil)	UD	6.4 [bc]	39	0.7	10.0
	AB	10.3 [a]	43	3.0	16.5
	HG	2.8 [d]	75	0.7	6.9
	MG	4.0 [cd]	76	0.6	9.4
	CW	4.2 [cd]	32	2.3	6.1
	NT	4.8 [cd]	58	2.1	10.6
	CT	7.8 [ab]	29	4.0	11.4

[1]UD: Undisturbed; AB: Abandoned from cultivation; HG: Highly grazed; MG: Moderately grazed; CW: Winter wheat; NT: No-till cotton; CT: Conventional till cotton;

[2]For each property means not marked with the same letter(s) are significantly different (*LSD* test, $P \leq 0.05$); CV: Coefficient of variation.

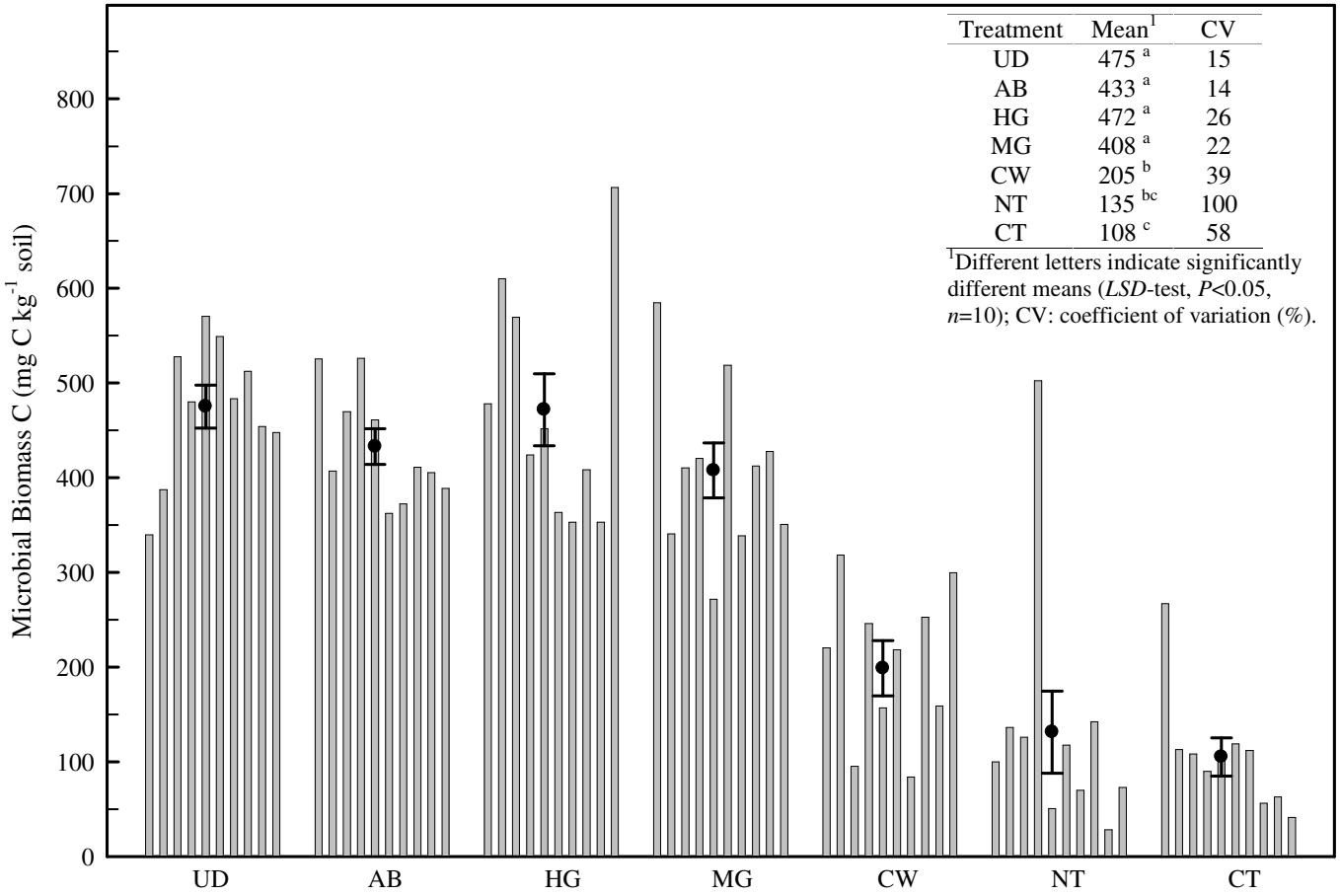

Fig. 1. Soil microbial biomass C in soil samples taken every 0.2 m along 2 m transects from different management systems: UD: Undisturbed; AB: Abandoned; HG: Highly grazed; MG: Moderately grazed; CW: Winter wheat; NT: No-till cotton; CT: Conventional till cotton. Columns are measurements, points are means, and error bars are mean standard errors.

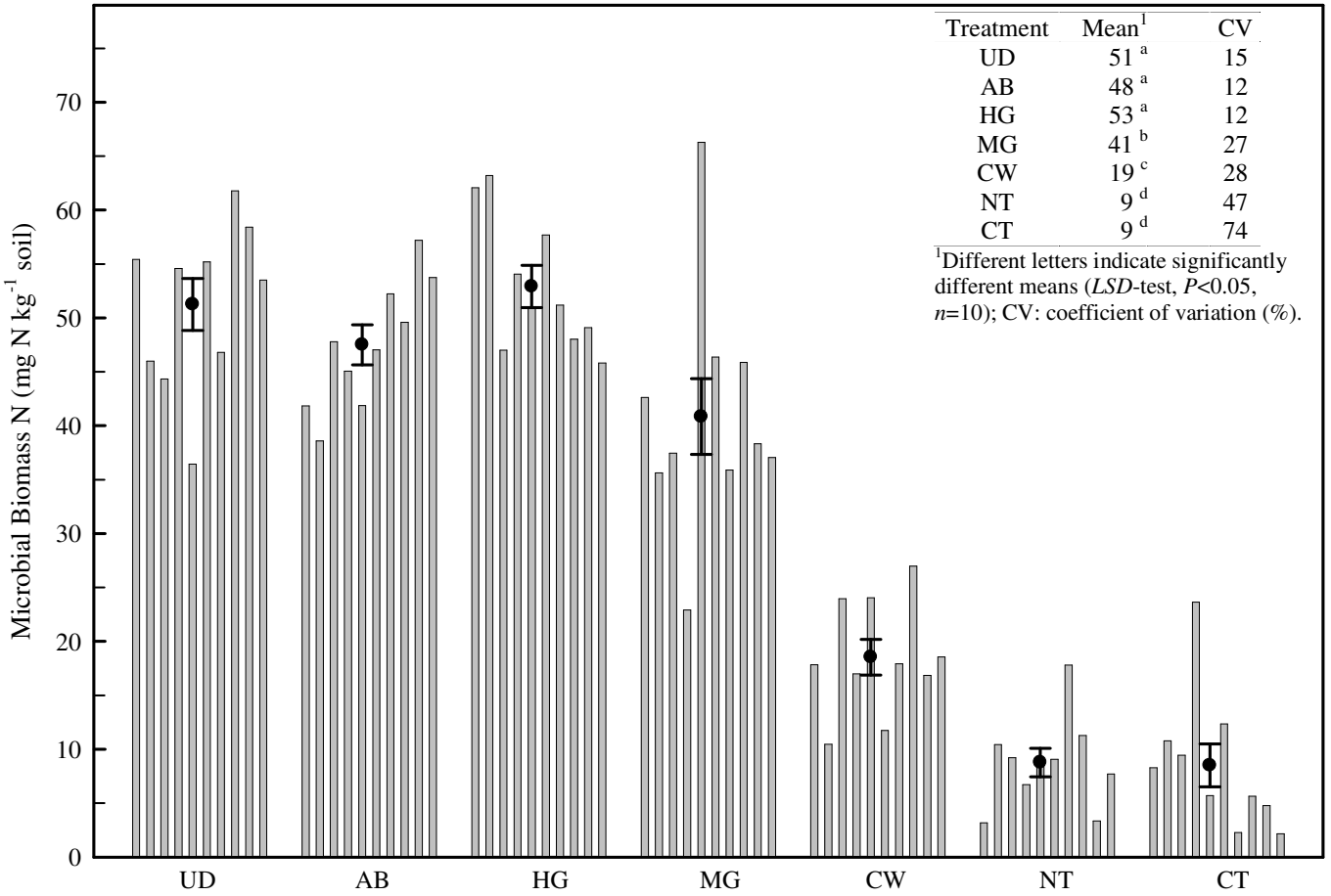

Fig. 2. Soil microbial biomass N in soil samples taken every 0.2 m along 2 m transects from different management systems: UD: Undisturbed; AB: Abandoned from cultivation; HG: Highly grazed; MG: Moderately grazed; CW: Winter wheat; NT: No-till cotton; CT: Conventional till cotton. Columns are measurements, points are means, and error bars are mean standard errors.

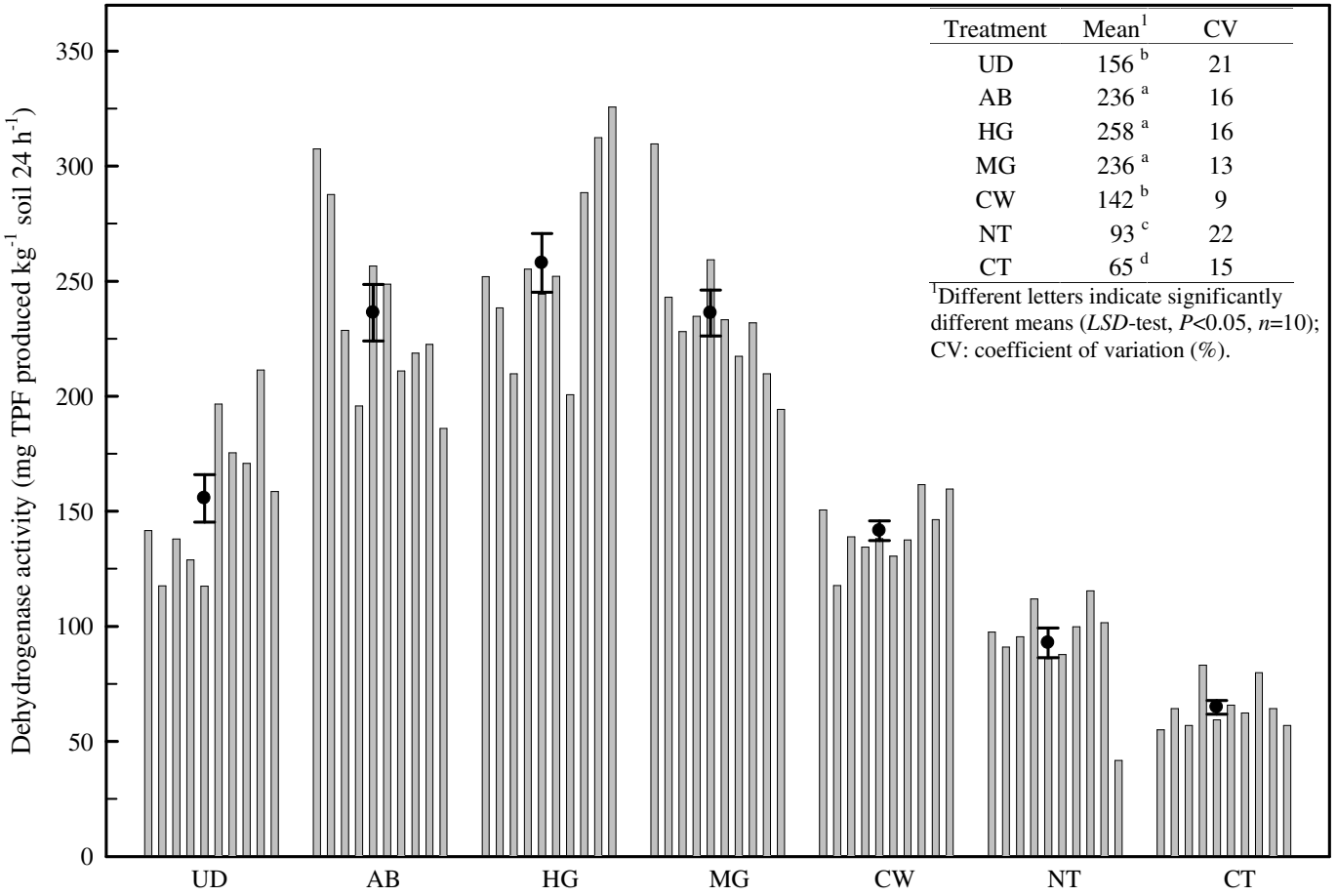

Fig. 3. Dehydrogenase activity in soil samples taken every 0.2 m along 2 m transects from different management systems: UD: Undisturbed; AB: Abandoned; HG: Highly grazed; MG: Moderately grazed; CW: Winter wheat; NT: No-till cotton; CT: Conventional till cotton. Columns are measurements, points are means, and error bars are mean standard errors.

Table 3. Ratios of selected soil and microbial parameters and their summary statistics.

Ratio[1]	Treatment[2]	Mean[3]	CV (%)	Min	Max
DOC : C_{org} (%)	UD	0.47 [c]	27	0.27	0.75
	AB	0.46 [c]	17	0.27	0.55
	HG	0.41 [c]	48	0.12	0.84
	MG	0.43 [c]	34	0.24	0.67
	CW	1.21 [b]	19	0.86	1.71
	NT	1.75 [a]	31	1.11	2.96
	CT	1.31 [ab]	29	0.78	2.26
C_{mic} : C_{org} (%)	UD	2.26 [a]	18	1.75	3.08
	AB	1.91 [a]	14	1.52	2.31
	HG	1.87 [a]	27	1.37	2.84
	MG	2.13 [a]	16	1.62	2.70
	CW	2.27 [a]	39	1.02	3.81
	NT	2.46 [a]	91	0.69	11.10
	CT	2.48 [a]	57	1.21	6.85
C_{mic} : N_{mic}	UD	9.3 [a b]	28	6.1	15.7
	AB	9.3 [a b]	22	7.1	12.5
	HG	8.7 [b]	31	6.3	15.4
	MG	10.1 [ab]	34	4.1	18.3
	CW	10.6 [ab]	63	4.0	30.4
	NT	12.8 [a]	113	3.9	74.6
	CT	14.2 [ab]	75	3.8	48.7
DH : C_{mic}	UD	0.32 [c]	23	0.21	0.47
	AB	0.54 [b]	18	0.37	0.71
	HG	0.56 [b]	27	0.37	0.89
	MG	0.59 [b]	24	0.45	0.95
	CW	0.75 [ab]	50	0.37	1.64
	NT	0.90 [a]	84	0.22	3.61
	CT	0.67 [ab]	52	0.21	1.42

[1]Natural logarithm transformations used for mean comparisons; [2] UD: Undisturbed, AB: Abandoned from cultivation, HG: Highly grazed, MG: Moderately grazed, CW: Winter wheat, NT: No-till cotton, CT: Conventional till cotton; [3]For each property means not marked with the same letter(s) are significantly different (*LSD* test, $P \leq 0.05$); CV: Coefficient of variation. The rest of the abbreviations are listed in Table 1.

compared with the UD soils, grazing enhanced the metabolic activities of the microbial community, although this increase was not significant.

Most measured soil parameters were correlated, with Pearson's coefficient (*r*) for paired parameters ranging from 0.48^{***} to 0.93^{***} (n=70), except between DOC and C_{mic} (0.26^{*}), and N_{inorg} with all other parameters (Table 4). With a few exceptions, the derived ratios were negatively correlated to soil parameters, with *r* ranging from -0.26^{*} to -0.78^{***}. The capacity of C_{org} to support microbial life (C_{mic} to C_{org} ratios expressed as percentages) and the relative metabolic activity of microbial communities (DH to C_{mic} ratios) showed no significant correlation to soil pH. The percentages of C_{org} that were present as DOC correlated strongly and negatively with C_{mic}, and N_{mic}, with *r* around -0.80^{***}. Although DH correlated negatively with the percentages of C_{org} present as DOC (DOC to C_{org}), the relative metabolic activity (DH to C_{mic}) correlated positively with the DOC to C_{org} ratio.

3.2. Spatial variability

Of the parameters evaluated, N_{inorg}, DOC, and C_{mic} were the most variable, whereas pH was the most uniform along each transect (Table 2, Fig. 1). Ten-fold differences in C_{mic} values were observed for soil samples that were 0.20 m apart along a transect (Fig. 1). Often, the highest and lowest C_{mic} values in a transect were adjacent to each other (NT in Figure 1, and MG and HG in Figure 2). Long-term grazing increased variability of N_{inorg} along transects, with CV around 75%, compared to CV of 29 to 58% in other soils tested (Table 2). In general, cultivation significantly reduced variation of

Table 4. Correlation coefficients (r) of the linear relationships between soil parameters and selected ratios (n = 70).

Soil parameter or ratio	pH	C_{org}	DOC	N_{inorg}	C_{mic}	N_{mic}	DH	C_{mic}:N_{mic}	DH:C_{mic}
Parameters									
C_{org}	0.57***								
DOC	0.49***	0.49***							
N_{inorg}	-0.36**	-0.02	-0.10						
C_{mic}	0.56***	0.86***	0.26*	-0.08					
N_{mic}	0.58***	0.93***	0.48***	-0.03	0.78***				
DH	0.58***	0.87***	0.53***	-0.15	0.74***	0.76***			
Ratios									
DOC : C_{org}	-0.44***			-0.08	-0.81***	-0.78***	-0.69***	0.15	0.53***
C_{mic} : C_{org}	-0.11		-0.32**	-0.03		-0.31*	-0.26*		
C_{mic} : N_{mic}	-0.23	-0.33**	-0.33**	0.01			-0.28*		
DH : C_{mic}	-0.21	-0.40***	0.03	-0.10		-0.40***			

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. The abbreviations are explained in Table 1.

DOC, evidenced by CVs higher than 34% for UD, HG, and MG and lower than 25% for CW, NT and CT (Table 2). However, cultivation increased variation in soil microbial biomass along a transect. The CVs for CW, NT, and CT ranged from 39 to 100% for C_{mic}, and from 28 to 74% for N_{mic}, while those in UD, AB, HG, and MG ranged from 14 to 26% for C_{mic} and 12 to 27% for N_{mic} (Figs. 1 and 2). Among the microbial parameters determined, DH was the least variable along transects, with CVs ranging from 9 to 22% (Fig. 3).

3.3. Spatial structure and dependence

The variograms for the evaluated soil parameters are presented in Figure 4. DOC in the cultivated systems, and N_{inorg} and DH in both cultivated and uncultivated systems had small nugget effects with nuggets (C_o) of 0 to 0.13, representing 0 to 39% of the total variance (Table 5). Other measured soil variables showed considerable nugget effects, with nuggets ranging from 0.46 to 0.81, representing 19 to 50% of the total variance.

The structural variance (sill minus nugget) is a measure of the spatial dependence of a variable in the range predicted by the fitted models (Trangmar et al., 1985). Structural variance has been used to define three classes of spatial dependence for soil variables (Cambardella et al., 1994). Variables with structural variance of ≥0.75 were strongly spatially dependent; of ≤0.25 were weakly spatially dependent; and between 0.25 and 0.75 were moderately spatially dependent. Based on the parameters obtained from the spherical models, strong spatial dependence was detected for N_{inorg} in both cultivated and uncultivated systems, and for C'_{org} in uncultivated, DOC in the cultivated,

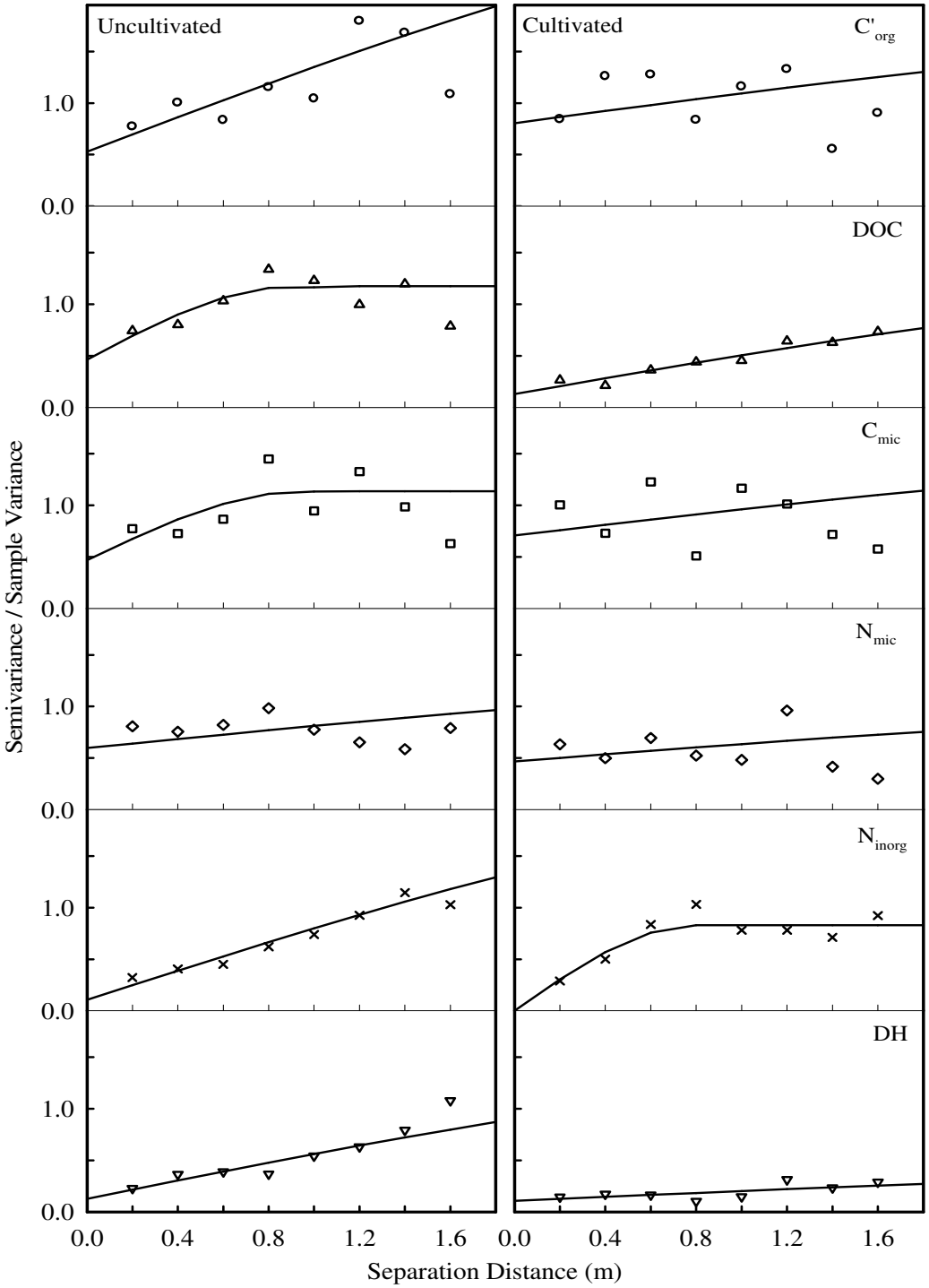

Fig. 4. Variograms of normalized organic C (C'_{org}), dissolved organic C (DOC), microbial biomass C (C_{mic}), microbial biomass N (N_{mic}), inorganic N (N_{inorg}) for uncultivated and cultivated soils. Points indicate sample semivariances, solid lines are fitted spherical models.

Table 5. Parameters of variogram models for the measured soil properties.

Soil Property	Treatment	Nugget (C_o)	Sill[1] (C_o+C)	Range[2] (m)	RSS	$C/(C_o+C)$
C_{org}[3]	Uncultivated	0.53	2.82	4.1	0.24	0.81
	Cultivated	0.81	1.61	4.1	0.70	0.50
DOC	Uncultivated	0.46	1.17	0.9	0.08	0.61
	Cultivated	0.13	1.09	3.7	0.02	0.88
N_{inorg}[4]	Uncultivated	0.11	2.04	4.1	0.02	0.95
	Cultivated	0.00	0.83	0.8	0.07	1.00
C_{mic}	Uncultivated	0.47	1.14	1.0	0.26	0.59
	Cultivated	0.71	1.42	4.1	0.52	0.50
N_{mic}	Uncultivated	0.60	1.19	4.1	0.22	0.50
	Cultivated	0.47	0.94	4.1	0.23	0.50
DH	Uncultivated	0.13	1.34	4.1	0.02	0.90
	Cultivated	0.11	0.37	4.1	0.02	0.71

C_o: Nugget variance; C: Structural variance; [1]Upper boundary of model variance; [2]Distance over which structural variance is expressed; RSS: residual sum of squares; $C/(C_o+C)$: Structural variance; [3]C_{org} normalized by dividing each value with treatment mean to remove local trends; and [4]Square root transformation. The rest of the abbreviations are listed in Table 1.

and DH in uncultivated systems (Table 5). Zero nugget effect was observed for N_{inorg} in the cultivated soils. The structural variances for N_{inorg} were 100% and 95% of the total variance in the cultivated and uncultivated systems, respectively (Table 5). Semivariances of N_{inorg} in the cultivated soils, and C_{mic} and DOC in the uncultivated treatments showed a well-structured spatial component of variance within the sampling distance (Fig. 4), with spatial dependence ranges of 0.8, 1.0, and 0.9 m, respectively (Table 3). For the rest of evaluated parameters, the models predicted spatial dependence ranges that exceeded the separation distance (lag) for which semivariance can be reliably calculated with data obtained in this study (1.60 m). There was little spatial structure shown for N_{mic} within the sampling distance.

Similarly, the spatial structure and dependence of the ratios DOC to C_{org}, C_{mic} to C_{org}, DH to C_{mic}, and C_{mic} to N_{mic} were evaluated. The proportion of C_{org} present as DOC in the cultivated soils was spatially well structured with structural variance 85% of the total (Fig. 5, Table 6,). Moderate spatial structure was observed for other tested ratios, with the exception of C_{mic} to N_{mic} ratios which showed no spatial structure as indicated by the 100% nugget. (Fig. 5).

4. Discussion

4.1. Effect of management on soil properties, and microbial biomass and activity

Management practices considerably affected soil C_{org}, C_{mic}, N_{mic}, and DH, as evidenced by 75 to 96% of the detected variation being accounted for by these

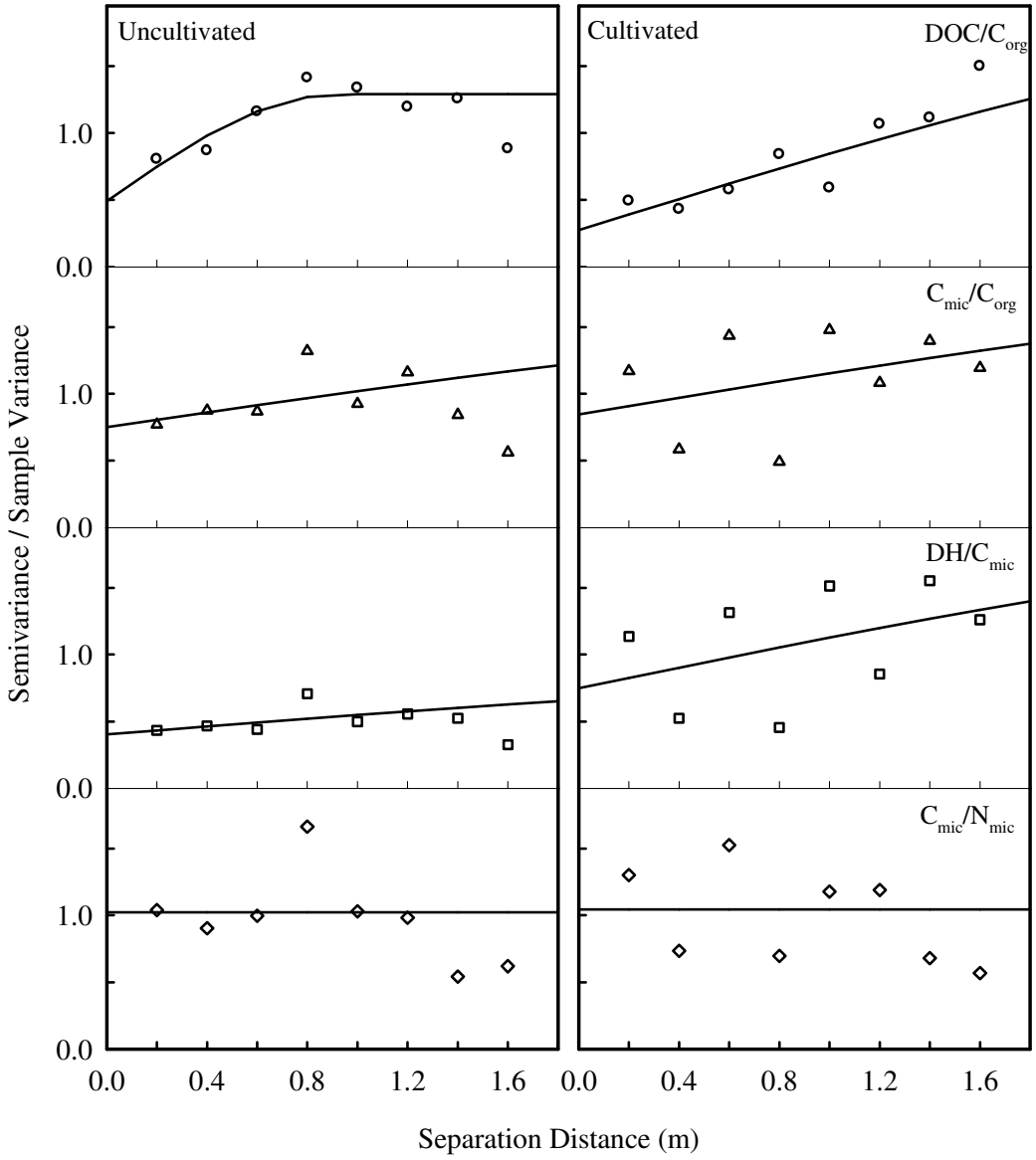

Fig. 5. Variograms of dissolved organic C (DOC) and microbial biomass C (C_{mic}) as percentages of organic C (C_{org}), metabolic activity (DH to C_{mic}), and community structure (C_{mic} to N_{mic}) for uncultivated and cultivated soils. Points indicate sample semivariances, solid lines are fitted models.

Table 6. Parameters of variogram models for calculated ratios of selected soil properties.

Ratio[1]	Treatment	Nugget (C_o)	Sill[2] (C_o+C)	Range[3] (m)	RSS	$C/(C_o+C)$
DOC : C_{org}	Uncultivated	0.49	1.29	0.9	0.05	0.62
	Cultivated	0.27	1.87	4.1	0.11	0.85
C_{mic} : C_{org}	Uncultivated	0.75	1.50	4.1	0.22	0.50
	Cultivated	0.85	1.71	4.1	0.88	0.50
DH : C_{org}	Uncultivated	0.40	0.81	4.1	0.05	0.50
	Cultivated	0.75	1.81	4.1	1.06	0.59
C_{mic} : N_{mic}	Uncultivated	1.02	N/A	N/A	0.66	0.00
	Cultivated	1.04	N/A	N/A	0.69	0.00

[1]Data were log transformed in order to normalize their distributions. Spherical models were fitted for the DOC to C_{mic}, C_{mic} to C_{org}, and DH to C_{org} ratios. Linear models fitted for the C_{mic} to N_{mic} ratio; [2]Upper boundary of model variance; [3]Distance over which structural variance is expressed; C_o: nugget variance; C: structural variance; RSS: residual sum of squares; $C/(C_o+C)$: structural variance; N/A: not applicable. The rest of the abbreviations are listed in Table 1.

treatments. These results show that cultivation reduced C_{org}, C_{mic}, N_{mic}, and DH, and are supported by several other studies (Hudson, 1994; Sherrod et al., 2003). This has been attributed to enhanced soil aeration and water infiltration by cultivation, which stimulated microbial growth and mineralization of soil organic matter (Paustian et al., 1997). The relatively high mineralization in the cultivated soils were further evidenced by increased DOC to C_{org} ratios (three-fold in the cultivated soils compared to uncultivated soils), and higher C_{mic} to C_{org} ratios in the cultivated soils, and also by data reported in other studies (Kalbitz et al., 2000; Saviozzi et al., 2001).

Although cultivation reduced DH activity, the metabolic activity of the microbial community, as indicated by the DH to C_{mic} ratios, increased in the cultivated soils compared to the uncultivated soils. This suggested that microbes in the cultivated soils had to consume more energy for maintenance, possibly due to increased stress factors in these soil ecosystems (Mäder et al., 2002).

Cultivation also affected microbial community structure as evidenced by the increased C_{mic} to N_{mic} ratios, suggesting an increase in the proportion of fungi to bacteria (Moore et al., 2000). The observed changes in the composition of microbial community may be due to different nature of substrates in different management systems (Fließbach and Mäder, 2000). The C to N ratio is approximately 80 to 1 for wheat straw (Holland and Coleman, 1987), and 55 to 1 for cotton residues (Fritschi et al., 2005). Degradation of the lignin-rich wheat straw and woody cotton residues requires high fungal activity (Holland and Coleman, 1987). On the contrary, plant species in a typical mixed-grass rangeland are less woody with C to N ratios about 24 to 27 (Schuman et al., 1999). In addition, the slightly acidic environment of the cultivated soils may favor fungal growth.

That fungi are efficient degraders of more recalcitrant substances was evidenced in this study by the significant negative correlations between DOC and C_{mic} to N_{mic} ratios. These results support the hypothesis that the quality of available substrate is a dominant factor determining microbial community composition (Fließbach and Mäder, 2000).

Contents of C_{org} and DOC, microbial biomass, and DH activity in the abandoned soils were restored to levels that were similar to or higher than those in the adjacent undisturbed soils following more than 30 yr of retirement from cultivation. This is consistent with results reported by Elliott et al. (1994), who report that C_{org} and C_{mic} of abandoned grasslands were nearly restored to the level of adjacent grasslands following 50 yr of succession.

Unlike cultivation, grazing increased soil C_{org} contents and promoted the metabolic activity of the microbial community. The increase in C_{org} contents by grazing was consistent with other studies (Schuman et al., 1999). Grazing influences the storage of the above and belowground biomass by stimulating new plant growth. Compared to native grassland, grazing may double below-ground biomass accumulation and turnover rates (Milchulas and Lauenroth, 1989). Positive effects of grazing on DH were also reported by Banergee et al. (2000). That grazing promotes plant growth as well as energy and nutrient flow (Hamilton III and Frank, 2001), explains the enhanced metabolic activity of the microbial community in this study.

4.2. Effect of management on spatial variability

The lower spatial variability for C_{org} in the conventionally cultivated soils (CW and CT) and the highly grazed systems may reflect the mixing effects by agricultural operations (Nael et al., 2004; Röver and Kaiser, 1999) and the reduced "patchiness" of plant cover due to grazing (Böhme et al., 2004). Burke et al. (1999) found that the spatial variability of below ground organic matter in grassland ecosystems was minimally influenced by above ground herbivory. Despite the lower spatial variability for C_{org}, higher variability for DOC was observed for the highly grazed system in this study. As a dynamic part of soil organic matter, DOC may be more affected by the vegetation and plant rhizosphere (Hamilton III and Frank, 2001), and by the amount and kind of organic materials present in soil (Kalbitz et al., 2000). High spatial variability of DOC has also been reported by Cook and Allan (1992).

Although macroscopically the cultivated soil looked more uniform than the uncultivated, the highest spatial variability of C_{mic} and N_{mic} contents was observed in the cultivated soils. This indicated that higher variability of microbial properties was detected at the micro scale, made possible by the small sampling interval (0.20 m). Regularly spaced rows and regular planting on the row led to a unique, clearly defined spatial distribution (Robertson et al., 1997). Cavigelli et al. (2005) measured similar coefficients of variation for C_{mic} and N_{mic} in four different cultivated soil types. On the contrary, the uniform horizontal distributions of roots in long-term grazed systems (Milchulas and Lauenroth, 1989) may have led to a more uniform distribution of below ground microbial decomposers.

4.3. Effect of management on spatial structure

The structural variance indicates spatial dependence of a soil parameter (Trangmar et al., 1985). Strong spatial dependence may be controlled by intrinsic variation in soil characteristics, such as soil texture and mineralogy. Extrinsic variations, such as fertilizer application, tillage, management practices, may control the variability of weakly spatially dependent parameters (Cambardella et al., 1994; Robertson et al., 1997; Yanai et al., 2003). The reported spatial dependence of C_{mic} in the literature ranged from not detectable to strong, with estimated ranges of 0.3 to 3 m (Morris, 1999; Nael et al., 2004; Stark et al., 2004). In this study, the estimated range of spatial dependence for C_{mic} in the uncultivated soils was within these limits (1 m), but was not in the cultivated soils. The differences in spatial dependency among studies may reflect the influence of different soil types, topography, vegetation, or agricultural practices (Ritz et al. 2004). In some studies, the spatial dependency for C_{mic} was not detected possibly due to large sampling distances, such as 1 m (Nael et al., 2004).

The detected periodic variations in semivariance ("hole effect") for C_{mic}, and the ratios of C_{mic} to C_{org}, and DH to C_{mic} in the cultivated soils may suggest that these variables were more sensitive to perturbation of the soil ecosystem. Incorporation of wheat residues creates clusters of favorable conditions for microbial growth that are not continuous in space. Hole effect due to regular spaced plant growth also has been reported by Pyrcz and Deutsch (2003) and Robertson et al. (1997).

Although C_{mic} and N_{mic} had a strong positive linear relationship ($r = 0.78^{***}$), unlike C_{mic} the normalized semivariance for N_{mic} was nearly constant within the sampling

range, showing weak spatial structure. This is inconsistent with Stark et al. (2004) who showed spatial dependency for N_{mic} at the centimeter scale from samples taken from lysimeters under conventional and organic farming. The difference in spatial structure of C_{mic} and N_{mic} could be because N_{mic} has stronger temporal variation than C_{mic}, depending on the stage of cell growth (Brookes et al., 1985a) and on soil N availability (Corre et al., 2002). High temporal variation of N_{mic} could have masked the appearance of its spatial pattern, which was also evidenced by the poor fit of variogram models and high nugget variance. Further, pure nugget effects were observed for the spatial distribution of C_{mic} to N_{mic} ratios, indicating that the microbial community structure was not spatially dependent at the evaluated spatial scale. Nugget variance could be due to micro scale variability, suggesting that structural dependence may exist below the sampling scale.

The high structural variance of DH indicated strong spatial dependence and structure, especially in uncultivated soils. There is little reported information about the spatial structure of DH in soil ecosystems. Cambardella et al. (1994) found spatial dependency for DH at a range of 51 m in a no-till field. On the contrary, Nunan et al. (2002) showed spatial dependence for living soil microbes at the micrometer scale, since living microbes may vary temporally and express spatial dependency at different and sometimes nested scales (Trangmar et al., 1985). In this study, the spatial structure of DH was not affected by cultivation in the same way as C_{mic}. One possible explanation is that DH provides a measure of active cells (Casida et al., 1964), while C_{mic} is a measure of microbial biomass without distinction among living, dormant and dead microbial cells. Because C_{mic} turnover can be as long as 5 yr (Dalal, 1998), it is not surprising that the spatial dependence and structure for DH and C_{mic} were different.

Although the predicted ranges for C'_{org} were identical in both cultivated and uncultivated soils, there was weak or no spatial relationship in the cultivated treatments, while a clear spatial relationship was detected in the uncultivated soils. The nugget effect was significantly higher in the cultivated fields compared to the uncultivated fields. Because a well established method was used for the C_{org} measurements, the higher nugget effects in the cultivated fields were likely due to a larger range of spatial dependence than could be detected based on a 2 m transect. Ranges of spatial dependence higher than 28 m have been reported for cultivated fields (Cambardella et al., 1994; Robertson et al. 1993; Robertson et al., 1997; Röver and Kaiser, 1999).

The stronger spatial dependence of DOC compared to C_{org} in cultivated soils may be indicative of the dynamic nature of DOC. C_{org} has slower turnover rate, thus is less sensitive to short-term perturbations. (Trangmar et al., 1985). According to the correlations among microbial and soil properties, microbial activity was one of the predominant contributors that shaped the spatial structure of DOC and C_{mic}.

The high spatial dependence of N_{inorg} in both cultivated and uncultivated systems indicated lower intrinsic variability and dominance by few, long-range processes (Trangmar et al., 1985). As an easily bioavailable form of N, plant uptake of N_{inorg} would be an important factor dictating its spatial distribution, leading to markedly different ranges of spatial dependence (0.8 versus 4.1 m) in cultivated and uncultivated soils.

4.4. Conclusions

Intensively grazed land may sustain greater microbial biomass and activity than land in its undisturbed, natural state. Grazed systems in general sustain higher levels of organic matter and microbial biomass than cultivated systems, making them more effective C storage pools. Returning land to grassland or rangeland after long-term cultivation may slowly restore key soil parameters at similar or higher levels than those in the undisturbed soils. Microbial biomass C and N were the most spatially variable, especially in cultivated soils, indicating that the factors that control these parameters operate at a micro scale. Generally, microbial C varied more than organic C, indicating the dynamic nature of microbial C. Dissolved organic C varied most in grazed systems, possibly due to plant distribution and patchiness. In the cultivated treatments, soil parameters such as soluble C and N that are more sensitive to perturbations showed higher spatial dependence than less dynamic parameters such as organic C. The spatial structure of microbial biomass in cultivated soils revealed a periodicity caused by cultivation operations.

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CHAPTER III

EFFECT OF MANAGEMENT ON MICROBIAL BIOMASS AND ACTIVITIES OF CARBON-TRANSFORMING ENZYMES

Abstract

Microbial biomass and enzyme activities are sensitive to management disturbances. Changes in these soil microbiological and biochemical properties in response to management practices reflect changes in functional capacity of soils under various land uses. The objectives were to evaluate effects of cultivation and grazing on different soil C pools and activities of C-transforming enzymes, including α- and β-glucosidases, α- and β-galactosidases, cellulase, and invertase, in semiarid prairie soil ecosystems. Soils were sampled from undisturbed (UD), abandoned (AB, set-aside from cultivation), heavily grazed (HG), moderately grazed (MG), and cultivated (CL) with winter wheat (*Triticum aestivum* L.). Results showed that C-transforming enzymes were sensitive in discriminating soil ecosystems under various land uses and can be used as indicators for detecting impact of soil management practices on the capacity of soil to cycle C at various stages of ecological succession. Long-term cultivation decreased total organic C, microbial biomass, and activities of C-transforming enzymes, but increased dissolved organic C content significantly. Cultivation also led to development of a

microbial community with decreased C use efficiency and enhanced metabolic activity. Grazing, especially at moderate intensity, did not lessen soil capacity to support microbial life and cycle C. When compared with the UD and cultivated systems, the intermediate status of the soil chemical, microbiological, and biochemical properties in the set-aside form cultivation system suggested that the soil ecosystem is restoring its capacity to sequester C and support microbial life through secondary succession. However, the impact of cultivation to the soil ecosystem was detectable following more than 30 years of conservation.

1. Introduction

The long-term productivity and sustainability of a soil ecosystem are closely related to its capacity to support a functioning microbial community. Microbiological and biochemical activities are the driving force for litter degradation, organic matter transformation, and nutrient cycling, and consequently control the size of C pools in the soil. Management practices such as grazing and cultivation may disturb the soil microbial community and affect the cycling of energy and matter in the soil ecosystem (Doran and Parkin, 1994).

Microbial biomass represents both a sink and source of soil C (Nannipieri et al., 2002). Because of its rapid turnover rate (1 to 2 yr) and its close relationship to soil processes, microbial biomass has been shown to be an early indicator for the effect of management practices on the soil ecosystem (Anderson and Domsch, 1989). Studies have shown that microbial biomass C declines when forest or grassland soils are

cultivated, and increases with crop rotations, reduced tillage practices, and applications of plant residues and other organic amendments (Carpenter-Boggs et al., 2003; Saviozzi et al., 2001). However, reports in the literature about effects of grazing on soil microbial biomass are not consistent. Microbial biomass C contents were increased, decreased, or remained unchanged following long-term grazing (Banergee et al., 2000; Bardgett et al., 1997; Holt, 1997; Sankaran and Augustine, 2004; Tracy and Frank, 1998).

Soil enzyme activities are the driving force in nutrient cycling and have also been suggested as indicators in detecting changes or disturbances of the soil ecosystem (Naseby and Lynch, 2002). Activities of enzymes are higher in grasslands and uncultivated soils than in cultivated soils, and increase with organic amendment applications (Acosta-Martinez et al., 2003; Bandick and Dick, 1999). Grazing and cultivation often lead to decline of soil organic matter (Abril and Bucher, 1999; Davinson and Ackerman, 1993), and loss of organic matter has been reported to decrease soil enzyme activities (Deng and Tabatabai, 1996a, 1996b, 1997).

Both microbial biomass and enzyme activities carry crucial functions in a soil ecosystem and regulate C cycling. Macromolecular C degradation requires a synergistic interaction of many enzymatic reactions acting in concert. Cellulases depolymerize polysaccharides to oligosaccharides and simple sugars, while glucosidases, galactosidases, and invertase break disaccharides to monosaccharides (Kiss et al., 1978). Meantime, extracellular polymeric substances synthesized and released by soil microbial community are major components of stable organic substances with residence time 10 to 100 yr (Gleixner et al., 2006). Studies showed evidence that soil organic matter shares a common “formation” process that is independent from the quality of input materials but

related to soil organisms (Gleixner et al., 2006; Gleixner et al., 2001). Therefore, disturbance of a soil ecosystem may impact microbial biomass, enzyme activities, and the quantity and quality of organic matter, which may impact ecosystem function and sustainability.

In a semiarid environment, limited water supply, despite relatively high temperatures, regulates microbial activities and slows down organic matter degradation. Consequently, soil C in semiarid ecosystems is retained relatively longer than in other ecosystems such as forest (Gifford, 1994). Limited studies have been conducted to understand these unique and widespread ecosystems in relation to sustainable management. Therefore, the objective of this study was to evaluate the effect of grazing and cultivation on microbial biomass and enzyme activities involved in C cycling in the semiarid prairie soils. Activities of six C-degrading hydrolases, including α- and β-glucosidase, α- and β-galactosidase, invertase, and cellulose, were evaluated.

2. Materials and methods

2.1. Site description

Soil samples were taken from the rolling upland mixed prairie in the Southern United States. The soils are classified as Cordell silty clay loam, shallow, somewhat excessively drained, weathered from hard siltstone. The vegetation is typical of the southern mixed prairie, dominated by perennial grasses with variable statures. Detailed

description of the location, the vegetation types, and the management history were reported by Fuhlendorf et al. (2002).

The treatments that were sampled for this study were: Undisturbed (UD), no grazing or cultivation for more than 50 yr; abandoned (AB), set-aside from cultivation for at least 30 yr and grazed since 1996; moderate grazing (MG), 25 animal unit days (AUD) per hectare; heavy grazing (HG), 50 AUD ha^{-1}; and cultivated with continuous winter wheat (CL). No fertilizers or pesticides have been applied to the UD, AB, MG, and HG treatments. The CL treatment received annual application of 46 kg N ha^{-1} (in the form of urea and mono-ammonium phosphate) and 16 kg P ha^{-1} (mono-ammonium phosphate) in early September. At the time of sampling (May 2005) the wheat was at the hard dough stage. In the rest of treatments many of the herbaceous plants were in bloom.

2.2. Sampling and analysis

For each treatment, nine plots (71 m x 71 m, 0.5 ha) were randomly selected to serve as field replications. Within each plot, a composite sample (35 to 45 cores, 0 to 0.10 m depth) was taken along the diagonal. The samples were kept on ice during transportation. Within 48 h following sampling, the field-moist soil samples were sieved (2-mm sieve), mixed thoroughly, and stored in sealed plastic bags at 4^{o}C. A portion of each processed sample was air-dried for textural and chemical analysis. A portion of air-dried sample was ground to pass an 80-mesh (180 μm) sieve for organic C and total N determinations.

Clay and sand contents were measured with the hydrometer method (Gee and Or, 2002). Soil pH was measured using a combination glass electrode (1 part soil to 2.5 parts 0.01 M $CaCl_2$). Soil organic C (C_{org}) was determined after digestion with $K_2Cr_2O_7$ and concentrated H_2SO_4 (Walkley-Black method, Nelson and Sommers, 1982), and total N (N_t) with Kjeldahl digestion (Bremner and Mulvaney, 1982).

Soil microbial biomass C (C_{mic}) and N (N_{mic}) were both determined with fumigation-extraction method (Brookes et al., 1985a and b; Vance et al., 1987). Contents of C and N extracted with 0.5 M K_2SO_4 from the unfumigated soils were used to indicate dissolved organic C (DOC) and soluble N (N_{sol}) (Haynes, 2005). Dehydrogenase activity (DH) was determined according to Casida et al. (1964). Activities of enzymes involved in C cycling were determined using methods listed in Table 1. All analyses were conducted in duplicate except for DH, which was measured in triplicate. Results were expressed on a moisture free basis. Soil moisture content was determined gravimetrically after drying at 105^{o}C for 48 h. The DOC to C_{org}, and C_{mic} to C_{org} ratios were calculated to assess relationships between C pools and the relative contributions of the more dynamic fractions of C to the total soil organic C. Similarly, the ratios of N_{sol} to N_t and N_{mic} to N_t were calculated. The ratio of DH to C_{mic} indicates the metabolic activity of a microbial community, and the C_{mic} to N_{mic} ratio may indicate nutrient availability to the soil microorganisms as well as microbial community composition (Khan and Joergensen, 2006; Muhammad et al., 2006).

Table 1. Methods used for the assays of C-transforming enzyme activities.

Class/EC number	Enzyme name	Reaction	Substrate	Reference
1.2.1.20	α-glucosidase	Hydrolysis of terminal non-reducing α-D-glucose residues → α-D-glucose	*p*-Nitrophenyl-α-D-glucopyranoside	Eivasi and Tabatabai (1988)
1.2.1.21	β-glucosidase	Hydrolysis of terminal non-reducing β-D-glucose residues → β-D-glucose	*p*-Nitrophenyl-β-D-glucopyranoside	Eivasi and Tabatabai (1988)
1.2.1.22	α-galactosidase	Hydrolysis of terminal non-reducing α-D-galactose residues → α-D-galactose	*p*-Nitrophenyl-α-D-galactopyranoside	Eivasi and Tabatabai (1988)
1.2.1.23	β-galactosidase	Hydrolysis of terminal non-reducing β-D-galactose residues → β-D-galactose	*p*-Nitrophenyl-β-D-galactopyranoside	Eivasi and Tabatabai (1988)
3.2.1.4 3.2.1.91 1.2.1.21	Cellulase system	Cellulose → glucose, cellobiose or higher molecular weight oligosaccharides	Cellulose	Deng and Tabatabai (1994)
3.2.1.26	Invertase	Sucrose +H_2O→ glucose + fructose	Sucrose	Frankenberger and Johanson (1983)

2.3. Statistical methods

The frequency distributions of the measured properties, the normality of residuals, and the equality of variances among treatments were tested. Significant differences among treatments were determined using one-way analysis of variance. Comparison of treatment means was done according to the least significant difference test (*LSD*, $P \leq 0.05$). Natural logarithm transformations were used for the comparisons of the ratios of C_{mic} to C_{org}, galactosidases to glucosidase, and enzyme activities to microbial biomass C among the treatments, because the residuals of the data and/or the variances were not equal. Pairwise correlations between soil chemical and biological properties as well as with enzyme activities were calculated. Principal component analysis (PCA) was applied to reduce the dimensionality of the six-variable system of enzyme activities and plotted by JMP® start statistics software (Sall et al., 2005). Because enzyme activities were expressed in different units, PCA was performed using the correlation rather the covariance matrix (Jolliffe, 1986). Analysis of variance for the principal component scores of the first two principal axes (PC1 and PC2) was performed to test the significance of separations between the management systems.

3. Results

3.1. Soil chemical and microbial properties

Soils of all treatments had neutral to alkaline pH values (7.2 to 7.6) and textures that varied from Loam to Silt loam (Table 2). Significant differences were observed in the mean C_{org} and N_t contents in soils tested (Table 2). The UD and MG soils had the highest C_{org} and N_t contents, while the CL had the lowest. When compared to the UD system, cultivation decreased C_{org} by 55%, and N_t by 45%. The HG and AB soils were intermediate in C_{org} and N_t contents between the UD and CL systems. Opposite trends were observed across the treatments for the soluble C and N pools. The CL soils had significantly higher concentrations of DOC and N_{sol} (Table 2), which led to significantly higher ratios of DOC to C_{org}, and N_{sol} to N_t for these soils.

About 1% of organic C and 2% of total N were present in soluble forms in CL soils, while these values were only 0.5 and 1% in the uncultivated treatments (Table 3). The soil C to N ratio was reduced significantly by cultivation (Table 3). The grazed soil ecosystems were not significantly different from the UD in the C_{mic} and N_{mic} contents. The HG system had similar DH activity to the UD, and both had significantly higher DH activities than the rest of the soils (Fig. 1). The CL system had the least microbial biomass and DH activity, showing 55% to 65% reduction when compared with the UD soils. Microbial biomass C accounted for about 3.1% of total C in the grazed and UD soils, while its contribution in the CL system was only 2.0% (Table 3). The N_{mic} was about 2.3% of total N for the grazed and UD systems, and only 1.5% for the CL system.

Table 2. Effect of management practices on soil properties and C and N pools (n = 9).

Soil Property	Treatment[1]					*LSD* ($P \leq 0.05$)
	UD	AB	MG	HG	CL	
pH	7.2	7.6	7.5	7.4	7.5	0.2
Sand (%)	33	30	27	39	32	7
Silt (%)	49	51	51	44	49	6
Clay (%)	18	19	22	17	19	3
C_{org} (g C kg^{-1} soil)	21.5	15.8	20.9	17.7	9.7	3.3
N_t (g N kg^{-1} soil)	2.2	1.6	2.1	1.9	1.2	0.3
DOC (mg C kg^{-1} soil)	96	81	92	106	117	20
N_{sol} (mg N kg^{-1} soil)	23	17	24	17	25	1

[1]UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. C_{org}: Organic C; N_t: Total N; DOC and N_{sol}: C and N extracted with 0.5 M K_2SO_4.

Table 3. Elemental and eco-physiological ratios between selected soil chemical and microbial properties (n = 9).

Ratio	Treatment[1]					*LSD* ($P \leq 0.05$)
	UD	AB	MG	HG	CL	
C_{org} : N_t	9.7	9.6	9.8	9.3	7.9	0.9
DOC : C_{org} (%)	0.5	0.5	0.4	0.6	1.2	0.2
N_{sol} : N_t (%)	1.0	1.1	1.1	1.0	2.1	0.2
C_{mic} : C_{org} (%)	2.8	2.9	3.3	3.2	2.0	0.6
N_{mic} : N_t (%)	2.2	2.3	2.3	2.4	1.5	0.3
C_{mic} : N_{mic}	12.6	12.2	14.0	12.7	11.2	2.5

[1]UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. C_{org}: Organic C; N_t: Total N; DOC and N_{sol}: C and N extracted with 0.5 M K_2SO_4; C_{mic} and N_{mic}: Microbial biomass C and N. Mean comparisons for C_{mic} to C_{org} ratio were based on natural logarithm transformed data.

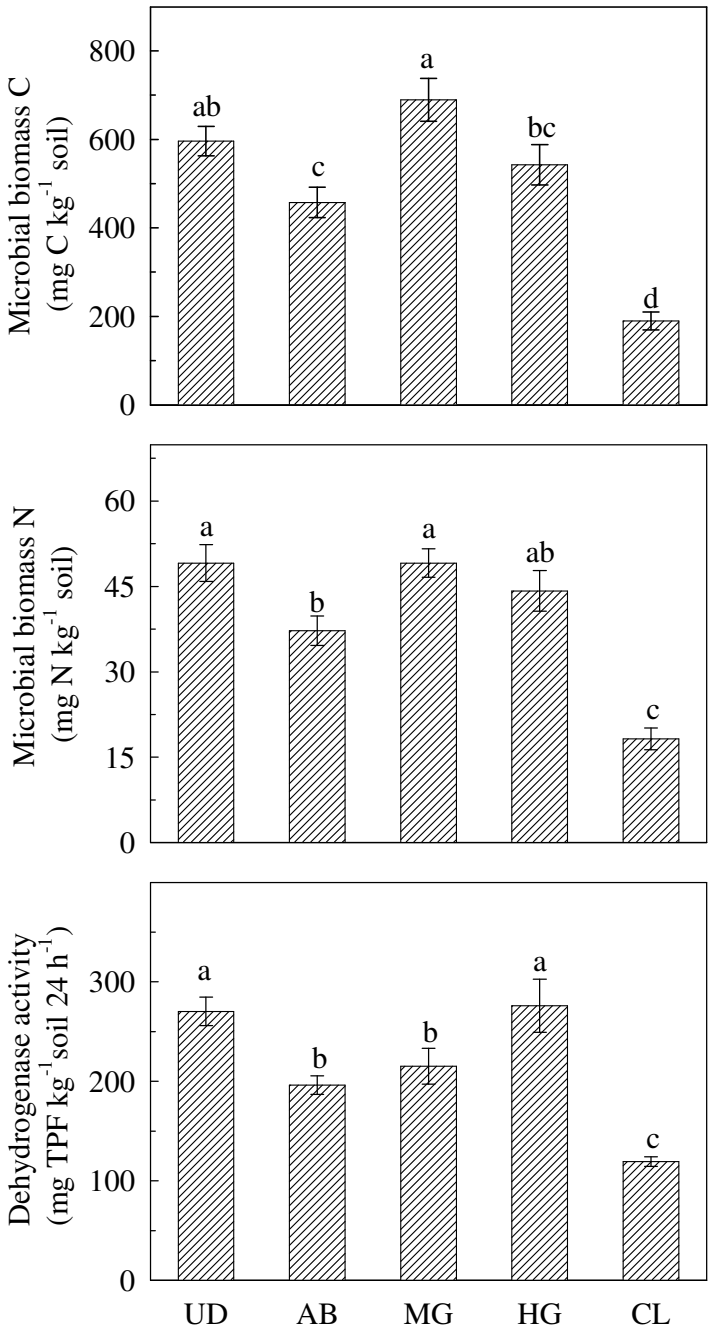

Fig. 1. Treatment effect on microbial biomass C and N contents and dehydrogenase activity. UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

Soil organic C, total N, and microbial biomass C and N contents were highly correlated with each other, with correlation coefficients (r) ranging from 0.69^{***} to 0.95^{***} (Table 4). However, these chemical and microbiological properties were not strongly correlated to either DOC or N_{sol}, with r values ranging from -0.22 to 0.22.

3.2. Enzyme activities

With the exception of invertase, the effect of management practices to the tested C-transforming enzymes was similar (Fig. 2). Grazing did not significantly affect activities of C-transforming enzymes, while cultivation led to marked reduction of these enzyme activities. For each of these five enzymes, the activity in the CL soils was on average 44% of that in the UD soils. The activities for these five enzymes in the AB soils were higher than those in CL soils, but lower than those in the UD, MG, and HG soils. Of the soils tested, invertase activity was the lowest in the UD soils and highest in the MG soils. When compared with the UD soils, cultivation more than doubled invertase activity. Grazing increased invertase activity and grazing intensity regulated the degree of this increase. When compared with the UD soils, MG tripled invertase activity. HG increased invertase activity compared to the UD, but resulted in one half of the activity in the MG soils. The activities of invertase in the AB system were not significantly different from those detected in the CL soils.

Significant positive correlations were observed between activities of most of the tested enzymes, and between these enzyme activities and soil organic C, total N, or microbial biomass C and N contents (Table 5). The trend for invertase was different,

Table 4. Correlation coefficients (*r*) between chemical and microbial soil characteristics (*n* = 45).

Variable	C_{org}	N_t	DOC	N_{sol}	C_{mic}
N_t	0.95^{***}				
DOC	-0.13	-0.06			
N_{sol}	-0.06	0.01	0.22		
C_{mic}	0.88^{***}	0.88^{***}	-0.22	-0.12	
N_{mic}	0.87^{***}	0.88^{***}	-0.12	-0.16	0.87^{***}

C_{org}: Organic C; N_t: Total N; DOC and N_{sol}: C and N extracted with 0.5 M K_2SO_4; C_{mic} and N_{mic}: Microbial biomass C and N; DH: Dehydrogenase activity; $^{***}P \leq 0.001$.

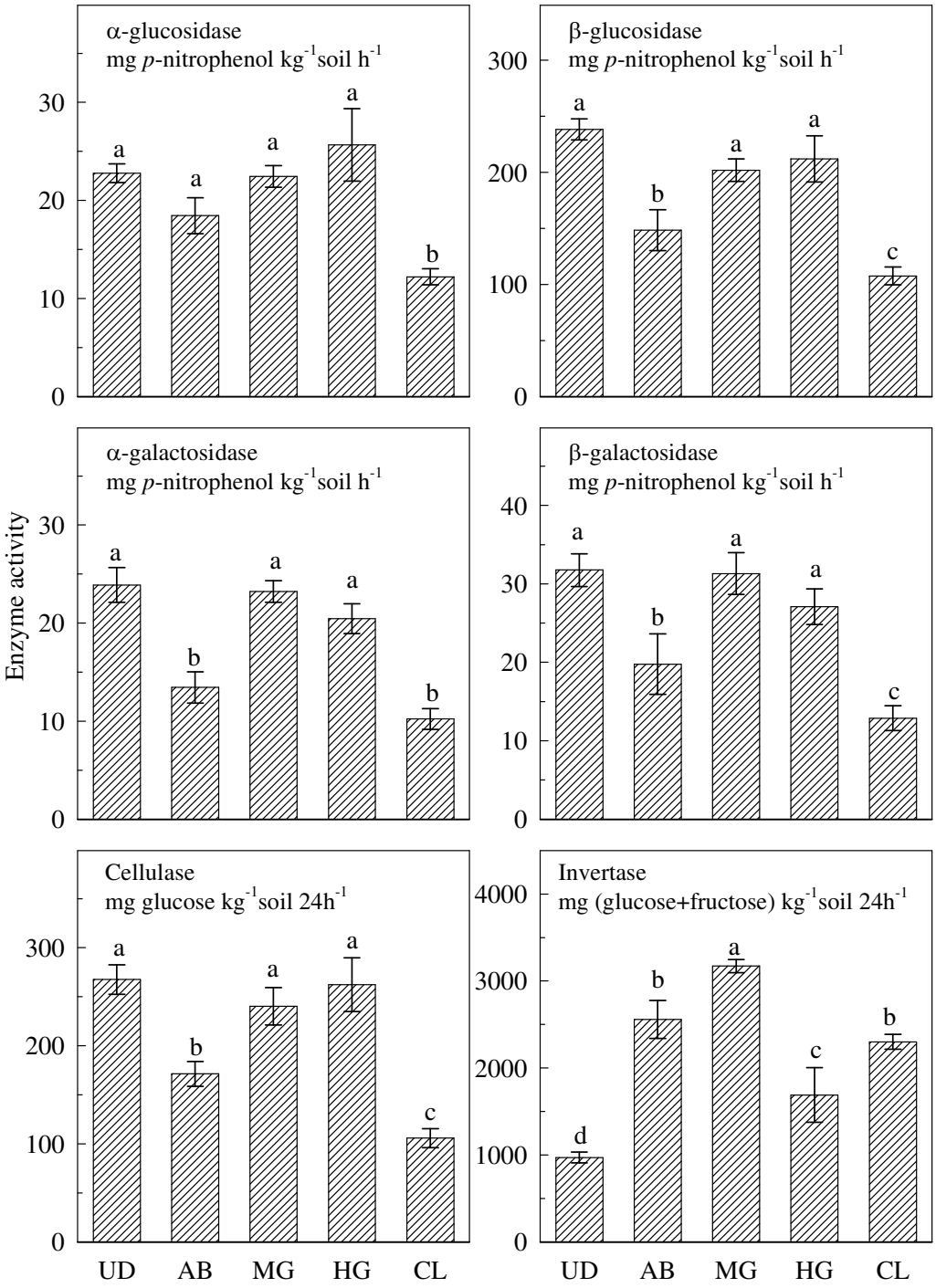

Fig. 2. Effect of grazing and cultivation on hydrolytic enzyme activities involved in C cycling. UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

Table 5. Correlation coefficients (r) between C-transforming enzyme activities and chemical/microbial soil properties (n = 45).

Variable	C_{org}	N_t	DOC	N_{sol}	C_{mic}	N_{mic}	DH	α-GLS	β-GLS	α-GAL	β-GAL	CELL
DH	0.69***	0.77***	-0.01	-0.31*	0.71***	0.80***						
α-GLS	0.80***	0.77***	0.04	-0.17	0.75***	0.78***	0.75***					
β-GLS	0.91***	0.89***	-0.08	-0.10	0.77***	0.80***	0.75***	0.79***				
α-GAL	0.90***	0.86***	-0.14	-0.00	0.78***	0.75***	0.61***	0.70***	0.90***			
β-GAL	0.86***	0.82***	-0.23	-0.02	0.74***	0.74***	0.58***	0.70***	0.91***	0.88***		
CELL	0.83***	0.79***	-0.05	-0.12	0.73***	0.82***	0.72***	0.80***	0.86***	0.81***	0.75***	
INV	-0.25	-0.25	-0.27	0.01	-0.05	-0.21	-0.37*	-0.33*	-0.40**	-0.26	-0.20	-0.42**

C_{org}: Organic C; N_t: Total N; DOC and N_{sol}: C and N extracted with 0.5 M K_2SO_4; C_{mic} and N_{mic}: Microbial biomass C and N; DH: Dehydrogenase activity, α- and β-GLS: α- and β-glucosidase; α- and β-GAL: α- and β-galactosidase; CELL: Cellulase; INV: Invertase; $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

showing little correlation or significant negative correlations to these tested chemical and microbial properties. These enzyme activities were not strongly correlated with the soluble forms of C (DOC) and N (N_{sol}).

Principal component analysis revealed that activities of enzymes involved in C transformations were clustered according to management practices (Fig. 3). The small angles between the biplot rays of β-glucosidase, α- and β-galactosidases, and cellulase activities with the PC1 axis indicated their high contribution to PC1, which accounted for 73% of the variance in the data. According to PC1, the CL was differentiated from the AB, and both of them were separated from all other treatments. PC2 values differentiated the MG from all the other treatments, and the UD from the AB treatment (Fig. 3).
The ratio of the combined activities of galactosidases to that of glucosidases ranged from 0.19 to 0.24, and was higher in the MG system and lower in the AB and CL soils when compared with those in the UD system. Wide variations were detected for the ratios of cellulase to invertase, ranging from 0.05 to 0.28 (Table 6). Cultivation reduced this ratio significantly, but the effect of grazing was inconsistent. Grazing at high intensity did not alter this ratio significantly, while grazing at moderate intensity decreased this ratio significantly and led to ratios similar to those detected in the CL and AB soils.
Cultivation resulted in significantly higher metabolic activity of the microbial community as indicated by changes in the DH to C_{mic} ratios. Cultivation also led to significant increase in enzyme activities per unit of C_{mic} with the largest increase for invertase activity per unit of C_{mic}.

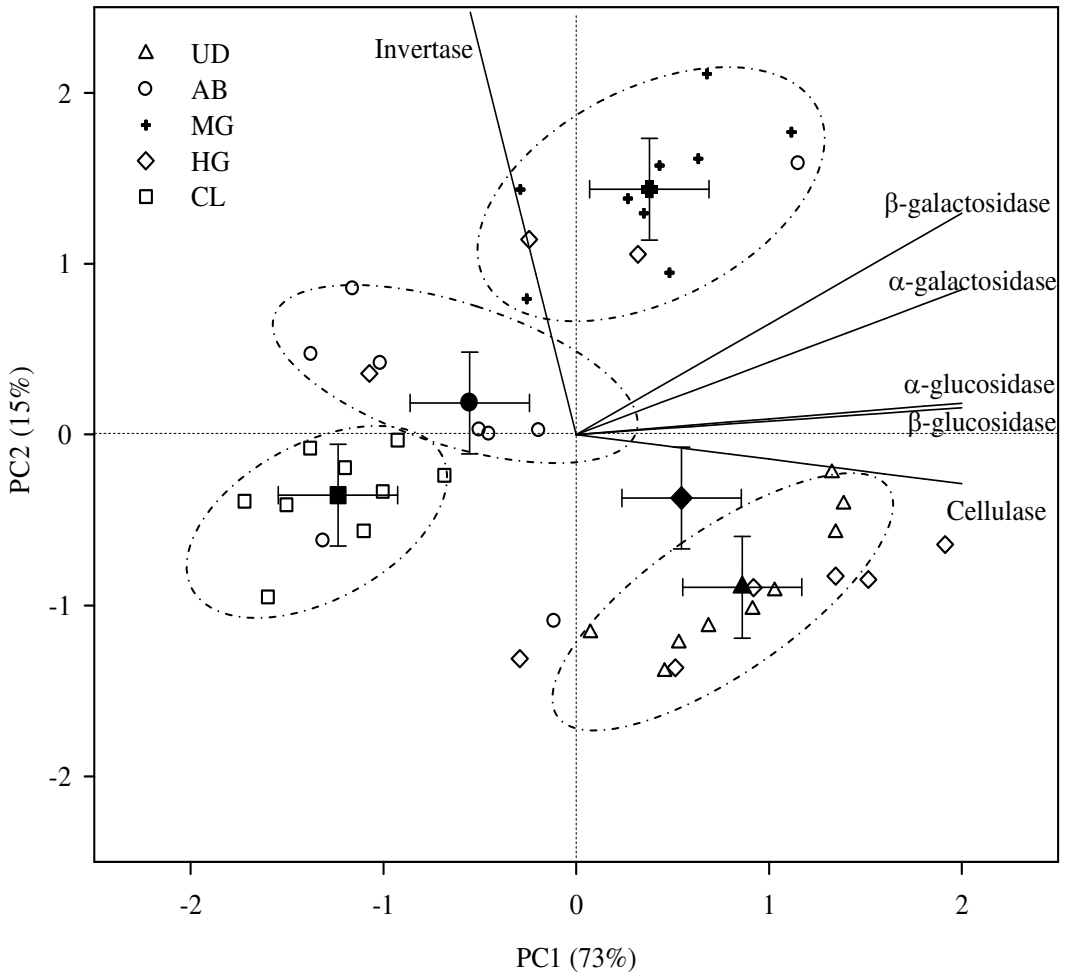

Fig. 3. Gabriel biplot of enzyme activities against the first two principal components (PC1 and PC2). Rays that have small angle with a PC axis contribute more to that PC. Rays that have small angles with each other are positively correlated. Filled symbols represent treatment means (n = 9). Horizontal and vertical error bars are based on 95% confidence intervals for the mean PC1 and PC2, respectively. Overlapping bars indicate that two means are not significantly different at the 95% confidence level. UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat.

Table 6. Treatment effects on enzyme activity ratios and ratios of enzyme activities to microbial biomass C (n = 9)[1].

Ratio	Treatment					*LSD* ($P \leq 0.05$)
	UD	AB	MG	HG	CL	
Galactosidases : Glucosidases	0.21	0.19	0.24	0.20	0.19	0.02
Cellulase : Invertase	0.28	0.07	0.08	0.21	0.05	0.06
DH : C_{mic}[2]	0.46	0.44	0.31	0.52	0.68	0.11
α-glucosidase : C_{mic}[3]	0.04	0.04	0.03	0.05	0.07	0.01
β-glucosidase : C_{mic}[3]	0.41	0.32	0.30	0.40	0.63	0.13
α-galactosidase : C_{mic}[3]	0.04	0.03	0.04	0.04	0.06	0.02
β-galactosidase : C_{mic}[3]	0.05	0.04	0.05	0.05	0.08	0.02
Cellulase : C_{mic}[4]	0.46	0.38	0.36	0.49	0.60	0.13
Invertase : C_{mic}[5]	1.66	5.89	4.75	3.51	13.14	2.24

[1]UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. Mean comparisons for the ratio galactosidases to glucosidases and for the enzyme activities to C_{mic} ratios were based on natural logarithm transformed data. [2]mg triphenyl formazan mg^{-1} C_{mic} $24h^{-1}$; [3]mg *p*-nitrophenol mg^{-1} C_{mic} h^{-1}; [4]mg glucose mg^{-1} C_{mic} $24h^{-1}$; [5]mg (glucose+fructose) mg^{-1} C_{mic} $24h^{-1}$.

4. Discussion

4.1. Soil chemical and microbial properties

When compared with the undisturbed system, cultivation resulted in significant reduction in soil organic C, total N, and microbial C and N, as reported by numerous other studies (Caldwell et al., 1999; Gupta and Germida, 1988; Waldrop et al., 2000). However, the degree of impact to the C and N pools varied. This reduction was 55% for organic C but only 45% for total N. The higher retention of N relative to C is, in part, due to the addition of N fertilizer that supplemented N nutrition for the microbial community and accelerated degradation of soil organic matter in the cultivated soils. Although long-term cultivation led to significant reduction of soil organic C and total N, it increased concentrations of dissolved organic C and soluble N significantly. As a result, the percentages of soluble C or N in total C or N increased due to cultivation, showing values exceeding those reported for typical agricultural soils. Following review of 12 studies that included data obtained from prairie soil ecosystems, Haynes (2005) found that most agricultural soils contained 0.05 to 0.40% dissolved organic C in soil organic C and 0.15 to 0.19% dissolved organic N in total N. Contents of soluble organic C and N have been reported to increase from spring to summer in winter cereal cultivated fields, while the release of soluble nutrients in rangeland often peaks in late summer and early fall (Haynes, 2005). Soils used in this study were sampled in late spring, which may explain the high soluble C and N contents in the cultivated soils. Moreover, the

reduced microbial biomass and activity by cultivation would also reduce consumption of readily available nutrients, leading to their accumulation in these soils.

Cultivation led to decreases in soil microbial biomass C and N, and dehydrogenase activity, and development of a microbial community with enhanced metabolic activity (increased DH to C_{mic} ratios) and reduced C use efficiency as evidenced by decreased percentages of organic C present as microbial C. Reduced residue inputs in the cultivated soils limited C availability and suppressed microbial growth. The increased metabolic activity suggested that the microbial community in the cultivated system invested more energy for maintenance rather than for growth (Khan and Joergensen, 2006; Mäder et al., 2002). The ratio of microbial C to organic C from long-term management systems was suggested to represent C equilibrium in the system (Anderson and Domsch, 1989). The percentages of organic C present as microbial C also indicate the capacity of soil to support microbial life (Deng et al., 2006) and are expected to be affected by the complexity of organic matter. Following evaluations of plant communities varying from monoculture to 32 plant species, Spehn et al. (2000) showed that reduction in plant species richness decreased microbial biomass and the ratio of microbial to organic C. Although the percentage of organic C present as microbial C varies considerably across soil ecosystems (Wardle, 1992), this value averaged about 2.3 in agricultural soils (Anderson and Domsch, 1989, Deng et al., 2006). The significantly higher percentages of organic C present as microbial C in the uncultivated soils of this study compared to cultivated ones indicated the higher nutrient use efficiency of the microbial community in the uncultivated soils. In an ecosystem that approaches equilibrium, the microbial community would exploit the use of available resources to

maximize its growth capacity. Cultivation disturbed soil C equilibrium, led to changes in microbial community structure (C_{mic} to N_{mic} ratio), and affected C and other nutrient availability to the microbial community.

Contrary to the effect of cultivation, grazing, especially at moderate intensity, did not alter the size of organic C pool and retained the capacity of the soil ecosystem to support microbial life at levels that were similar to the undisturbed soils. Although grazing has shown to increase bare areas in rangeland (Fuhlendorf et al., 2002), grazing has also been shown to stimulate plant growth (Hamilton III and Frank, 2001). With as much as 60 to 99% of ingested nutrients by cattle returning back to soil as organic waste (Haynes and Williams, 1993), grazing may promote nutrient cycling (Hamilton III and Frank, 2001) but may not change the nutrient pool sizes significantly. In this study, organic C pool was significantly reduced by grazing at high intensity. It has been reported that long-term heavy grazing reduced root biomass (Mawdsley and Bardgett, 1997) and increased mineralization rates (Holland and Delting, 1990). Both altered soil processes contributed to significant reduction of organic C.

When compared with the undisturbed soils, grazing did not alter the tested microbial properties, soluble C and N content, and elemental and eco-physiological ratios significantly. This is somewhat surprising. Fuhlendorf et al. (2002) conducted a study at the same site and reported that grazing reduced tallgrass coverage from 19.3% in the ungrazed site to 1.4% in the heavily grazed site. Since the aboveground plant community and belowground soil microbial community select each other (Callaway et al., 2004; Klironomos, 2002), the marked change in the composition of plant community was expected to lead to significant changes in the soil microbial community. It is possible

that the tested microbial properties did not sensitively reflect the changes that occurred in the microbial community. Alternatively, grazed and undisturbed systems provided similar nutritional environments, or microbial communities adapted to exploit organic materials of different quality. Grazing, however, did increase C use efficiency by the microbial community, as evidenced by the higher percentages of organic C present as microbial C.

Abandonment from cultivation, in the effort of ecosystem conservation and restoration, resulted in soil chemical and microbial property values greater than the cultivated but lower than the undisturbed soils. Successional development of vegetation that occurred on land no longer intensively managed, and on land that was being reclaimed or restored, was accompanied by slow build up of organic matter (Wali, 1999; Zak et al., 1990) and microbial biomass (Sparling et al., 1994). Similar trend was clearly shown in this study. Impact of cultivation on soil microbial community in the abandoned soils was detectable, suggesting that 30 years of conservation was not long enough to erase human impact on a soil ecosystem. On the other hand, this type of ecosystems are widespread and have been considered as a long-term (>100 yr) C sink (Fuhlendorf et al., 2002), implying their importance in regulating the global C cycle.

In both grazed and undisturbed systems, the higher microbial C to N ratio indicated lower N availability than in the cultivated system (Khan and Joergensen, 2006), as well as changes in microbial community composition (Muhammad et al., 2006). The higher ratio of microbial biomass C to N suggested the higher contribution of fungi, which use available substrate more efficiently than bacteria (Holland and Coleman, 1987;

Ohtonen et al., 1999), also evidenced by the higher microbial to organic C ratios compared to the cultivated soils.

4.2. Enzyme activities

Activities of enzymes clustered according to management systems, suggesting that enzyme activities are sensitive indictors of ecosystem disturbance. The effects of management practices on most C-transforming enzyme activities were similar to those on organic C, total N, and microbial biomass and activity, demonstrating the intimate relationship between the soil habitat and its inhabitants. Cultivation led to significant reduction of enzyme activities because of reduction in microbial biomass, which supports the hypothesis that soil enzymes are primarily of microbial origin (Tabatabai, 1994). The little correlations between invertase activity and microbial biomass and negative correlations with activities of other tested C-transforming enzymes suggested that invertase in these soils may predominantly be of plant origin. Invertase tends to increase under vegetation, and its activity often correlates with plant density and composition (Skujins, 1976).

The significant positive correlations between organic C and the enzyme activities suggested that the soil organic matter plays an important role in protecting and maintaining accumulated soil enzymes in their active forms, as supported by several studies (Acosta-Martinez et al., 2003; Deng and Tabatabai, 1996a, 1996b, 1997; Jordan et al., 1995). Again, invertase behaved differently. The little correlation between invertase activity and organic C may suggest that plant derived invertase was not stabilized in soil

by forming complexes with organic matter. Moreover, invertase activity was weakly correlated with clay content ($r = 0.32$, data not shown), which is another proposed protective mechanism that stabilizes enzymes in soil (Ruggiero et al., 1996). Therefore, activity of invertase does not persist in the soil.

Because most of the tested enzymes were of microbial origin, enzyme synthesis would be enhanced in the environment that favored establishment of microbial community. Cultivation may physically disrupt microhabitats and negatively impact the microbial community. Fungal mycelium may be broken and lose its ability to explore and exploit nutrient patches. Further, most bacteria are limited to movement within the water films on soil particles and along roots (Caldwell et al., 1999). On the other hand, the year-round presence of natural vegetation and the widespread root system of perennial grasses in the uncultivated sites enhanced the rhizosphere effect and promoted microbial community establishment when compared to cultivated soils (Bandick and Dick, 1999). Therefore, removal from cultivation led to partial restoration of functions in the abandoned ecosystem, evidenced by its significantly higher microbial biomass, and most of the tested C-transforming enzyme activities when compared to cultivated soils.

Soil microbial communities adjust their enzyme activities to the environment to efficiently exploit C resources (Dilly and Nannipieri, 1998), and consequently their metabolic activity can be evaluated by determining the ratios of intracellular enzyme activities to microbial C. Changes of enzyme activity to microbial C ratio following treatments that significantly affect microbial activity would also reflect the status and persistence of enzymes in soil (Deng et al., 2006). Following a treatment that significantly reduced microbiological activity, increase in this ratio would suggest that

the enzyme activities were originated predominantly from accumulated enzymes that were free of microbial cells and persisted in the soil environment. In this study, cultivation significantly reduced microbial activity, but increased the ratios of enzyme activities to microbial C, suggesting that the tested enzymes were dominated by accumulated enzymes.

Ratios between enzyme activities may indicate differences in the substrate composition in the tested soil environment (Caldwell, 2005). The higher galactosidases to glucosidases ratio detected in moderately grazed soils suggested higher contribution of galactosides (melibiose and lactose) than of glucosides (maltose and cellobiose) in the disaccharide C pool. The undisturbed system had the highest cellulase to invertase ratio, indicating a higher relative contribution of complex carbohydrates than disaccharides. On the contrary, disaccharides contributed more to the carbohydrate C pool than polysaccharides in the moderately grazed, abandoned, and cultivated systems. Changes in the aboveground plant community composition (Fuhlendorf et al., 2002) may have resulted in changes in the composition of belowground C pools in these soils.

4.3. Relationships among enzymes

The strong positive correlations among most enzyme activities were because many enzyme activities correlate with organic C (Deng and Tabatabai, 1996a, 1996b, 1997), and because of their specificity for similar organic substrates (Sparling et al., 1986). Although cultivation effects on enzyme activities were similar, and most of the enzyme activities tested were significantly correlated with each other, the sensitivity in

response varied. According to principal component analysis, β-glucosidase, α-galactosidase, and cellulase activities contributed most to PC1. Thus, activities of these enzymes are most sensitive to disturbance by cultivation, which is consistent with observations by Caldwell et al. (1999). Invertase activities showed little correlation to activities of other tested enzymes and contributed most to PC2. Results obtained suggested that the tested hydrolytic C-transforming enzymes revealed differences in soil processes and C partitioning in a wide range of soil ecosystems at various stages of ecological succession.

Results obtained in this study showed that ecological succession is a slow process. Impacts of cultivation on ecosystem functional diversity of C-transforming enzyme activities, microbial biomass, and various C and N pool sizes were not restored to levels of the undisturbed soils following over 30 years of ecosystem conservation effort. These findings are consistent with those reported by Booth (1941) who surveyed 106 fields at various stages of plant succession in Oklahoma and Kansas, where fields were removed from cultivation for 31 years and comparisons were made with nearby native prairie. Compared to organic C content, enzyme activities are more sensitive indicators of ecosystem disturbance that may be used to evaluated effectiveness of ecosystem management in preserving ecosystem health and maintaining soil quality.

4.4. Conclusions

Long-term cultivation decreased total organic C, microbial biomass, and activities of C-transforming enzymes, but increased dissolved organic C content significantly.

Cultivation also led to development of a microbial community with reduced C use efficiency and enhanced metabolic activity. Grazing, especially at moderate intensity, did not lessen the soil capacity to support microbial life and cycle C. When compared with the undisturbed and cultivated systems, the intermediate status of the soil chemical, microbiological, and biochemical properties in the set-aside form cultivation system suggested that the soil ecosystem is restoring its capacity to sequester C and support microbial life through secondary succession. However, the impact of cultivation to the soil ecosystem was detectable following more than 30 years of conservation. Carbon-transforming enzymes were sensitive in discriminating soil ecosystems under various land uses and can be used as indicators for detecting differences in the capacity of soil to cycle C among a wide range of management practices and among systems at various stages of ecological succession.

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CHAPTER IV

EFFECT OF MANAGEMENT ON NITROGEN-TRANSFORMING ENZYMES

Abstract

Land uses and management practices affect dynamics of N cycling in the soil ecosystem. The objective was to evaluate activities of enzymes involved in N cycling and the sizes of N pools in semiarid prairie soil ecosystems under long-term (more than 30 years) management treatments. Soils were sampled from undisturbed, set-aside from cultivation, grazing at moderate and high intensities, and cultivated with winter wheat (*Triticum aestivum* L.). Total N, microbial biomass N, and soluble N were quantified. Activities of amidohydrolases (L-asparaginase, L-glutaminase, urease), protease, β-glucosaminidase, and nitrate reductase were determined. When compared with the undisturbed system, long-term cultivation decreased total N and microbial N pools, but increased the concentrations of soluble N. Grazing, in particular at moderate intensity, did not lessen the capacity of the soil ecosystem to transform nitrogenous compounds and cycle N. However, grazing at high intensity did reduce the total N pool. Of the soils tested, cultivated soils had the lowest activities for N-transforming enzymes, except for protease activity which was increased significantly by long-term cultivation and grazing. When compared with the undisturbed soils, grazing at different intensities did not alter

asparaginase and urease activities significantly, and stimulated activities of glutaminase, β-glucosaminidase, and protease at levels higher than those in the undisturbed system. Grazing at moderate intensity reduced nitrate reductase activity significantly, resulting in activity levels that were not significantly different from those in the cultivated soils. With the exception of protease activity, N-transforming enzyme activities in the set-aside from cultivation system were intermediate between the cultivated and undisturbed systems. Of the soils tested, protease activity was the highest in the set-aside from cultivation system. Most of the tested enzyme activities were positively correlated with soil chemical and microbial properties, with correlation coefficients ranging from 0.39^{*} to 0.91^{***} ($P \leq 0.05$). However, activities of these enzymes were not strongly correlated with the labile C and N contents. Data obtained suggested that grazing did not lessen the capacity of the soil ecosystem to transform nitrogenous compounds. Long-term cultivation and associated soil disturbance significantly decreased the sizes of total N and microbial N pools, and led to development of a microbial community with reduced N use efficiency. Based on the evaluation of different soil N pools and N-transforming enzyme activities, ecosystem conservation for more than 30 years stimulated secondary succession that consequently permitted the soil ecosystem to regain its physiological capacity to recycle N, support microbial life, and evolve towards the functioning capacity of the undisturbed soil ecosystem. However, the distinct clustering of N-transforming enzymes according to management practices illustrated that 30 years of conservation did not completely erase the impact of cultivation. This study demonstrated that N-transforming enzymes were sensitive in revealing differences among soil ecosystems and therefore can be useful indicators for detecting impacts of management practices and for

evaluation on effectiveness of conservation efforts in restoring and maintaining functional diversity of soils at various stages of ecological succession.

1. Introduction

Amidohydrolases are hydrolytic enzymes that break C-N bonds and release NH_4-N. Protease breaks peptide bonds that also lead to the release of NH_4-N. Since 97 to 99% of soil N is in organic forms, and ammonification is the first step to their mineralization, amidohydrolases and protease play crucial roles in N cycling. Activities of amidohydrolases and protease are affected by cultivation and crop species (Asmar et al., 1994; Caravaca et al., 2002), cropping systems and residue management (Deng and Tabatabai, 1996; Dodor and Tabatabai, 2003; Klose and Tabatabai, 2000), fertilizer and manure application of different types at different rates (Bandick and Dick, 1999; Deng et al., 2006; Kang and Lee, 2005), successional stage (Tscherko et al., 2003), and degree of soil erosion (Garcia et al., 1994). As a common practice in semiarid prairie ecosystems (Burke et al., 1997), grazing also influences amidohydrolase activities and impacts soil N mineralization-immobilization through consumption and waste deposition by animals (Singh et al., 1991), stimulation of above ground biomass production (Schuman et al., 2002) and root exudation (Schuman et al., 1990), which alter microbial community structure and activity and impact soil enzyme activity (Wardle et al., 2004). However, reports about the impact of grazing on soil microbiological and biochemical properties are not consistent. Soil microbial biomass decreased by intensive grazing in semiarid grassland soils (Holt, 1997; Sankaran and Augustine, 2004). In another case, however,

chronic grazing by herds of large herbivores did not deplete microbial biomass (Tracy and Frank, 1998), while long-term exclusion of grazing from grassland resulted in significant reductions in microbial biomass and activity in the surface soil (Bardgett et al., 1997).

As a crucial element in biological systems, N cycling and dynamics are regulated by microbial and biochemical processes. Understanding the changes of amidohydrolase and protease activities in response to grazing and cultivation are important in developing strategies to enhance the capacity of soil to cycle N and ecosystem function. As sensitive indicators of environmental perturbations (Dick, 1992), activities of enzymes involved in N transformations indicate the soil potential to transform N (Burns, 1982), and reveal information on N dynamics and effects of management on soil ecosystem function. Therefore, the objectives were to evaluate the effects of different management practices on the sizes of different soil N pools, and on the activities of L-asparaginase, L-glutaminase, urease, protease, β-glucosaminidase, and nitrate reductase in semiarid prairie ecosystems.

2. Materials and methods

The site and location of the research area were described in detail in Chapter II. Soils were sampled in May 2005 from five long-term treatments, including undisturbed (UD), abandoned from cultivation (AB), moderate grazing (MG), heavy grazing (HG), and cultivated with winter wheat (CL). The sampling strategy for this study was described in Chapter III. Briefly, composite soil samples (35 to 45 cores, 0 to 0.10 m

depth) were obtained from nine randomly selected plots (0.5 ha each) of each treatment to serve as field replications.

Preparation and analysis of the soil samples and results for clay and sand content, soil pH, total soil organic C (C_{org}), total N (N_t), dissolved organic C (DOC), inorganic N (N_{sol}), soil microbial biomass C (C_{mic}) and N (N_{mic}), and dehydrogenase activity (DH) were described in Chapter III. Briefly, soils tested had neutral to alkaline pH values (7.2 to 7.6) with textures varying from Loam to Silt loam. The UD and MG soils had the highest but similar C_{org}, N_t, C_{mic}, and N_{mic} contents, while the CL soil had the least. Intensive grazing resulted in significantly lower C_{org} and N_t contents than the UD system, but not C_{mic} and N_{mic}. Of all systems tested, the CL system had the highest contents of DOC and N_{sol}. In the uncultivated soils, N_{mic} accounted for more than 2.0% of N_t, but only 1.5% in the CL system.

Enzyme activities involved in N transformations were determined using field-moist soil samples (particle size < 2 mm) by methods listed in Table 1. All analyses were conducted in duplicate. Results were expressed on a moisture free basis. Soil moisture content was determined gravimetrically after drying at 105 ^{o}C for 48 h.

Statistical analysis was performed as described in Chapter III and included analysis of variance, mean comparisons (*LSD* test, $P \leq 0.05$), pairwise correlations between tested properties, and principal component analysis (PCA) of enzyme activities. JMP® start statistics software (Sall et al., 2005) was used for PCA and data presentation. Enzyme activity per unit C_{mic} was calculated to evaluate impact of cultivation and

Table 1. Methods used for the assays of N-transforming enzyme activities.

Class/EC number	Enzyme name	Reaction	Substrate	Reference
3.5.1.1	L-asparaginase	L-asparagine + $H_2O \rightarrow$ L-aspartic acid + NH_3	L-asparagine	Frankerberger and Tabatabai (1991a)
3.5.1.2	L-glutaminase	L-glutamine + $H_2O \rightarrow$ L-glutamic acid + NH_3	L-glutamine	Frankerberger and Tabatabai (1991b)
3.5.1.5	Urease	$NH_2CONH_2 + H_2O \rightarrow CO_2 + 2NH_3$	Urea	Tabatabai and Bremner (1972)
3.6.1.1	Protease	Proteins or polypeptides + $H_2O \rightarrow$ amino acids	Casein	Ladd and Butler (1972)
3.2.1.30	β-glucosaminidase	Hydrolysis of terminal non- reducing *N*-acetyl-β-D-glucosamine residues	*p*-Nitrophenyl-N-acetyl-β-D-glucosamine	Parham and Deng (2000)
1.7.99.4	Nitrate reductase	$NO_3^- \rightarrow NO_2^-$	5 mM KNO_3	Abdelmagid and Tabatabai (1987)

grazing on metabolic activity of the microbial community. Natural logarithm transformations were used for the comparisons of the ratios of L-asparaginase, protease, and β-glucosaminidase to C_{mic}, because the residuals of the data and/or the variances were not equal. Ratios between enzyme activities were calculated to assess impact of cultivation and grazing on enzyme substrate compositions that reflect changes in the composition of soil organic N pool (Caldwell, 2005).

3. Results

3.1. Enzyme activities

Of the soils tested, cultivated soils had the lowest activities for N-transforming enzymes, except for protease (Fig. 1). Most notably, when compared to the UD soils, activities of asparaginase and urease decreased by more than 70% in the CL system. Smaller, but significant reductions were observed for glutaminase, β-glucosaminidase, and nitrate reductase, whereas protease activity in the CL system was 47% higher than in the UD.

Compared to UD soils, grazing at different intensities did not significantly alter asparaginase and urease activities. Grazing stimulated activities of glutaminase, β-glucosaminidase, and protease, resulting in activity levels higher than those in UD system. Interestingly, grazing at moderate intensity reduced nitrate reductase activity significantly, resulting in activity levels that were not significantly different from those in the CL soils.

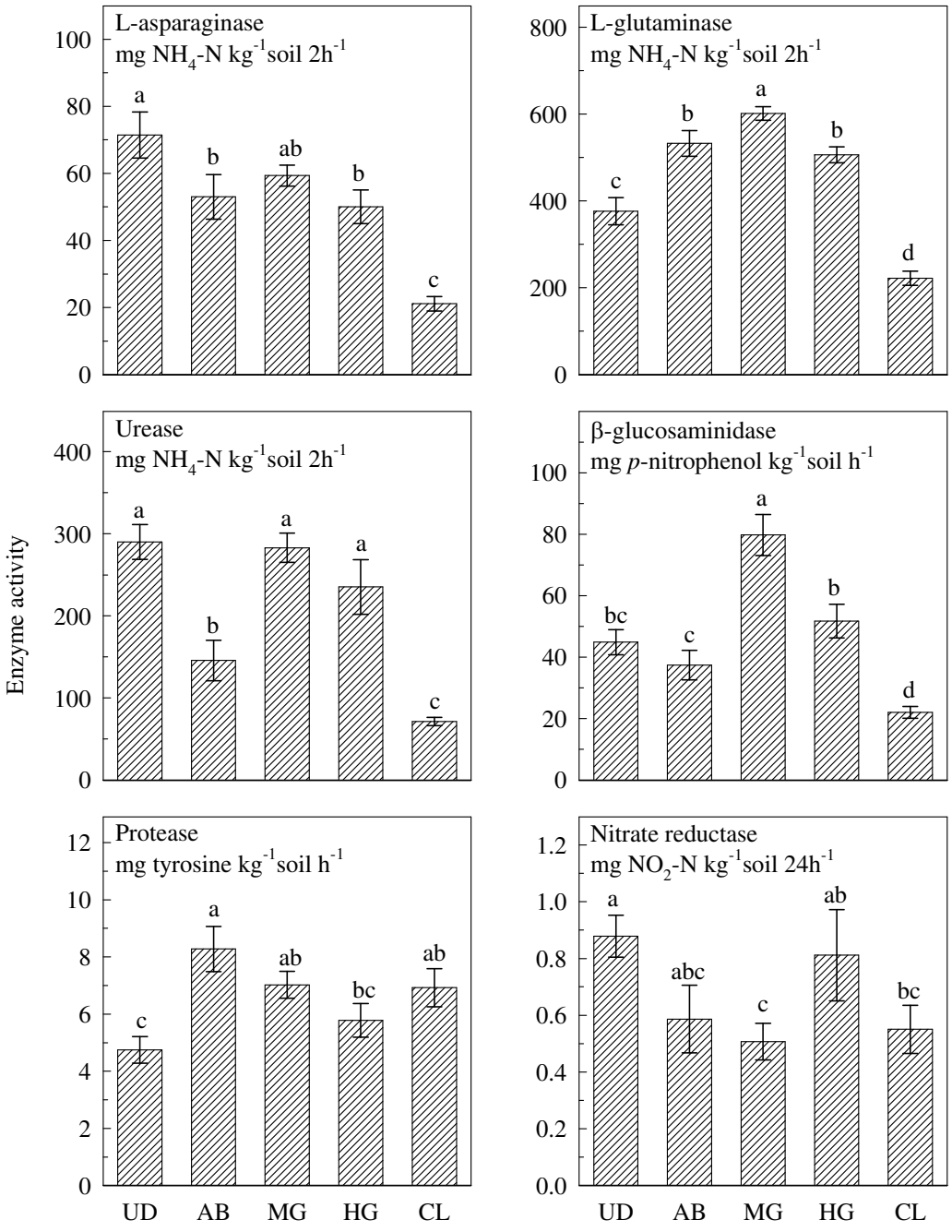

Fig. 1. Effect of management on N-transforming enzyme activities. UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test (n = 9, $P \leq 0.05$).

With the exception of protease activity, N-transforming enzyme activities in the AB system were intermediate between the CL and UD systems. Protease activity was highest in the AB system.

Most of the tested enzyme activities positively correlated with soil chemical and microbial properties, with correlation coefficients ranging from 0.39^{*} to 0.91^{***} (Fig. 2 and Table 2). However, activities of these enzymes were not strongly correlated with the labile C (DOC) and N (N_{sol}) contents. Correlations between β-glucosaminidase and DH activity or between nitrate reductase and C_{mic} were not significant, while little correlations were observed between protease activity and other soil properties and enzyme activities tested.

According to substrates used, four categories of N-transforming enzymes were tested, including enzymes that hydrolyze amino acids (asparaginase and glutaminase), amino sugars (β-glucosaminidase), urea, and proteins. The ratios between different enzyme activities are indicators of relative quantity of different nitrogenous compounds present in the soil N pools (Caldwell, 2005), and indicative of the relative potential for each management system to hydrolyze various organic N compounds. When compared to the UD system, cultivation and grazing did not affect the ratio of amino acid hydrolases to β-glucosaminidase activities, but increased the ratios of amino acid hydrolases or β-glucosaminidase to urease activities (Table 3). Activity ratios of protease to the other three categories of enzymes were increased by cultivation, but not significantly affected by grazing. When enzyme activities were expressed per unit of C_{mic}, the activities of asparaginase and urease were not significantly affected by cultivation or grazing. The activities of protease, β-glucosaminidase, and nitrate reductase

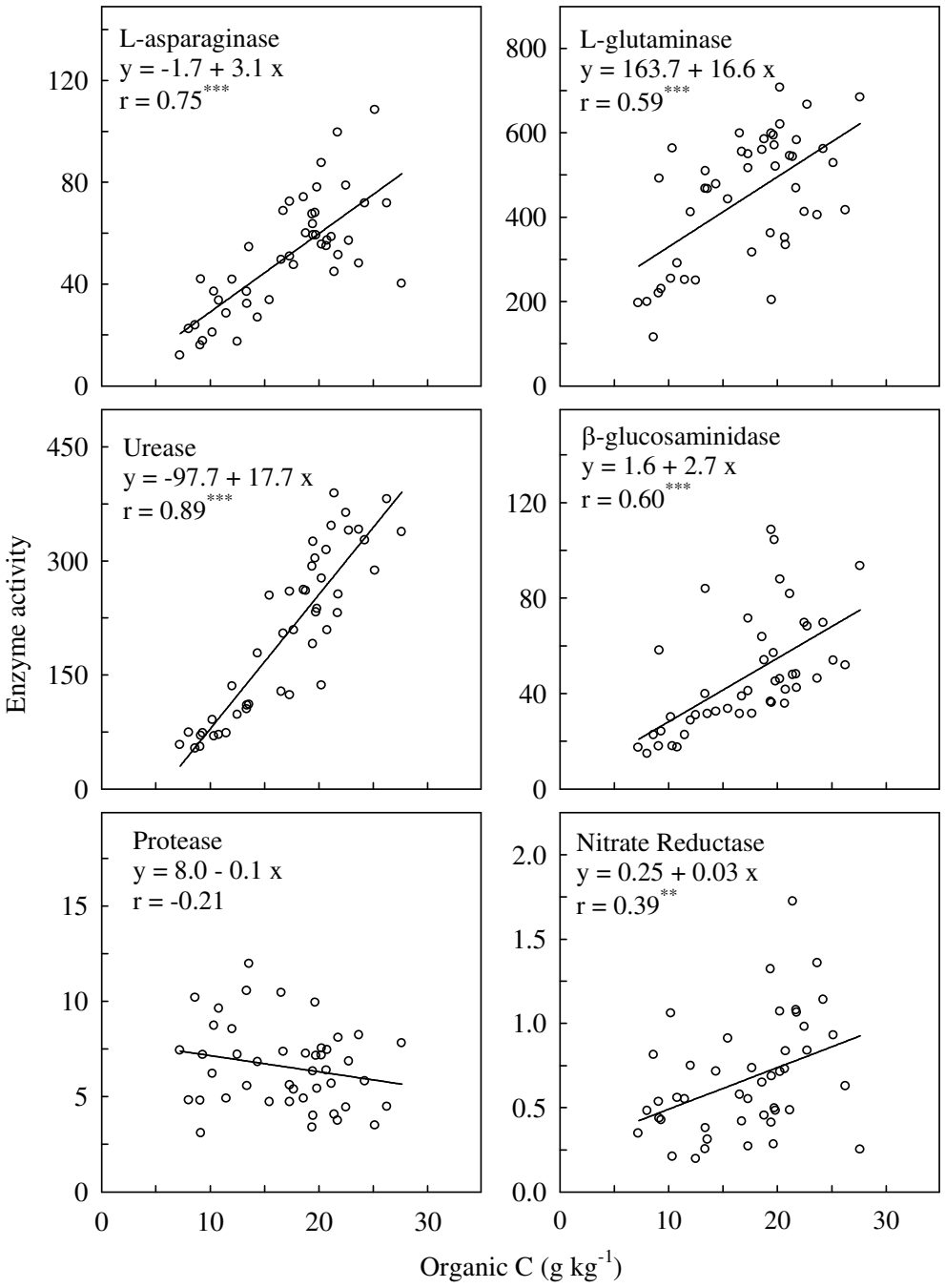

Fig. 2. Relationship between soil organic C contents and N-transforming enzyme activities in soils under different management systems. Activities of L-asparaginase, L-glutaminase, and urease are expressed as mg NH_4-N kg^{-1} soil $2h^{-1}$, of protease as mg tyrosine kg^{-1} soil h^{-1}, of β-glucosaminidase as mg *p*-nitrophenol kg^{-1} soil h^{-1}, and of nitrate reductase as mg NO_2-N kg^{-1} soil $24h^{-1}$ ($n = 45$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$).

Table 2. Correlation coefficients (*r*) between soil properties, and N-transforming enzyme activities (*n* = 45).

Variable	N_t	DOC	N_{sol}	C_{mic}	N_{mic}	DH	L-asp	L-glu	Urease	Protease	β-GSM
L-asparaginase	0.65^{***}	-0.20	-0.08	0.66^{***}	0.68^{***}	0.46^{**}					
L-glutaminase	0.48^{***}	-0.26	-0.45^{**}	0.67^{***}	0.60^{***}	0.33^{*}	0.56^{***}				
Urease	0.91^{***}	-0.14	-0.01	0.85^{***}	0.81^{***}	0.74^{***}	0.58^{***}	0.43^{**}			
Protease	-0.19	0.07	-0.08	-0.18	-0.19	-0.15	-0.28	0.08	-0.31^{*}		
β-glucosaminidase	0.54^{***}	-0.34^{*}	-0.06	0.63^{***}	0.52^{***}	0.25	0.43^{**}	0.65^{***}	0.53^{***}	-0.16	
Nitrate reductase	0.38^{**}	-0.04	0.03	0.25	0.43^{**}	0.47^{**}	0.28	0.00	0.46^{**}	-0.33^{*}	-0.02

N_t: Total N; DOC and N_{sol}: C and N extractable with 0.5 M K_2SO_4; C_{mic} and N_{mic}: Microbial biomass C and N; DH: Dehydrogenase activity; L-asp: L-asparaginase; L-glu: L-glutaminase; β-GSM: β-glucosaminidase. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Table 3. Treatment effects on enzyme activity ratios and ratios of enzyme activities to microbial biomass C (n = 9).

Ratio	Treatment[1]					*LSD* ($P \leq 0.05$)
	UD	AB	MG	HG	CL	
Enzymes						
(L-asp + L-glu) : β-GSM	0.10	0.06	0.12	0.10	0.09	0.03
(L-asp + L-glu) : Urease	1.61	4.70	2.41	3.06	3.45	1.27
Protease : (L-asp + L-glu)	0.01	0.01	0.01	0.01	0.03	0.01
Protease : Urease	0.02	0.07	0.03	0.03	0.10	0.03
Protease : β-GSM	0.11	0.26	0.09	0.12	0.33	0.08
β-GSM : Urease	0.16	0.27	0.30	0.30	0.31	0.14
Enzyme to Microbial biomass C[2]						
L-asparaginase : C_{mic}[3]	0.12	0.12	0.09	0.09	0.12	0.03
L-glutaminase : C_{mic}[3]	0.64	1.20	0.90	0.99	1.30	0.29
Urease : C_{mic}[3]	0.49	0.31	0.41	0.43	0.42	0.12
Protease : C_{mic}[4]	0.01	0.02	0.01	0.01	0.04	0.01
β-glucosaminidase : C_{mic}[5]	0.08	0.08	0.12	0.10	0.13	0.04
Nitrate reductase : C_{mic}[6]	0.001	0.001	0.001	0.001	0.003	0.001

[1]UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. (L-asp + L-glu): Sum of L-asparaginase and L-glutaminase activities; β-GSM: β-glucosaminidase; [2]Mean comparisons for L-asparaginase, protease and β-glucosaminidase to C_{mic} ratio were based on natural logarithm transformed data; [3]mg NH_4-N mg^{-1} C_{mic} $2h^{-1}$; [4]mg tyrosine mg^{-1} C_{mic} h^{-1}; [5]mg *p*-nitrophenol mg^{-1} C_{mic} h^{-1}; [6]mg NO_2-N mg^{-1} C_{mic} $24h^{-1}$.

per unit of C_{mic} increased significantly by cultivation. Glutaminase activity per unit of C_{mic} increased by both cultivation and grazing.

3.2. Relationships among enzymes

Significant positive correlations were found between most of the enzymes involved in N transformations, with correlation coefficients ranging from 0.43^{**} to 0.65^{***} (Table 2). However, protease activity had little correlation with other enzyme activities. Nitrate reductase had weak positive correlation with urease and weak negative correlation with protease.

To reduce the dimensionality of the six-variable system of enzyme activities, principal component analysis (PCA) was applied. Enzyme activities in the coordinate system of Figure 3 defined by the first two principal axes (PC1 and PC2) were represented by Gabriel biplot rays. These analyses further illustrated that the tested enzyme activities were correlated with each other, and that urease and asparaginase made marked contribution to PC1. The PC1 accounted for 46% of the variance in the data, while PC1 and PC2 together accounted for 70%. Activities of enzymes involved in N transformations were clustered according to management practices. PC1 separated all treatments except for the grazed systems from the UD, while PC2 differentiated the AB and grazed systems from the UD, and also the CL system from the AB and MG.

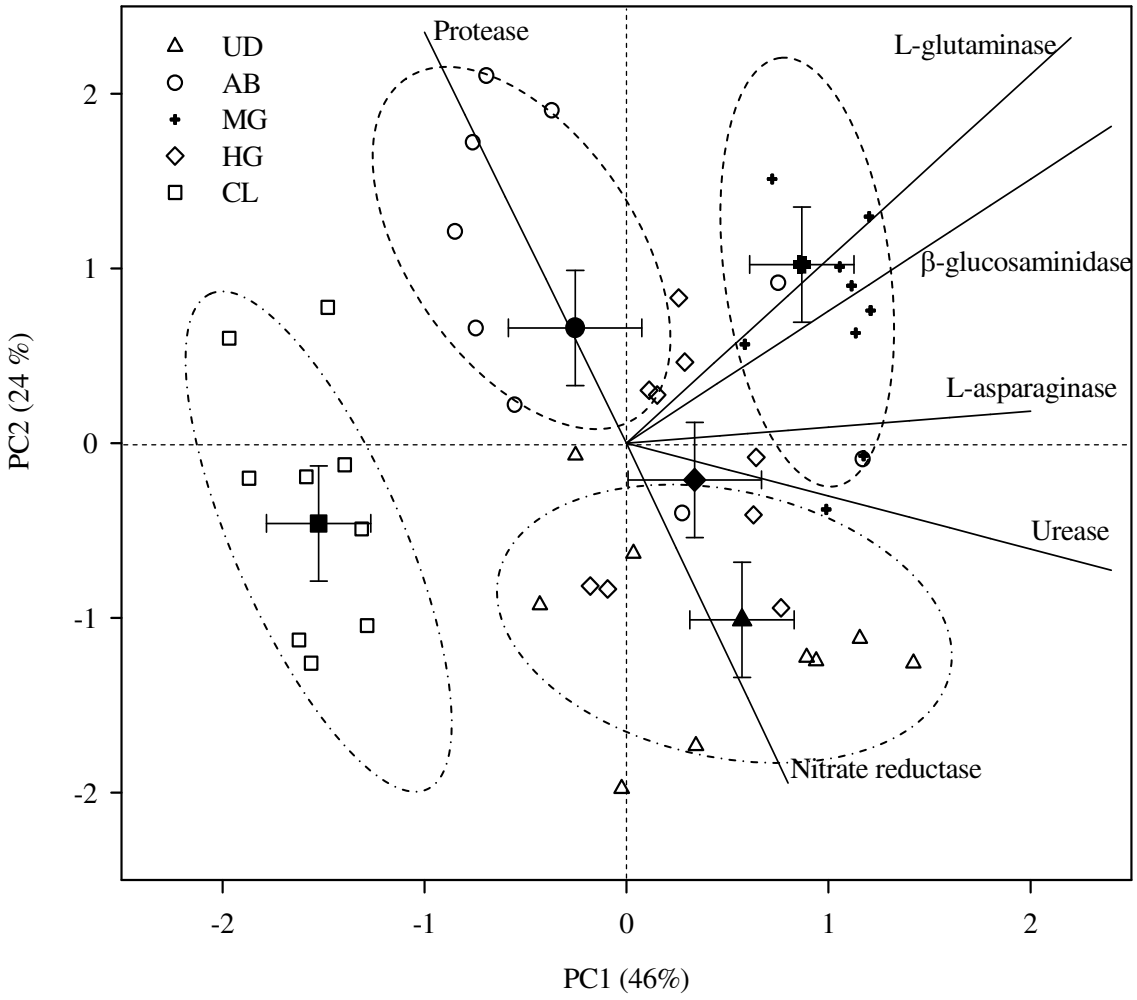

Fig. 3. Gabriel biplot of enzyme activities against the first two principal components (PC1 and PC2). Rays that have small angle with a PC axis contribute more to that PC. Rays that have small angles with each other are positively correlated. Filled symbols represent treatment means (n = 9). Horizontal and vertical error bars are based on 95% confidence intervals for the mean PC1 and PC2, respectively. Overlapping bars indicate that two means are not significantly different at the 95% confidence level. UD: Undisturbed; AB: Abandoned from cultivation; HG: Highly grazed; MG: Moderately grazed; CL: Winter wheat.

4. Discussion

4.1. Enzyme activities

The similarity in the responses of the tested N-transforming enzymes and soil chemical and microbial properties to cultivation demonstrated the interdependence among these properties, the importance of organic matter in protecting and maintaining soil enzymes in their active forms, and the dependence of enzymatic degradation processes on the soil microbial community.

Of the ecosystems evaluated, grazing, and especially moderate grazing, enhanced the activities of most N transforming enzymes and potential of soil to cycle N. Animal excreta, the year-round presence of vegetation, and the widespread fibrous root system of perennial grasses increased rhizosphere-microbe interaction and promoted activities of soil biota (Bandick and Dick, 1999). When compared to the cultivated soils, the set-aside from cultivation soil ecosystem had partially restored its capacity to support the microbial community and its ability to cycle N.

Although the cultivated system received annual applications of urea fertilizer, it had the lowest urease activity of the soils tested. As an enzyme that hydrolyzes urea and releases NH_4-N, urease activity was not increased by urea fertilizer application, suggesting that the cultivated soils are stressed environments for microbial growth, possibly due to limited C availability as suggested in Chapter III. Relatively small size of the microbial community is evidenced by microbial C and N contents. The close relationship between urease activity and microbial biomass indicated that urease activity

in soil was predominantly intracellular and of microbial origin. This is also evidenced by the significant correlation between urease activity and dehydrogenase activity, a known intracellular enzyme. In addition, the cultivated system received monoammonium phosphate that slowly released NH_4-N and contributed to soluble N. The increased N in soil solution could suppress urease activity, a hypothesis supported by studies of Burket and Dick (1998) and McCarty et al. (1992).

As an important form of organic N, soil amino acid composition differs between cultivated and uncultivated soils (Warman and Isnor, 1991). Because asparaginase and glutaminase are mostly inducible enzymes (Skujins, 1976), the significantly higher glutaminase activities indicated the dominant presence of glutamine versus asparagine in all soils tested. Cultivated systems had lower asparaginase activities than the uncultivated systems, possibly due to reduced microbial activity and inorganic fertilizer additions that suppressed asparaginase and glutaminase activities (Kang and Lee, 2005).

Activity of β-glucosaminidase is involved in both C and N cycling (Ekenler and Tabatabai, 2002; Parham and Deng, 2000), and was enhanced by grazing but lessened by cultivation. Studies showed that constitutive β-glucosaminidase was produced by fungi other than chitinolytic bacteria and actinomycetes (Miller et al., (1998). The grazed systems in this study were dominated by microbes with higher microbial C to N ratios (Chapter III), indicative of higher proportion of fungal biomass. The shift of the microbial community towards fungal dominance was also demonstrated by Fatty Acid Methyl Ester (FAME) analysis (Chapter VI).

The relatively low protease activity and weak correlations between its activity and soil organic C and clay content (data not shown) for all soils tested, support the concept

that proteases do not persist in the soil environment. Studies showed that extracellular protease did not stay active in soils for longer than six to seven days (Watanabe and Hayano, 1996). Protease activity in soil was attributed to living microorganisms and root hairs (Badalucco et al., 1996). In dryland sweet potato fields and lowland rice paddy soil, the dominant source of protease was *Bacillus* spp. (Watababe and Hayano, 1993 and 1994). In this study, protease activity was not correlated with microbial biomass or dehydrogenase activity, suggesting that this enzyme is predominantly of plant origin. Low protease activities may be the rate-limiting factor for N mineralization (Asmar et al., 1994), because protease breaks peptide bonds and releases amino acids for subsequent degradation that ultimately lead to the release of NH_4-N. The relatively high protease activities in the set-aside from cultivation and the moderately grazed system indicated greater potential for these systems to cycle N through protein degradation and ammonification.

The grasslands of the Southern Great Plains in the United States emit an estimated 0.42 kg N_2O-N ha^{-1} yr^{-1} through nitrification and denitrification processes, while the mean flux in the USA is estimated to be 0.28 kg N_2O-N ha^{-1} yr^{-1} (Mummey et al., 2000). However, the activities of nitrate reductase in this study were generally low in all management systems, due to the prevailing semiarid conditions in the study area; therefore the potential for N losses through denitrification would be limited. The undisturbed, highly grazed and set aside from cultivation systems had the highest and similar nitrate reductase activity, and possibly similar potential for denitrification. The relatively high nitrate reductase activity in these systems was attributed to the absence of soil disturbance and the presence of litter accumulation and grazing activity that may

promote formation of anaerobic microsites. On the other hand, synthesis of nitrate reductase may be limited in the cultivated soils because of the increase aeration due to tillage.

4.2. Effect of soil chemical and microbial properties on enzyme activities

Regardless of whether the enzyme activities decreased or increased by cultivation, activities per unit of microbial biomass C were unchanged or increased following conversion of grassland to winter wheat. Cultivation introduced stress factors absent from the undisturbed soils, which promoted metabolic activity of the microbial community and enhanced enzyme synthesis. Several studies have reported that environmental stress induced high metabolic activity of the inhabitant microbial community (Allison and Vitousek, 2005; Harder and Dijkhuizen, 1983). The enhanced metabolic activity is also evidenced by the increased dehydrogenase activity per unit of microbial C (Chapter III). Moreover, increase of an enzyme activity per unit of microbial C following treatments that significantly reduced microbial activity would suggest that activity of this enzyme originated predominantly from accumulated enzymes that were free of microbial cells and persisted in the soil environment (Deng et al., 2006). Cultivation reduced the activities of glutaminase, β-glucosaminidase, and nitrate reductase but increased their activities per unit of microbial biomass C, suggesting that these enzyme activities were either dominated by accumulated enzymes that persisted in the soil or they were intracellular. Specifically, syntheses of glutaminase and β-glucosaminidase are known to be inducible (Deng et al., in press; Peterbauer et al., 1996;

Skujins, 1976), and their activities are dominated by extracellular enzymes, suggesting that these enzymes persisted in cultivated soils. Nitrate reductase activity in the cultivated soils originated predominantly from intracellular enzymes, which is also evidenced by the significant correlation between activities of this enzyme and dehydrogenase. The activities of asparaginase or urease per unit of microbial C were not affected significantly by cultivation or grazing, indicating that synthesis of these two enzymes were not sensitive to environmental conditions, and activities of these two enzymes were dominated by intracellular enzymes.

As discussed above, proteases are mostly of plant origin, and their activities do not persist in the soil environment for longer than six to seven days (Watanabe and Hayano, 1996). The significant increase of protease activity by cultivation and grazing indicate synthesis and release of substantial quantity of protease by plants. Protease activity per unit of microbial C indicates plant and microbe interactions in the changing environment. The significant higher ratios in cultivated soils than the grazed soils suggest that protease degradation rates were lower in the cultivated or once cultivated soils. This is probably due to the reduction in microbial biomass (Nunan et al., 2000), evidenced by the negative correlation between the activities of protease and dehydrogenase. Although the synthesis of both asparaginase and glutaminase have been suggested to be inducible by the presence of their respective substrates, responses of their activities or activities per unit of microbial C to management practices were different, reflecting varied substrate input due to changes in the above ground plant community composition.

4.3. Relationships among enzymes

Most enzyme activities were significantly and positively correlated with each other and with organic C contents, as shown by numerous other studies (Deng and Tabatabai, 1997). The low correlation between protease activity to activities of other tested enzymes and soil organic C revealed the distinctly different source and status of this enzyme in the soil environment, as discussed above. The wide angles between the activity of nitrate reductase or protease on principal component axes indicated their limited contributions to the treatment separation. Of the enzymes tested, urease contributed most to PC1, followed by asparaginase and β-glucosaminidase, whereas nitrate reductase and protease contributed most to PC2. These results showed that activities of amidohydrolases revealed differences in N transformations processes among a wide range of management practices and among systems at various stages of ecological succession.

Despite the supplementation of readily available N, the microbial community in the cultivated soils showed greater ability to decompose complex nitrogenous compounds (amino acids, amino sugars, and proteins) rather than simpler organic N forms (e.g., urea), as evidenced by the increased ratios of protease to other N-transforming enzymes. The enhanced N availability by fertilizer application may have induced C limitation, driving the microbial community to break down complex organic compounds for C acquisition (Allison and Vitousek, 2005). Zhang et al. (1999) reported 54% lower amino sugar concentration in cultivated soils compared to native pastures. The lower ratio of microbial C to N and lower fungi proportion in the cultivated system compared to the rest

of the systems tested (Chapter VI) suggest lower contribution of amino sugars such as fungal chitooligosaccharides in the N pool, implying dependence on more complex organic N sources.

In the grazed systems, the ratios of amino acid transforming enzymes to urease and protease were lower than in the cultivated system, suggesting that soil N pools contained more urea versus amino acids and proteins. Microorganisms acquired more N from urea in the grazed systems from animal urine, preferring to metabolize the substrate that supports the highest rate of growth, whereas enzymes for the metabolism of other substrates remain repressed (Harder and Dijkhuizen, 1983). According to enzyme ratios, glutamine and asparagine contributed more to the organic N pool than amino sugars (higher ratio of glutaminase and asparaginase to β-glucosaminidase), and amino sugars contributed more than proteins (lower protease to β-glucosaminidase ratio). Because of the complex nature of nitrogenous compounds present in soil, evaluation of changes in enzyme activity ratios provides insight in revealing major differences in the composition of soil N pools that were affected by management practices.

4.4. Conclusions

When compared with the undisturbed system, long-term cultivation decreased total N and microbial N pools, increased the concentrations of soluble N, and resulted in a microbial community with reduced N use efficiency. Activities of most N-transforming enzymes were lower in the cultivated systems. Grazing, in particular at moderate

intensity, did not lessen the capacity of the soil ecosystem to transform nitrogenous compounds and cycle N. However, grazing at high intensity did reduce the total N pool.

Based on evaluation of different N pools and the N transforming enzyme activities, ecosystem conservation for more than 30 years stimulated secondary succession that consequently permit the soil ecosystem to regain its physiological capacity to recycle N, support microbial life, and evolve towards the functioning capacity of the undisturbed soil ecosystem. However, the distinct clustering of N-transforming enzymes according to management practices illustrated that 30 years of conservation did not completely erase the impact of cultivation.

Nitrogen-transforming enzymes were sensitive in revealing differences between soil ecosystems and therefore, can be useful indicators for detecting impacts of management practices and for evaluation on effectiveness of our conservation effort in restoring and maintaining functioning diversity of soils at various stages of ecological succession.

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CHAPTER V

EFFECTS OF MANAGEMENT ON SOIL PHOSPHORUS POOLS AND PHOSPHATASE ACTIVITIES

Abstract

Understanding factors that regulate P cycling and dynamics in soil ecosystems are important in developing management practices that sustain and enhance ecosystem function. The objectives were to evaluate the effect of long-term (more than 30 years) land use and management practices on the P pool sizes and phosphatase activities in semiarid prairie soil ecosystems. Treatments included undisturbed, set-aside from cultivation, heavily grazed, moderately grazed, and winter wheat (*Triticum aestivum* L.). Total, organic, labile, and microbial P were quantified. Activities of acid and alkaline phosphomonoesterase, phosphodiesterase, and inorganic pyrophosphatase were determined. In general, phosphatase activities were more sensitive to cultivation than grazing. Soil disturbance by grazing or cultivation did not lead to significant change in soil total P. When compared to the undisturbed soils, grazing did not alter the size and composition of the tested P pools nor lessen the capacity of the soil to cycle P. However, long-term cultivation led to significant increase in soil Olsen-P and significant reduction of microbial biomass P, while fertilizer P application suppressed phosphatase activities.

As a result, cultivation led to organic P accumulation and reduced the capacity of soil to cycle P. Of the P pools tested, the major quantitative shift induced by cultivation occurred in the inorganic P. Moreover, cultivation led to the development of a microbial community with significantly reduced P use efficiency, while grazing maintained a soil microbial community with relatively high P use efficiency. Abandonment from cultivation allowed the soil ecosystem to regain its capacity to cycle P and to slowly evolve towards the P cycling capacity of the native soil.

1. Introduction

As a crucial component of biological systems that enable the existence of life, phosphorus in the environment receives increasing attention. Soils usually contain between 100 to 3000 mg P kg^{-1} soil, and organic forms of P can range from 30 to 65% of total soil P (Harrison, 1987). The size and dynamics of different pools of soil P are regulated by microbiological and biochemical processes (Parton et al., 2005; Richardson, 1994). Understanding P cycling in the soil environment is important in assessing ecosystem functions that may be directly linked to land use and management practices.

Extracellular phosphatases govern the transformation of organic P to orthophosphate (McGill and Cole, 1981), and thus are key players of P cycling. Activities of phosphatases are affected by vegetation type and density (Caldwell et al., 1999; Waldrop et al., 2000), and vary with ecosystem successional stage (Tscherko et al., 2003), soil erosion (Garcia et al., 1994), and agricultural practices, including cropping

systems, tillage, fertilizer applications, and grazing intensity (Deng et al., 2006; Deng and Tabatabai, 1997; Singh and Ral, 2004).

Microbial biomass P is an active form of the soil organic P that responds rapidly to changes in the soil environment and affects P availability to plants in natural and managed soil ecosystems (Oberson and Joner, 2005). The annual rate of P cycling through microbial biomass was approximately 2 to 11 for arable soils and 7 to 40 kg P ha^{-1} yr^{-1} for grassland soils in the UK (Brookes et al., 1984). In New Zealand pastures, microbial P fluxes were between 14 and 36 kg ha^{-1} yr^{-1} (Chen et al., 2003; Sparling et al., 1994). These findings demonstrate that microbial biomass P plays a key role in P cycling in grassland ecosystems and is affected by management practices. Factors that affect substrate availability for microorganisms, such as the quantity and quality of organic matter inputs, tillage, crop rotation, and soil pH, also influence microbial biomass P (Aarons et al., 2004; Balota et al., 2003). However, limited information is available on the processes that determine P cycling dynamics and availability (Frossard et al., 2000).

In semiarid prairie ecosystems, cultivation and grazing are common agricultural practices (Burke et al., 1997), and P availability is second to N as a limiting nutrient for primary productivity (Cole et al., 1977). Few studies were conducted to understand the role of phosphatase activities and microbial biomass P in regulating organic P transformations in such environments. Therefore, the objective was to evaluate the effect of different management practices on the soil P pools and activities of phosphatases (acid and alkaline phosphomonoesterase, phosphodiesterase and inorganic pyrophosphatase) in prairie soils under semiarid conditions.

2. Materials and methods

The site and location of the research area were described in detail in Chapter II. Soils from five long-term (conducted for at least 30 years) treatments, including undisturbed (UD), set-aside from cultivation (AB), moderate grazing (MG), heavy grazing (HG), and winter wheat (CL) were sampled in May 2005. The sampling scheme was described in Chapter III. Briefly, composite soil samples (35 to 45 cores, 0 to 0.10 m depth) were obtained from nine randomly selected plots (0.5 ha each) from each treatment, which served as field replications.

Preparation and analysis of the soil samples for soil chemical and microbial properties were performed as described in Chapter III. Calcium carbonate contents ($CaCO_3$) were measured with the titrimetric method (Soil Survey Staff, 1996) and ranged between 3 and 6%. Soils of all treatments had neutral to alkaline pH values (7.2 to 7.6), and textures that varied from Loam to Silt loam. Of the treatments evaluated, the UD and MG soils had the highest or similar organic C (C_{org}), total N (N_t), microbial biomass C (C_{mic}) and N (N_{mic}) contents, while the CL soil had the least (Fig. 1 and Table 3, Chapter III). More intensive grazing (HG) resulted in significantly lower C_{org} and N_t contents than the UD and MG systems, while microbial biomass C and N were not different from those in the UD system. Of all systems tested, the HG system had the highest dehydrogenase activity (DH), and the CL system had the highest contents of dissolved organic C (DOC) and inorganic N (N_{sol}) (Table 3, Chapter III). Total P was measured after digestion with $HClO_4$ acid (Olsen and Sommers, 1982). Organic P (P_{org}) was determined by sequential extraction of soil with concentrated HCl and NaOH (Mehta et

al., 1954). In this procedure, inorganic P (P_{inorg}) was determined directly by colorimetric method (Murphy and Riley, 1962), while total P (P_t) was determined following $HClO_4$ digestion of the extracts. Organic P was calculated as the difference between total and inorganic P. Soil microbial biomass P (P_{mic}) was determined after fumigation-extraction with 0.5 M $NaHCO_3$, pH: 8.5, at 1:2 soil:$NaHCO_3$ ratio as described by Brookes et al. (1982). Contents of P extracted from the unfumigated soils were used as a measure of available P (P_{olsen}). Enzymes involved in organic P transformations were determined in the field-moist samples by methods listed in Table 1. All analyses were conducted in duplicate. Results are expressed on a moisture free basis. Soil water content was determined gravimetrically after drying at 105 ^{o}C for 48 h.

Statistical analysis was performed as described in Chapter III and included analysis of variance, mean comparisons (*LSD* test, $P \leq 0.05$), and principal component analysis of enzyme activities. Principal component analysis (PCA) was applied to reduce the dimensionality of the six-variable system of enzyme activities and performed by JMP® start statistics software (Sall et al., 2005). The percentages of P_{org}, P_{inorg} and P_{mic} in P_t, and of P_{mic} in P_{org} were calculated to indicate their relative contribution to the P_t and P_{org} pools. The ratio of P_{inorg} to P_{org} was calculated to indicate relative relationship between those P pools. The ratio of C_{org} to P_{org} was calculated to demonstrate changes in organic matter composition, and the C_{mic} to P_{mic} ratio to indicate P availability to the soil microorganisms and changes in microbial community composition (Anderson and Domsch, 1980; Muhammad et al., 2006). Enzyme activity per unit C_{mic} was calculated to evaluate treatment effect on metabolic activity of the microbial community. Ratios between enzyme activities were

Table 1. Methods used for the assays of P-transforming enzyme activities.

Class/EC number	Enzyme name	Reaction	Substrate	Reference
EC 3.1.3.2	Acid phosphomonoesterase	Orthophosphoric monoester + H_2O→ alcohol + orthophosphate	*p*-Nitrophenyl phosphate	Tabatabai and Bremmer (1969)
EC 3.1.3.1	Alkaline phosphomonoesterase	Orthophosphoric monoester + H_2O→ alcohol + orthophosphate	*p*-Nitrophenyl phosphate	Eivazi and Tabatabai (1977)
EC 3.1.4.1	Phosphodiesterase	Orthophosphoric diester + H_2O→ orthophosphate + alcohol or phenol or nucleoside	*Bis*(*p*-Nitrophenyl) phosphate	Browman and Tabatabai (1978)
EC 3.6.1.1	Inorganic pyrophosphatase	Pyrophosphate + H_2O → 2 orthophosphates	Sodium pyrophosphate	Dick and Tabatabai (1978)

calculated to assess impact of management practices on enzyme substrate compositions that reflect changes in the composition of soil organic P pools (Caldwell, 2005).

3. Results

3.1. Soil phosphorus pools

Soil total P contents determined by the two methods were similar. To avoid confusion, total soil P data from the extraction method were used in the analysis and presented. The mean concentration of total soil P was about 0.7 g P kg^{-1} soil, and was not significantly affected by land use and management practices (Fig. 1). The relative P pool sizes, however, were altered by the treatments evaluated. Long-term cultivation led to significant reduction in P_{mic}, and accumulation of P_{org} and P_{olsen}. Soil conservation by removal from cultivation for over 30 years resulted in P_{mic} levels that were significantly higher than in the cultivated soils, but significantly lower than in the UD and grazed ones. Removal from cultivation accelerated break down of P_{org} and led to P_{org} levels that were not only significantly lower than the cultivated soils, but also significantly lower than the grazed and noticeable lower than the UD soils.

The relationships among different P pools reflect P partitioning in the tested soil systems (Table 2). Although grazing did not alter the relative composition of organic and inorganic P in the system, organic P increased from 21% of the total P in the UD soils to 40% in the cultivated ones. Interestingly, in soils that were removed from cultivation for over 30 years, organic P constituted only 14% of total P, the lowest of all soils tested.

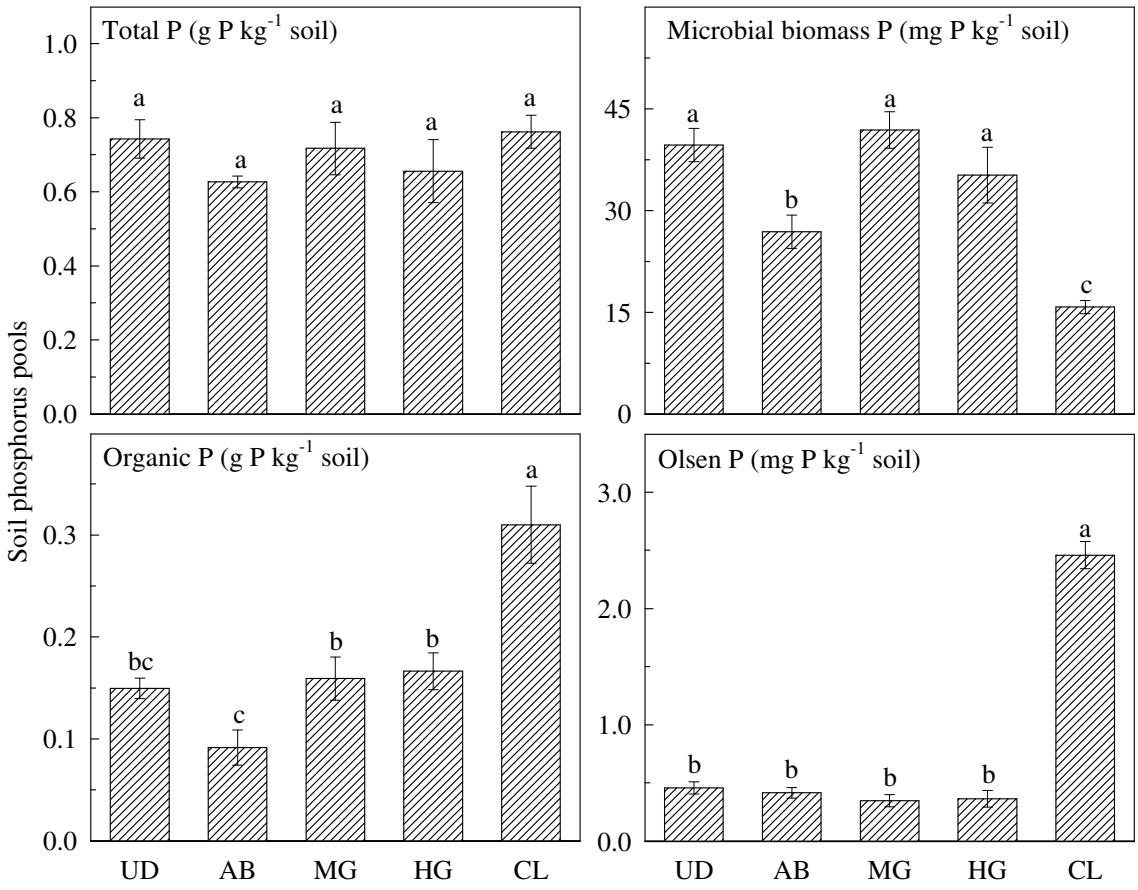

Fig. 1. Effects of land use and management practices on the sizes of soil phosphorus pools. UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

Table 2. Calculated elemental and eco-physiological ratios between selected soil chemical and microbial properties (n = 9).

Ratio[1]	Treatment[2]					*LSD* ($P \leq 0.05$)
	UD	AB	MG	HG	CL	
P_{org} : P_t (%)	21	14	22	27	40	7
P_{inorg} : P_t (%)	79	86	78	83	60	7
P_{inorg} : P_{org}	4.1	8.4	3.8	2.9	1.6	2.7
P_{mic} : P_t (%)	5.6	4.3	6.1	5.8	2.1	1.3
P_{mic} : P_{org} (%)	27	33	29	22	6	10
P_{mic} : P_{inorg}	0.07	0.05	0.08	0.08	0.04	0.02
P_{mic} : P_{olsen}	102	68	142	122	9	43
C_{org} : P_{org}	149	233	150	109	35	73
C_{mic} : P_{mic}	15	17	17	16	12	3

[1]P_{org}: Organic P; P_t: Total P; P_{inorg}: Inorganic P; P_{olsen}: P extracted with 0.5 M $NaHCO_3$; C_{org}: Organic C; P_{mic} and C_{mic}: Microbial biomass P and C. [2]UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat.

The relative shifts between organic and inorganic P pools were amply demonstrated by the ratios between these two P fractions. This ratio varied from 4.1 in the UD soils, to 1.6 in the cultivated ones, and to 8.4 in the AB soils.

About 2 to 6% of total soil P was in the form of P_{mic} (Table 2). The relationship between these two P pools was not altered significantly by grazing. This percentage significantly less for cultivated soils. Six to 33% of organic P was found in microbial biomass, with the highest value in the AB soils, and the lowest in the cultivated ones. The ratio of P_{mic} to P_{inorg} was increased slightly by grazing, but reduced significantly by cultivation. Similar trends were found for the ratio of P_{mic} to P_{olsen}, the two most dynamic P pools. Cultivation reduced this ratio from 102 in the UD soils to 9 in the cultivated ones.

Major changes in organic matter composition are reflected in the changes of C_{org} to P_{org} ratios. Grazing did not significantly affect composition of soil organic matter, but the ratio of C_{org} to P_{org} varied from 149 in the UD soils to 35 in the cultivated ones (Table 2). Interestingly, this ratio in the AB soils was 233, significantly higher than the UD soils, and the highest of all soils tested. Microbial community structure often reflects the nutritional status of the growth environment, and can be evaluated by examining the relationships between major nutrients composing the microbial biomass. When compared to undisturbed soils, ratios between C_{mic} to P_{mic} were significantly lower in the cultivated soils and slightly higher in grazed soils.

The relationships between soil P pools and between soil P and other chemical and microbial soil properties were further evaluated by correlations between tested properties (Table 3). Total P was weakly correlated with C_{org}, N_t, microbial biomass (C_{mic}, N_{mic}, and

Table 3. Correlation coefficients (*r*) between chemical and microbial soil properties (*n* = 45).

Variable	pH	$CaCO_3$	C_{org}	N_t	DOC	N_{sol}	C_{mic}	N_{mic}	DH	P_t	P_{org}	P_{inorg}	P_{olsen}
P_t	0.25	0.34^{*}	0.17	0.29^{*}	0.34^{*}	0.40^{**}	0.12	0.21	0.33^{*}				
P_{org}	0.15	-0.18	-0.27	-0.20	0.47^{**}	0.54^{**}	-0.34^{*}	-0.31^{*}	-0.24	0.59^{***}			
P_{inorg}	0.07	0.54^{***}	0.39^{**}	0.49^{***}	0.10	0.13	0.38^{**}	0.47^{**}	0.51^{***}	0.83^{***}	0.02		
P_{olsen}	-0.38^{*}	-0.25	-0.34^{*}	-0.35^{*}	0.17	0.26	-0.50^{***}	-0.53^{***}	-0.41^{**}	0.06	0.34^{*}	-0.17	
P_{mic}	-0.17	0.07	0.91^{***}	0.87^{***}	-0.03	-0.02	0.90^{***}	0.86^{***}	0.66^{***}	0.21	-0.20	0.39^{**}	-0.46^{**}

P_t: Total P; P_{org}: Organic P; P_{inorg}: Inorganic P; P_{olsen}: P extracted with 0.5 M $NaHCO_3$; C_{mic}, N_{mic}, and P_{mic}: Microbial biomass C, N and P; C_{org}: Organic C; N_t: Total N; DOC and N_{sol}: C and N extracted with 0.5 M K_2SO_4; DH: Dehydrogenase activity; $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

P_{mic}), and microbial activity (DH). Organic P was positively correlated with DOC, N_{sol} and P_{olsen}, was negatively correlated with C_{mic} and N_{mic}, and had little correlation with P_{mic}, DH, C_{org}, and N_t. Inorganic P positively correlated with C_{org}, N_t, and microbial biomass and activity (C_{mic}, N_{mic}, P_{mic}, and DH), and weakly with the soluble forms of C, N, and P (DOC, N_{sol}, and P_{olsen}). Olsen P was negatively correlated with C_{mic}, N_{mic}, and P_{mic}. As expected, microbial biomass P was positively correlated with soil total C and N, and microbial biomass and activity. Of the P pools evaluated, P_{mic} was weakly correlated with P_t, significantly and positively correlated with P_{inorg}, but negatively correlated with P_{org} and P_{olsen}.

3.2. Enzyme activities

When compared with the UD soils, phosphatase activities were either enhanced or not affected by grazing, but reduced significantly by cultivation (Fig. 2). Generally, phosphatase activities in CL soils were less than half of those in the UD soils. Activities of acid phosphomonoesterase, phosphodiesterase, and inorganic pyrophosphatase were highest in the MG system, followed by the UD, HG, and AB systems. Of the two phosphomonoesterases tested, activities of alkaline phosphomonoesterase were about three times the activities of acid phosphomonoesterase.

With the exception of inorganic pyrophosphatase, activities of phosphatases were correlated with soil C_{org}, N_t, and microbial properties (C_{mic}, N_{mic}, P_{mic}, DH), with correlation coefficients varying from 0.20 to 0.93*** (Fig. 3 and Table 4). Weak negative correlations were observed between phosphatase activities and P_t or P_{org}. In fact,

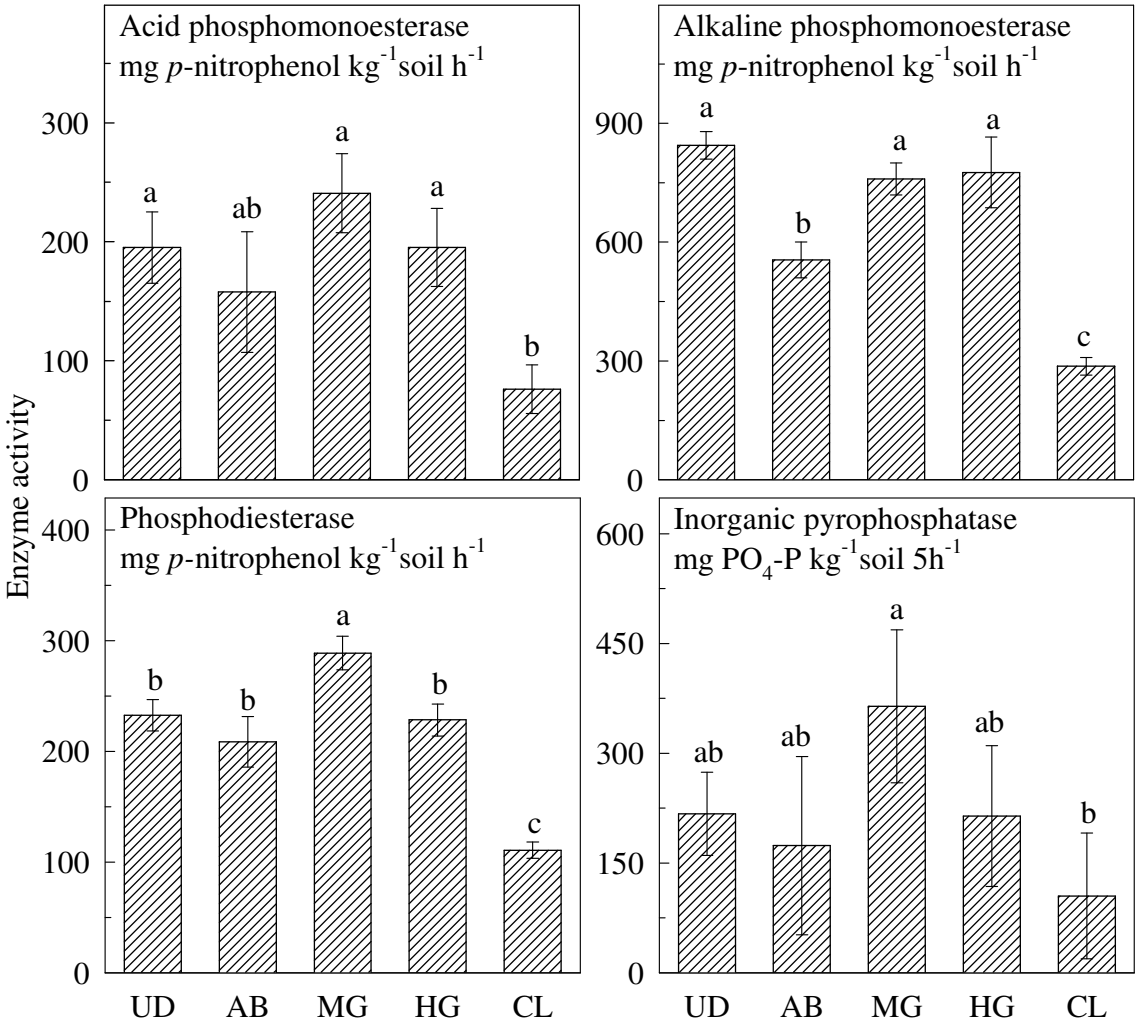

Fig. 2. Effects of land use and management practices on activities of hydrolytic phosphatases. UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. Mean comparisons and calculation for inorganic pyrophosphatase were based on natural logarithm transformed data. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

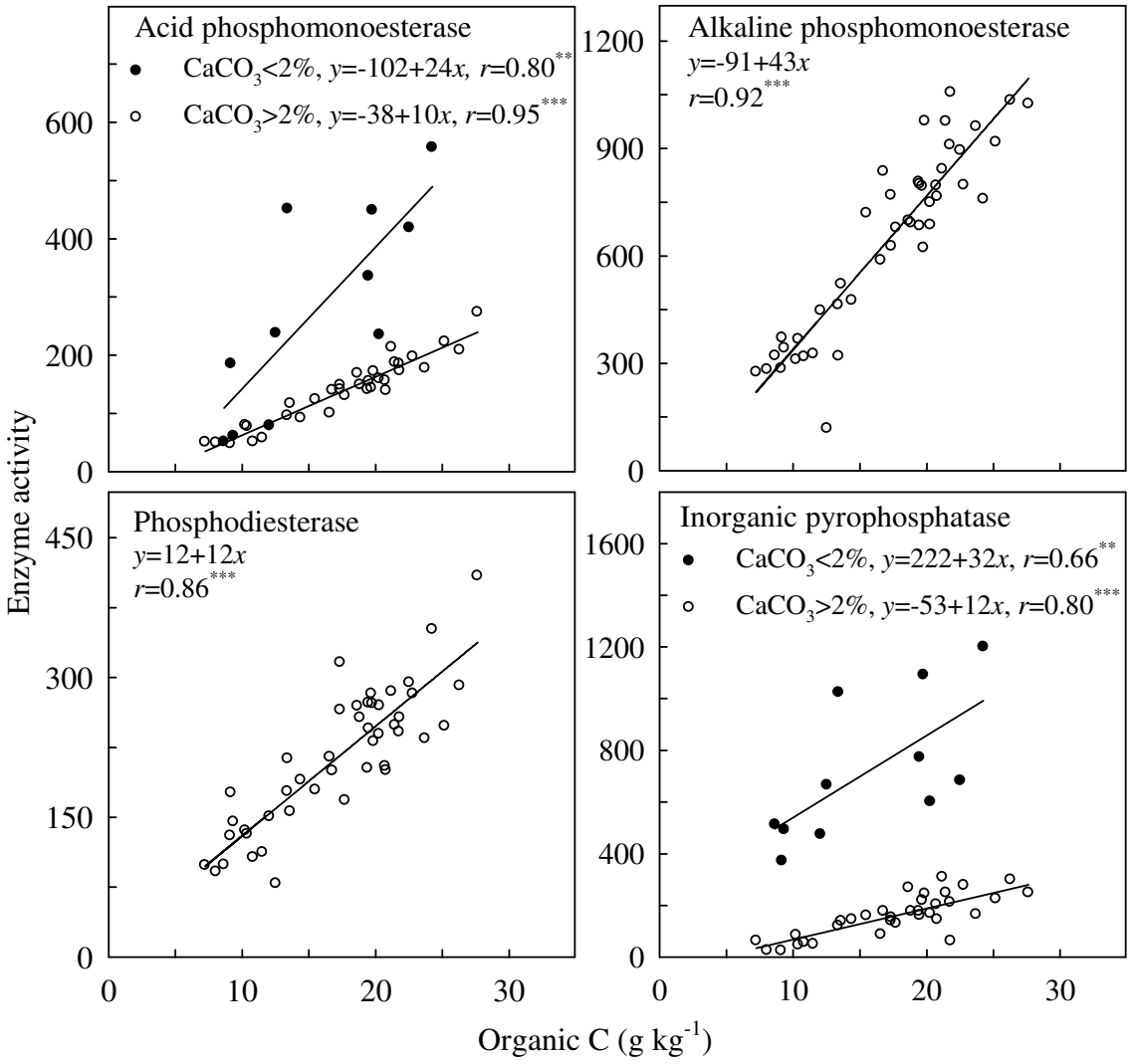

Fig. 3. Relationship between soil organic C contents and P-transforming enzyme activities in soils tested. Activities of acid and alkaline phosphomonoesterases and phosphodiesterase are expressed as mg *p*-nitrophenol produced kg^{-1} soil h^{-1}, of inorganic pyrophosphatase as mg PO_4-P produced kg^{-1} soil $5h^{-1}$ ($n = 45$; $^{**}P \leq 0.01$, $^{***}P \leq 0.001$).

Table 4. Correlation coefficients (*r*) between phosphatase activities and chemical/microbial properties, and between phosphatase activities with each other ($n = 45$).

Variable	N_t	P_t	P_{org}	P_{inorg}	DOC	N_{sol}	P_{olsen}	C_{mic}	N_{mic}	P_{mic}	DH	Acid-P	Alk-P	P-diest
Acid-P	0.44^{**}	-0.38^{**}	-0.28	-0.27	-0.43^{*}	-0.20	-0.10	0.43^{**}	0.37^{*}	0.38^{**}	0.20			
Alk-P	0.90^{***}	0.23	-0.25	0.45^{**}	-0.02	-0.13	-0.50^{***}	0.88^{***}	0.89^{***}	0.93^{***}	0.79^{***}	0.34^{*}		
P-diest	0.82^{***}	0.03	-0.30^{*}	0.17	-0.23	-0.18	-0.48^{***}	0.90^{***}	0.80^{***}	0.83^{***}	0.59^{***}	0.63^{***}	0.79^{***}	
InPyr-P	0.08	-0.49^{***}	-0.24	-0.43^{**}	-0.45^{**}	-0.08	0.08	0.08	0.03	0.04	-0.13	0.84^{***}	-0.04	0.30^{*}

Acid-P and Alk-P: Acid and alkaline phosphomonoesterase; P-diest: Phosphodiesterase; InPyr-P: Inorganic pyrophosphatase. The rest of the abbreviations are explained in Table 3; $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

activities of acid phosphomonoesterase and inorganic pyrophosphatase were negatively correlated to concentrations of P_t and P_{inorg}. Most enzyme activities were negatively correlated with easily extractable forms of C, N, and P. There were significant negative correlations between activities of acid phosphomonoesterase or inorganic pyrophosphatase and DOC, and between activities of alkaline phosphomonoesterase or phosphodiesterase and P_{olsen} (Table 4). The tested phosphatases were separated into two distinct groups according to their relationship with dehydrogenase activities. Activities of acid phosphomonoesterase and inorganic pyrophosphatase strongly correlated with each other, but showed little correlation with DH activity. On the other hand, activities of alkaline phosphomonoesterase and phosphodiesterase correlated significantly and positively with each other, and showed strong correlations with DH activity.

To reduce the dimensionality of the four-variable system of phosphatase activities, principal component analysis (PCA) was applied. The first principal component (PC1) accounted for 62% of the variance, with the first two principal components incorporating 95% of the variance in the data (Fig. 4). The tested phosphatase activities were sensitive to cultivation induced changes in the ecosystems, distinctly clustered together, separating from other treatments evaluated. Phosphatase activities also clustered according to AB treatment, but the separation from UD and grazed systems was not significant. When compared to the UD soils, grazing did not significantly affect phosphatase activities tested. Of the enzymes studies, the activities of acid phosphomonoesterase and phosphodiesterase contributed most to PC1.

The impact of land use and management practices on the relationships between paired phosphatases and between phosphatase and microbial biomass were further

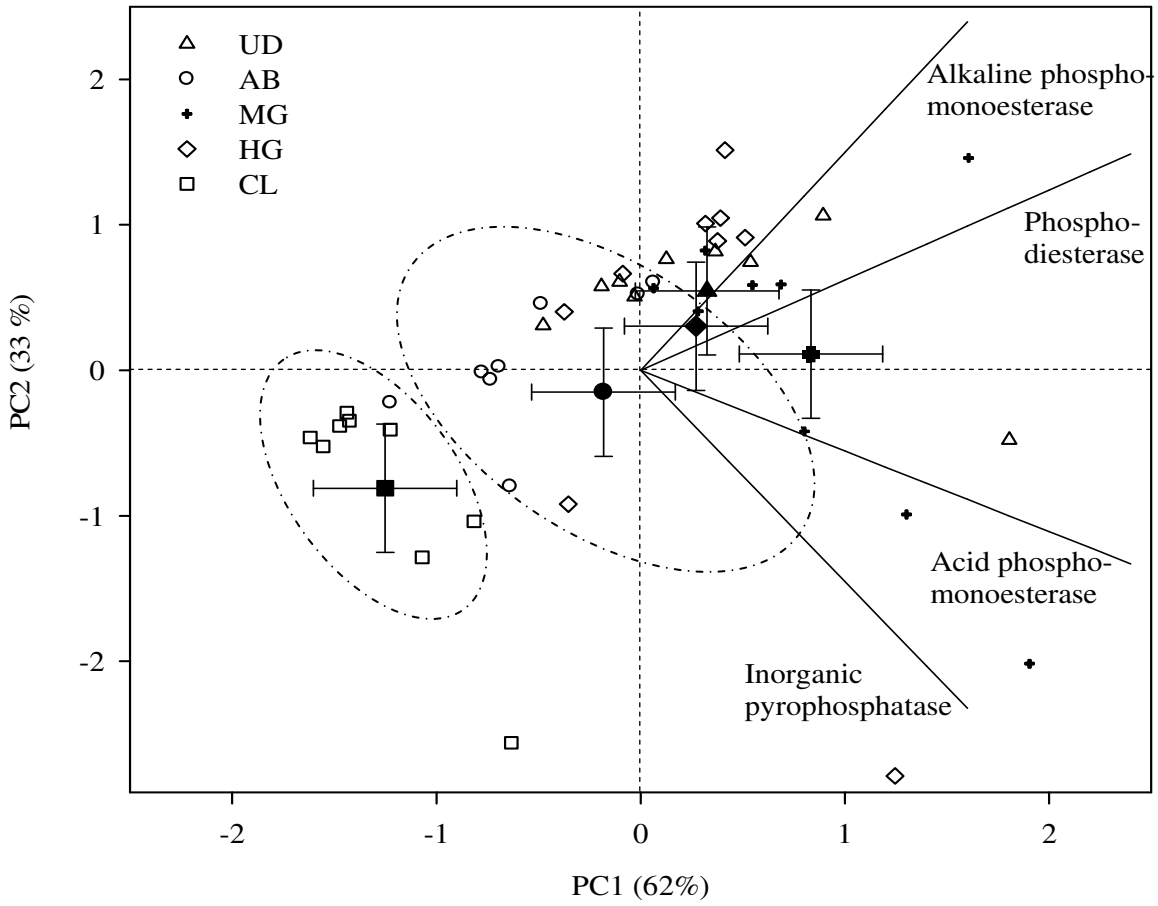

Fig. 4. Gabriel biplot of phosphatase activities against the first two principal components (PC1 and PC2). Rays that have small angle with a PC axis contribute more to that PC. Rays that have small angles with each other are positively correlated. Filled symbols represent treatment means (n = 9). Horizontal and vertical error bars are based on 95% confidence intervals for the mean PC1 and PC2, respectively. Overlapping bars indicate that two means are not significantly different at the 95% confidence level. UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat.

Table 5. Treatment effects on enzyme activity ratios and ratios of enzyme activities to microbial biomass C (n = 9).

Ratios[2]	Treatment[1]					*LSD* ($P \leq 0.05$)
	UD	AB	MG	HG	CL	
Enzymes						
P-diest : P-monoest	0.22	0.30	0.29	0.24	0.31	0.04
InPyr-P : P-monoest	0.23	0.35	0.44	0.35	0.59	0.39
P-diest : InPyr-P	1.11	1.44	0.93	1.41	1.87	0.99
Enzymes to Microbial biomass C						
Acid-P : C_{mic}[3]	0.33	0.31	0.38	0.41	0.48	0.31
Alk-P : C_{mic}[3]	1.44	1.22	1.11	1.42	1.59	0.28
P-diest : C_{mic}[3]	0.39	0.45	0.43	0.44	0.64	0.11
InPyr-P : C_{mic}[4]	0.42	0.56	0.73	0.68	1.27	0.91

[1]UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Winter wheat. [2]P-diest: Phosphodiesterase; Acid-P and Alk-P: Acid and alkaline phosphomonoesterase; P-monoest: Sum of Acid-P and Alk-P activities; InPyr-P: Inorganic pyrophosphatase; [4]mg *p*-nitrophenol mg^{-1} C_{mic} h^{-1}; [5]mg PO_4-P mg^{-1} C_{mic} $5h^{-1}$.

evaluated by changes of relative enzyme activities. The ratio of phosphodiesterase to the sum of phosphomonoesterase activities ranged from 0.22 to 0.31, and was significantly lower in UD and HG than in the rest of the systems (Table 5). Cultivation also increased the ratios of inorganic pyrophosphatase to phosphomonoesterases and phosphodiesterase to inorganic pyrophosphatase. When phosphatase activities were expressed per unit of C_{mic}, the CL system had significantly higher phosphatase activities per unit of C_{mic} than the rest of the evaluated systems.

4. Discussion

4.1. Soil phosphorus pools

Grazing did not alter any of the tested P pools, but cultivation led to accumulation of organic P and easily extractable P, and significant reduction of inorganic P and microbial biomass P. These results suggested that grazing did not alter P partitioning among different P pools although it stimulated P cycling. Fertilizer input influenced not only P cycling but also P partitioning in the soil ecosystem. Similar observations on P cycling and partitioning in grazed systems were reported by other studies (Marrs et al., 1989; van Oorschot and Robbemont, 1997). Grazing stimulates P cycling and redistribution in soils from animal excreta. It has been reported that in a grazed pasture which produces 15 Mg dry matter ha^{-1} yr^{-1}, approximately 34 kg P ha^{-1} with a substantial proportion of organic P annually returns via dung to replenish the total P pool (McDowell and Stewart, 2005; Williams and Haynes, 1995). In this study, total soil P levels in

grazed systems were similar to those of the undisturbed system, indicating minimal removal of P from the system. The undetected change of plant available P contents induced by grazing is consistent with finding reported by other studies (Peco et al., 2006; Singh and Ral, 2004).

Despite removal of P by harvest, annual addition of P fertilizer maintained total soil P in the cultivated system to levels comparable to the undisturbed system. Winter wheat in central Oklahoma produces an average grain yield of 2048 kg ha^{-1} yr^{-1}, based on 64 years of yield data, with a P content of 0.40% (Parham et al., 2002). Therefore, P output is estimated to be about 8 kg P ha^{-1} yr^{-1} from harvesting of grain, assuming the straw was returned to the soil. Since the cultivated soil received 16 kg P ha^{-1} yr^{-1} as fertilizer, the data obtained imply an excess of 8 kg P ha^{-1} yr^{-1} in the system, which may have been lost by leaching, runoff, or wind erosion..

The significant reduction of inorganic P by cultivation indicated that inorganic P was actively involved in P cycling and more active than the organic P. In fact, cultivation led to accumulation of organic substances that are rich in P, as evidenced by the significant decrease of organic C to P ratios in the cultivated soils compared to the undisturbed soils. These results reflect two possibilities, greater degradability of carbohydrates, or C was the most limiting factor for microbial growth in the cultivated soils. High ratios of soil organic C to organic P (more than 200) are associated with soils deficient in P, while ratios less than 100 indicate soils well supplied with P with respect to crop productivity (McGill and Cole, 1981). According to this ratio, the cultivated system in this study was more adequately supplied with soil P (C_{org}:P_{org} = 35:1), while the set aside from cultivation was not (C_{org}:P_{org} = 233:1). Return of cultivated land to

prairie permitted restoration of ecosystem functions and the capacity to cycle nutrients, which led to accelerated degradation of organic P. Since the set-aside from cultivation system showed accumulation of organic C, these data imply that a significant portion of organic P that was accumulated during cultivation was more labile, and its degradation was driven predominantly by microbial and biochemical processes. This would suggest that monoester P compounds are dynamic components of the organic P pool, because up to 99% of the increase in soil organic P following inorganic fertilization was in the form of monoesters (Condron et al., 1985). Monoesters are important components of inositol phosphates (phytate), and are among the more recalcitrant and abundant organic P forms in soils.

The relative changes of organic and inorganic P pools in the cultivated system were in part due to addition of inorganic fertilizer. Condron et al. (1985) showed that addition of 33 kg inorganic P ha^{-1} yr^{-1} for 20 yr increased soil organic P content by 21% relative to an adjacent unfertilized field. The accumulation of organic P in cultivated soils may also be related to lower phosphatase activities that limited organic P mineralization processes. Because cultivation led to reduction of microbial biomass and activity, consumption of readily available P was reduced, resulting in accumulation of Olsen P. This is consistent with results reported by Colvan et al. (2001) and Parham et al. (2002), and may suggest that microbial growth in the cultivated soils was not limited by P nutrition.

The microbial biomass P determined for the grassland soils of this study (40 mg P kg^{-1} soil) was within the range reported in the literature. Microbial biomass P contents in grassland soils vary from 4 to more than 100 mg P kg^{-1}, and increase with increasing

organic matter content (Oberson and Joner, 2005). Although grazing has been reported to increase microbial biomass C, N, and P (Singh et al., 1991), in this study grazing did not seem to affect microbial biomass P.

Percentages of microbial biomass P in total soil P from 0.5 to 11.8% have been reported, and varied depending on soil type, organic matter content, and land use (Brookes et al., 1984; Chen et al., 2003; Oberson et al., 1999). In this study, microbial P comprised 4 to 6% of total soil P in the uncultivated soils, but only 2% in the cultivated ones. This suggested that the microbial community of the uncultivated systems had higher P use efficiency. Because microbial biomass P is more labile than most soil organic P fractions (Brookes et al., 1984), high proportions of microbial P in organic P indicate that microbial P is a substantial sink and source of labile P. Higher percentages of microbial biomass P (about 28% of organic P) and lower Olsen P contents in the uncultivated compared to the cultivated system suggested that microbial biomass P was a predominant source of available P for the soil biological community. In the cultivated system, only 6% of the organic P was part of microbial biomass, which further supports the hypothesis that organic P in cultivated soils accumulates in relatively stable forms.

The microbial C to microbial P ratio reflects P availability to soil microorganisms, as well as microbial community composition (Anderson and Domsch, 1980; Muhammad et al., 2006). Chauhan et al. (1981) reported that microbial C to P ratio ranged from 12:1 when P was readily available, to 45:1 when P was in limited supply. In this study, microbial C to P varied from 12:1 to 17:1, indicating adequate P supply for the microbial community in all systems tested. A decrease in the microbial biomass C to P ratio indicated shifts in the microbial community structure (Muhammad et al., 2006), which is

also supported by the positive correlation between the ratios of microbial C to P and fungi to bacteria in this study ($r = 0.39^{**}$, Chapter VI).

The positive correlation between microbial biomass P and organic C contents and the weak negative correlations between microbial biomass P and organic P suggested that C was the limiting factor affecting microbial growth and activity in soils tested, whereas P was not a major limiting nutrient for microbial growth and activity. This finding may indicate that chemical processes other than biological and biochemical processes controlled P supply in these soils. Studies showed that inositol phosphates, which often constitute >50% of soil organic P, are stabilized by precipitation or adsorption onto soil colloids (Turner et al., 2002). In this study, inorganic P positively correlated to microbial biomass P ($r = 0.39^{**}$), suggesting that mechanisms such as dissolution and desorption play an important role in supplying P for microbial growth. The negative correlations between microbial biomass and Olsen P further revealed the significance of microorganisms in immobilizing soil available P. Studies demonstrated that microbial P immobilization led to low soil solution P concentrations (Kandeler et al., 1999; Ross et al., 1999).

4.2. Enzyme activities

High phosphatase activities imply great capacity for a soil to mineralize complex P compounds through biochemical processes. In this study, grazing, especially at moderate intensity, did not lessen the capacity of soil to cycle P, but cultivation reduced this capacity significantly. The reductions in tested P-transforming enzyme activities

were accompanied by reductions in organic C, total N, inorganic P, and microbial biomass and activity. These findings further support the hypothesis that soil properties are interdependent (Aon and Colaneri 2001; Deng and Tabatabai, 1997). Lower phosphatase activities in the cultivated soils led to accumulation of organic P, suggesting that mineralization of organic P was dominated by enzymatic processes. Annual applications of inorganic P fertilizer in the cultivated system increased the concentration of orthophosphates in the soil solution which suppressed phosphatase activities. This is also evidenced by the weak or negative correlations between available P and phosphatase activities, and supported by other studies (Juma and Tabatabai, 1977; Kandeler et al., 1999). However, three of four phosphatases tested showed activities in the set-aside from cultivation system at levels that were not significantly different from the undisturbed soils. This indicated that some ecosystem functions can be restored by removing from cultivation for at least 30 years.

The strong positive correlations between phosphatase activities and soil organic C underlines the role of soil organic matter in stabilizing soil enzymes (Deng and Tabatabai, 1997) and providing C and energy sources for microbial community. Inorganic pyrophosphatase activity was weakly correlated with organic C ($r = 0.18$). By separating the samples according to $CaCO_3$ content (Fig. 3), the correlations between inorganic pyrophosphatase and organic C were significant ($r = 0.80^{***}$ and 0.66^{**}). Studies showed that the relative concentrations of Mg^{2+} and Ca^{2+} affected pyrophosphoatase activity; Mg^{2+} is required for activation of pyrophosphate (PPi) to produce MgPPi (that active substrate that can be hydrolyzed by pyrophosphatase) (Tabatabai and Dick, 1979). It has long been recognized that Ca^{2+} can combine with the

PPi and inhibit formation of the active substrate. Studies showed inhibition by Ca^{2+} of pyrophospatase purified from yeast and *Escherichia coli* (Butler and Sperow, 1977; Josse, 1966). This was further evidenced by the negative correlation between inorganic pyrophosphatase activity and $CaCO_3$ content obtained in this study (r = -0.68***, data not shown). Similar relationship with organic C was shown for acid phosphatase activity, suggesting that Ca^{2+} may also affect the activity of this enzyme. In addition, concentrations of $CaCO_3$ affect soil pH, which could indirectly affect acid phosphatase activity. Presumably acid phosphomonoesterase is predominant in acid soils (Eivazi and Tabatabai, 1977; Juma and Tabatabai, 1977). This is also evidenced by the significant negative correlation between activity of this enzyme and soil pH (r = -0.46**, data not shown).

The tested phosphatases separated distinctly into two major groups, with strong significant correlations within each group. The noticeably different relationships between each group and microbial biomass and dehydrogenase activity suggested that activities of these two groups originated predominantly from different type and status of enzymes. Activities of acid phosphomonoesterase and inorganic pyrophosphatase showed little relationship with microbial biomass or dehydrogenase activity, indicating their activities predominantly originated from accumulated enzymes that were free of microbial cells but were protected by forming complexes with soil organic matter. This is further evidenced by their strong significant correlations with organic C contents and the similarities that were exhibited from their relationship. Studies showed that the two enzymes were mostly of similar origin, from plant roots, and interacted similarly with the soil matrix (Dick and Tabatabai, 1978; Naidja et al., 2000). Activities of alkaline

phosphatase and phosphodiesterase showed strong significant correlations with microbial biomass and dehydrogenase activity, indicating that these enzymes are predominantly of microbial origin (Browman and Tabatabai, 1978; Eivazi and Tabatabai, 1977), and that their activities were dominated by intracellular enzymes associated with microbial cells. These two enzyme activities were also strongly and significantly correlated with soil organic C, indicating significant contributions of enzymes from heterotrophic microbes and limitation of organic C and energy sources in these soil ecosystems.

Weak negative correlations were observed between phosphatase activities and organic P when all data were included. However, by separating data from the cultivated systems, strong positive correlations were found between the activities of alkaline phosphomonoesterase and phosphodiesterase with organic P ($r = 0.66^{***}$ and 0.49^{***}, respectively, data not shown) in the uncultivated soils. This suggested that organic P mineralization was more dependent on biochemical processes in the uncultivated than the cultivated systems. In the cultivated soils, phosphatase activities were suppressed by the applied P fertilizer because phosphate is an end product of the enzymatic reaction (Juma and Tabatabai, 1977). The weak correlation between phosphatase activities and organic P in soils has been reported by other studies (Adams, 1992; Adams and Pate, 1992). It has been postulated that reduced P demand was the main cause of the reduced phosphatase activity.

The metabolic activity of microbial communities can be evaluated by determining the ratios of intracellular enzyme activities with respect to microbial C. Changes of this ratio following treatments that significantly affect microbial activity would also reflect the status and persistence of enzymes in soil (Deng et al., 2006). In the cultivated soils of

this study, the ratios of phosphatase activities to microbial C increased, despite significant reductions in microbial biomass and activity, suggesting that significant portion of the tested enzyme activities were from accumulated enzymes that were free of microbial cells and persisting in the soil environment. Correlations between phosphatase and dehydrogenase activities suggested that activities of alkaline phosphatase and phosphodiesterase were attributed mostly to intracellular enzymes, while those of acid phosphatase and inorganic pyrophosphatase attributed mostly to accumulated enzymes. This was also evidenced by significant positive correlations between the latter two enzyme activities and soil clay contents ($r = 0.47^{**}$ and 0.57^{***}, respectively; data not shown). Soil as a living system contains enzymes that stay active within microbial cells, and/or are accumulated through immobilization and stabilization by organic complexes and clay minerals. Enzyme activities detected from a soil include activities of all sources. Management affected not only total enzyme activities, but also activities contributed by different sources. In this study, the activities contributed by accumulated enzymes were generally higher in the cultivated than in uncultivated soils.

The complex relationships between enzymes and their integrated interactions with different soil environments were demonstrated by principal component analysis. Activities of tested enzymes clustered according to management systems, suggesting that different management practices placed different and unique stress and limiting factors to soil microbiological functions and their interaction with other soil properties. Unlike C- and N-transforming enzymes (Chapter III and IV), phosphatase activities did not show significant changes by grazing but only by cultivation. Similar to C- and N-transforming enzymes, the sensitivity in response varied among enzymes tested. Acid

phosphomonoesterase contributed most to PC1, followed by phosphodiesterase, while alkaline phosphomonoesterase contributed most to PC2, followed by inorganic pyrophosphatase.

Enzyme activity ratios may reflect the relative concentrations of the respective substrates in an environment (Caldwell, 2005). Therefore, changes in enzyme ratios imply changes in soil constituents. The lower phosphodiesterase to phosphomonoesterases and inorganic pyrophosphatase to phosphomonoesterases ratios in the undisturbed and grazed systems revealed dominance of orthophosphoric monoesters in the P pools. This is in agreement with studies by McDowell et al. (2005) and Turner et al. (2003) who reported that organic P in permanent pasture was dominated by orthophosphate monoesters as opposed to orthophosphate diesters and inorganic pyrophosphate. In contrast, the cultivated soils showed higher phosphodiesterase to phosphomonoesterases, and phosphodiesterase to inorganic pyrophosphatase ratios. Orthophosphate diesters constitute the majority of fresh organic P inputs to soil, and are more easily degradable than orthophosphoric monoesters (Turner and Haygarth, 2005). The relatively high orthophosphoric diester contents in the cultivated soils also implied unfavorable conditions for their mineralization. Condron et al. (1990) found that soils with the least favorable conditions for organic P mineralization had the highest proportion of orthophosphoric diesters. Although addition studies are needed to reveal the nature of organic P compounds in different soil environments, the shifts in enzyme activity ratios indicated differences in the composition of organic P pools under different management practices.

4.3. Conclusions

In general, phosphatase activities were more sensitive to cultivation than grazing. Soil disturbance by grazing or cultivation did not lead to significant change in soil total P. When compared with the undisturbed soils, grazing did not alter the size and composition of all tested P pools nor lessen the capacity of the soil to cycle P. However, long-term cultivation led to significant increase in soil Olsen-P and significant reduction of microbial biomass P, while fertilizer P application suppressed phosphatase activities. As a result, cultivation led to organic P accumulation and reduced the capacity of soil to cycle P. Of the P pools tested, the major shift in quantity induced by cultivation occurred in the inorganic P. Moreover, cultivation also led to the development of a microbial community with significantly reduced P use efficiency, while grazing maintained a soil microbial community with relatively high P use efficiency. Abandonment from cultivation allowed the soil ecosystem to regain its capacity to cycle P and to evolve slowly towards the P cycling capacity of the native soil.

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CHAPTER VI

RESPONSE OF MICROBIAL DIVERSITY AND COMMUNITY STRUCTURE TO MANAGEMENT PRACTICES

Abstract

A diverse community of microorganisms governs soil processes. Revealing changes in soil biota induced by management may help the development of management strategies to improve the productivity and sustainability of soil ecosystems. The main objective was to evaluate the effects of long-term management practices on the diversity and structure of the soil microbial communities as determined by Fatty Acid Methyl Ester (FAME) analysis. Five long-term (more than 30 years) treatments were evaluated, including undisturbed, set-aside from cultivation, moderately grazed, heavily grazed, and winter wheat (*Triticum aestivum* L.). The non-cultivated systems had the highest microbial biomass and the highest proportions of fungal and protozoan biomarkers. The undisturbed system had higher proportion of Gram-positive bacteria, while the grazed systems favored fast growing microorganisms such as Gram-negative bacteria. In the cultivated system, the microbial community also was dominated by Gram-negative bacteria, and higher proportions of cyclopropyl fatty acids that indicated nutritional stress. The correlations between enzyme activities and microbial biomass were stronger

than between enzyme activities and phenotypic groups of organisms (Gram-positive and Gram-negative bacteria, actinomycetes, fungi, and protozoa), suggesting that the size of the microbial community rather than its composition had more impact on the enzyme functional capacity of the soil ecosystem.

1. Introduction

As a driving force regulating soil processes, changes in microbial diversity, composition, and community structure may impact the functional capacity of a community to cycle nutrients and degrade organic substances. Waldrop et al. (2000) showed that compositional changes in soil microbial communities were accompanied by changes in their ability to degrade macromolecular C compounds. Direct linkage between changes in community structure and community function was demonstrated by Buyer and Drinkwater (1997).

Alterations in plant species composition strongly affected microbial community composition (Grayston et al., 2001; Singh et al, 2006). However, it is not clear how different management practices affect microbial diversity and community structure, and the associated soil processes. Cultivation may reduce microbial population and diversity (Øvreås and Torsvik, 1998). The microbial community composition of cultivated fields did not differ from fields abandoned from cultivation for nine years, but differed from fields that had never been cultivated (Buckley and Schmidt, 2001). Following elimination of agricultural activities and long-term successional development, soil ecosystems shifted

soil microbial communities from bacteria- to fungi-dominated (Ohtonen et al., 1999; Zhang et al., 2005)..

Because of vast diversity, and enormous population and limitations to culture microbes inhabiting in the soil environment, evaluation of microbial communities present an understated challenge. The species composition of soil microbial communities is still largely unknown. Of the numerous methodologies applied to the evaluation of microbial communities in the environment, Fatty Acid Methyl Ester (FAME) analysis provides a relatively unbiased view of soil microbial community structure (Zelles, 1999). Changes in the composition and abundance detected fatty acids using FAME reflect changes and variation among phylogenetic groups of prokaryotes and eukaryotes, as well as a unique "signature" of the evaluated community (Zelles et al., 1992).

It has been suggested that microbial diversity and community structure reveals the potential of ecosystem function, while functional diversity and capacity such as enzyme activities provide a direct measure of ecosystem function (Caldwell, 2005; Waldrop et al., 2000). Changes of soil enzyme activities in response to changes in soil microbial diversity and community structure, and the relationships between these soil properties are key elements in revealing soil ecosystem function. Therefore, the objective of this study was to evaluate the effect of long-term management practices on the diversity and structure of the soil microbial communities, and to link microbial community structure to its functional diversity expressed through soil enzymatic activity.

2. Materials and methods

2.1. Site description and soils

The site and location of the research area were described in detail in Chapter II. Five long-term (conducted for at least 30 years) treatments including undisturbed (UD), abandoned (set-aside from cultivation, AB), moderate grazing (MG), heavy grazing (HG), and winter wheat (CL) were sampled in May 2005. The sampling scheme was described in Chapter III. Briefly, composite soil samples (35 to 45 cores, 0 to 0.10 m depth) were obtained from nine randomly selected plots (0.5 ha each) which served as field replications for each treatment. The field-moist soil samples were sieved (2-mm sieve), mixed thoroughly, and stored in sealed plastic bags at 4^{o}C. A portion of each sample was freeze dried, sealed in glass vials and stored at -20^{o}C for Fatty Acid Methyl Ester (FAME) analysis.

Determinations of clay and sand contents, soil pH, $CaCO_3$, total soil organic C (C_{org}), total N (N_t), soil microbial biomass C (C_{mic}) and N (N_{mic}), and dehydrogenase activity (DH) are described in Chapter III. Determination of inorganic P (P_{inorg}), organic P (P_{org}), and soil microbial biomass P (P_{mic}) are described in Chapter V. Enzyme activities involved in organic C, N, and P transformations were assayed in the field-moist samples as described in Chapters III, IV and V. Soils of all treatments had neutral to alkaline pH values (7.2 to 7.6), $CaCO_3$ contents between 3 and 6%, and textures that varied from Loam to Silt loam (Chapter III). Results showed that management practices affected several abiotic and biotic properties of the prairie soil ecosystems. When

compared to the undisturbed system, moderate grazing did not affect organic C, total N and P contents, and microbial biomass and activity significantly. Values of these properties were followed by those in the highly grazed and set-aside from cultivation systems, and were lowest in the cultivated soils. Similar pattern was observed for most of the enzyme activities involved in C, N, and P cycling.

2.2. FAME analysis

Fatty acids were extracted from the soils using the procedure described for pure culture isolates as previously applied for soil analyses (Acosta-Martínez et al., 2004; Cavigelli et al., 1995). Briefly, the method consists of four steps: (i) saponification of fatty acids by treating the soil (3 g of <2 mm freeze dried soil) with 3 ml 3.75 M NaOH (methanol: water, 1:1) solution at 100^{o}C 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 fatty acid methyl esters (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 organic phase containing 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 H_2 as the carrier gas. The temperature program was ramped from 170°C to 250°C at 5°C min^{-1}. Peaks in a sample were identified by comparison to standard fatty acids (Microbial ID, Newark, Del.) and their relative peak areas (percentages over total detected areas) were determined with respect to other fatty

acids in a sample using the MIS Aerobe method of the Microbial Identification System (MIDI, MIS, Microbial ID, Inc., Newark, DE).

The fatty acids are reported as the number of atoms in the C chain, followed by a colon, the number of double bonds, and the position of the first double bond from the methyl (ω) end of the molecules; *cis* isomers are indicated by the suffix *c*, and branched fatty acids are indicated by the prefixes *i* and *a* for *iso* and *anteiso*, respectively. Interpretation of the FAME profiles was aided by the use of fatty acid markers that tend to be abundant in particular groups of organisms (Cavigelli et al., 1995; Zelles, 1999). Specifically, the terminaly branched saturated fatty acids *i*14:0, *i*15:0, *a*15:0, *i*16:0, 17:0, *i*17:0, *a*17:0, *i*19:0, and *i*20:0 were used to indicate Gram(+) bacteria; the monounsaturated and cyclopropyl fatty acids 14:1ω5*c*, 15:1ω6*c*, 16:1ω9*c*, 16:1ω5*c*, 17:1ω8*c*, 17:1ω6c, *cy*17:0, 18:1ω7*c*, 18:1ω9c, and *cy*19:0 were used to indicate Gram(-) bacteria; the mid-chain branched saturated fatty acids 10*me*16:0, 10*me*17:0, 10*me*18:0 to indicate actinomycetes; the fatty acids 18:1ω9*c*, 18:2ω6*c*, 18:3ω6*c*, 20:1ω9*c* and 20:2ω6*c* to indicate fungi. The fatty acids 18:1ω9*c*, 18:3ω6*c* were also used as mycorrhizal biomarkers; and the 20:4ω6*c* as protozoan biomarker. The ratio of cyclopropyl fatty acids to their precursors, were calculated as indicators of starvation stress (Bossio et al., 1998; White et al., 1996). The diversity of the microbial community was calculated using the Shannon index *H*, (Magurran, 1988; Shannon, 1948):

$$H = -\sum_{i=1}^{k} p_i \ln p_i$$

where $p_1, p_2, \ldots, p_k$ are the relative proportion of the *k* identified fatty acids.

2.3. Statistical methods

The central tendency and spread of the measured properties across different management systems were compared with ANOVA, based on the least significant difference test (*LSD*, $P \leq 0.05$). Correlations between FAME abundance (% x the total area detected) and soil chemical, microbial, and biochemical properties were calculated. Principal component analysis (PCA) was applied to reduce the dimensionality of the 7-dimensional data space, to identify the microbial groups that represented most of the variability, and to compare the microbial community structure among the management systems. PCA analysis was performed by using JMP® start statistics software (Sall et al., 2005).

3. Results

3.1. Microbial abundance, diversity and composition

According to total FAME areas detected, microorganisms were most abundant in the moderately grazed systems, followed by the highly grazed, the undisturbed, the abandoned from cultivation, and the cultivated system (Fig. 1). As an indicator of community richness (Collins and Cavigelli, 2003), the total number of fatty acids detected varied among soil ecosystems, with significantly lower number of FAME detected in the cultivated soils (Fig. 2). The numbers of fatty acids detected in the MG, HG, and UD treatments were not significantly different. The Shannon index indicates

both richness and diversity, showing the highest value in the UD, followed by the AB, MG, HG, and CL systems. Long-term cultivation led to significant reduction of microbial richness and diversity. Changes in the microbial richness and diversity were further evaluated by changes in the relative abundance of bacteria, fungi, and protozoa. In each of the evaluated microbial communities, the relative abundance of bacteria was significantly higher, while those of fungi and protozoa were significantly lower in the cultivated soils than the uncultivated ones (Fig. 3). When compared with the UD soils, grazing, especially at moderate intensity, led to significant increase in the relative abundance of fungi and actinomycetes in microbial communities, but did not affect the relative abundance of protozoa significantly.

The increase in the relative abundance of bacteria in the cultivated soils was attributed to increase in the relative abundance of Gram(-) bacteria (Fig. 4). In fact, increases in the relative abundance of Gram(-) bacteria were observed in all disturbed soil ecosystems. The increases were significant in the more intensively managed (CL and HG) systems. When the changes of bacterial communities were expressed as the ratios of Gram(-) to Gram(+) bacterial abundance, the observed changes were also significant for the MG system. The ratios of fungal to bacterial biomass were highest in the MG system, followed by AB, UD, HG, and CL systems.

Long-term cultivation increased nutritional stress in the soil environment, as evidenced by the significantly higher ratios of cyclopropyl fatty acids (*cy*17:0 and *cy*19:0) to their monoenoic precursors (16:1ω7*c* and 18:1ω7*c*) in the cultivated soils than the uncultivated ones (Fig. 5).

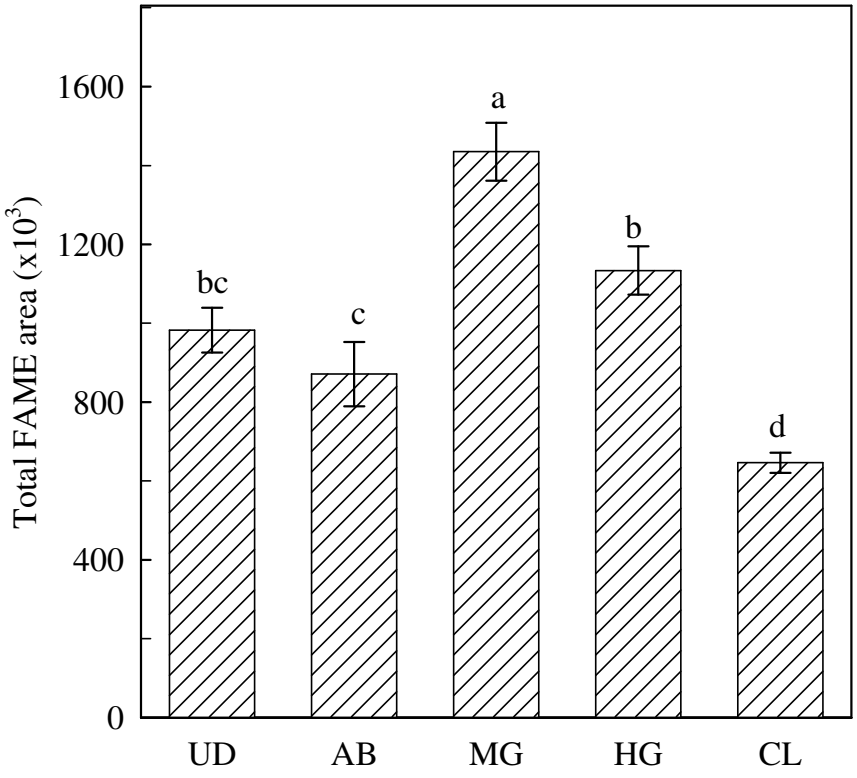

Fig. 1. Effect of management practices on the total area of detected fatty acids determined by FAME analysis. Undisturbed, AB: Abandoned from cultivation, MG: Moderately grazed, HG: Highly grazed, CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

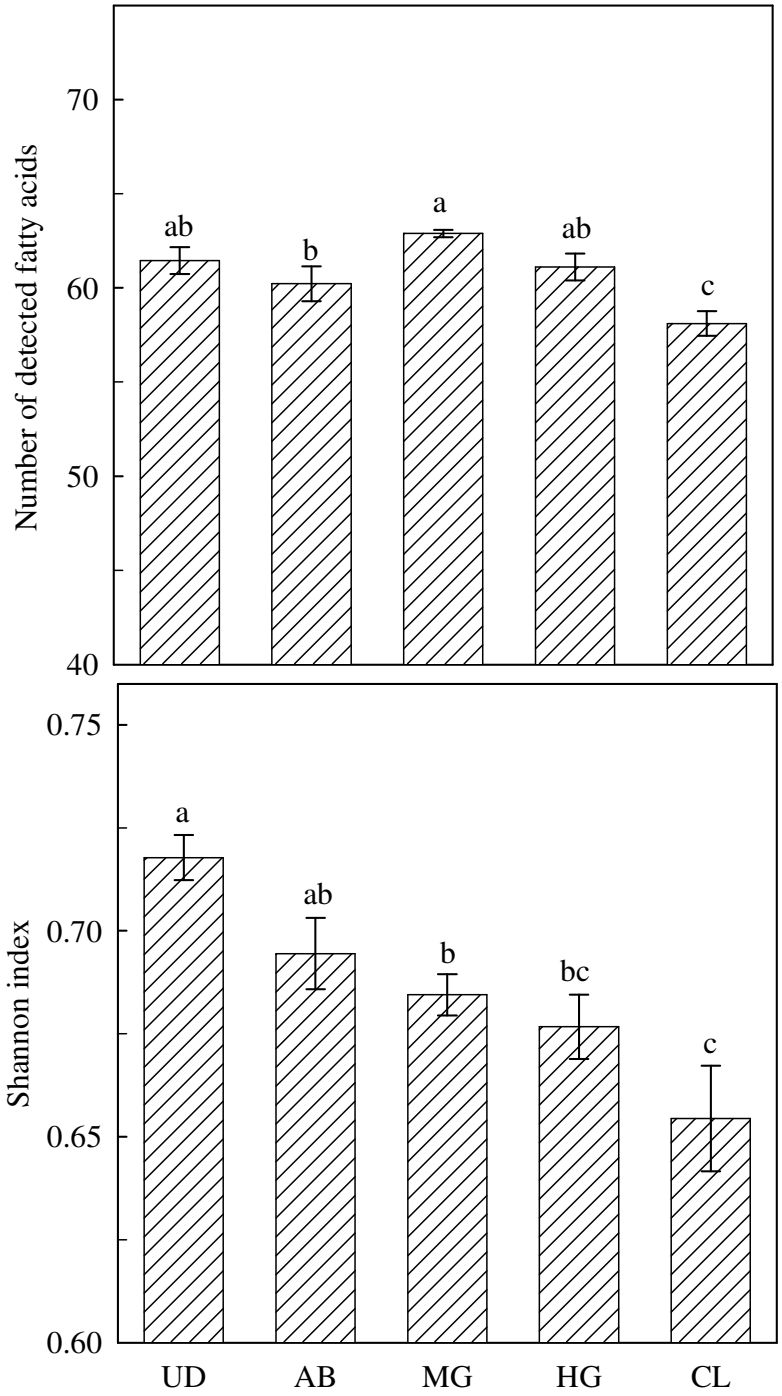

Fig.2. Effect of management practices on the number of detected fatty acids and on Shannon diversity index. Undisturbed, AB: Abandoned from cultivation, MG: Moderately grazed, HG: Highly grazed, CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

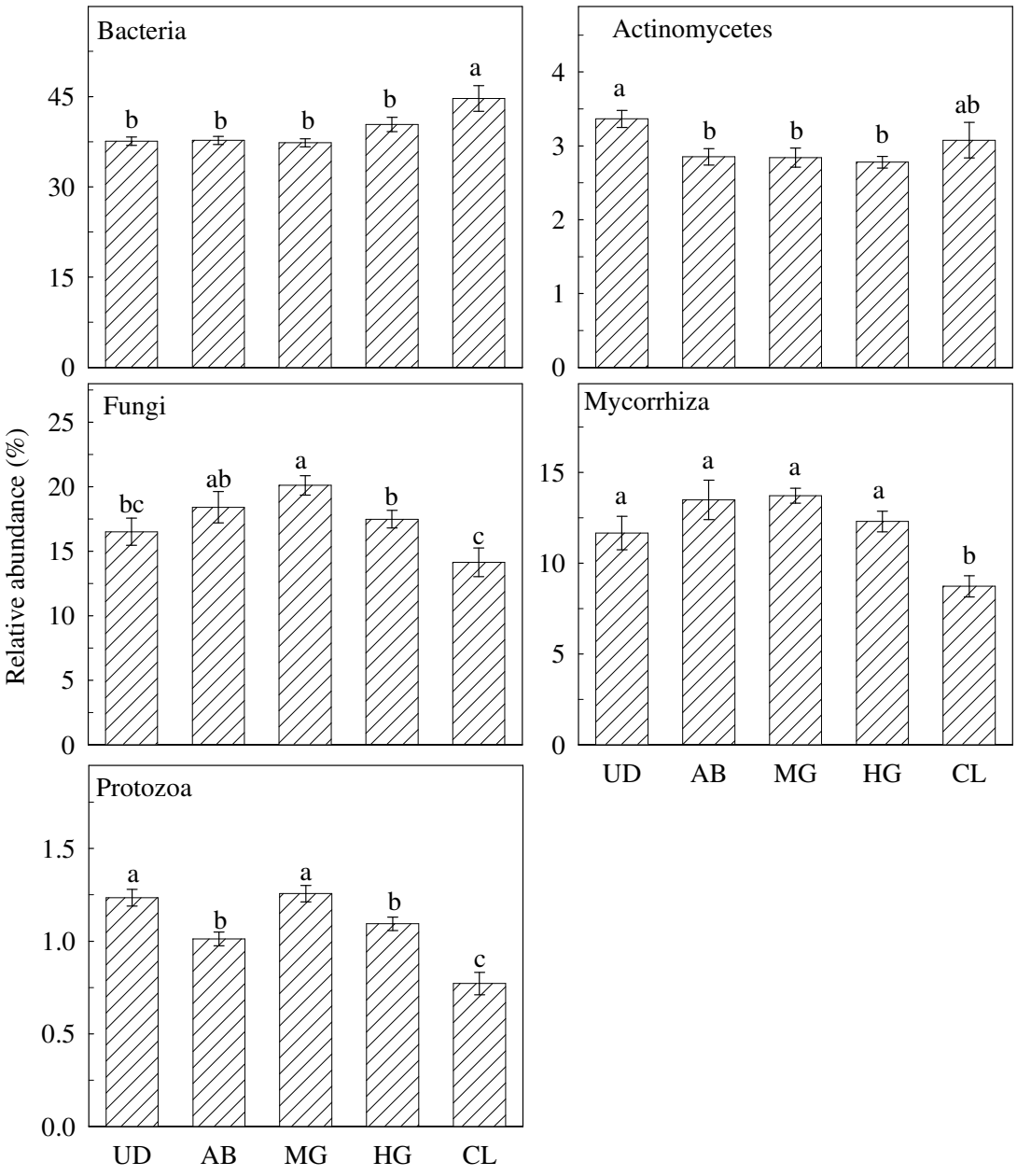

Fig.3. Effect of management practices on the relative abundance of phenotypic microbial groups. Undisturbed, AB: Abandoned from cultivation, MG: Moderately grazed, HG: Highly grazed, CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

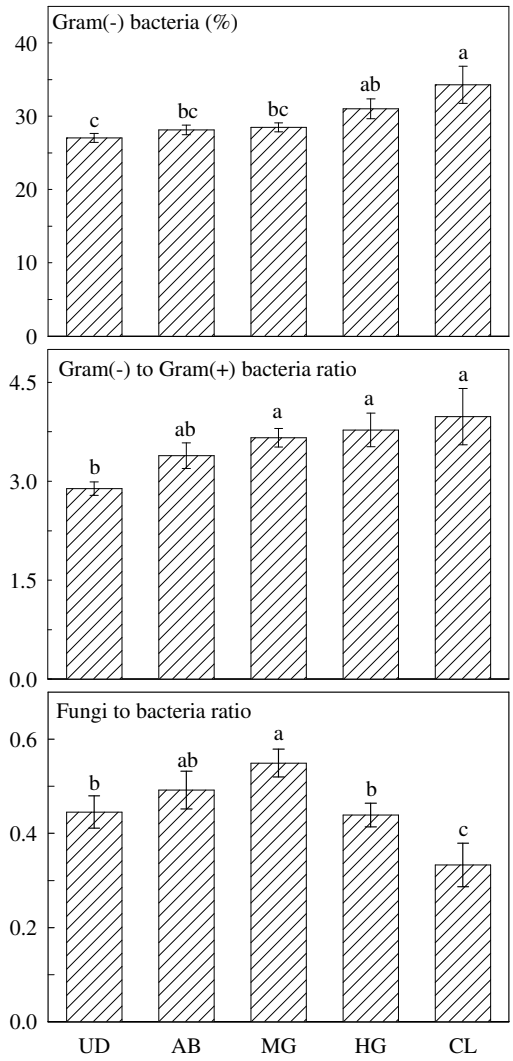

Fig. 4. Effect of management practices on microbial community composition. Undisturbed, AB: Abandoned from cultivation, MG: Moderately grazed, HG: Highly grazed, CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

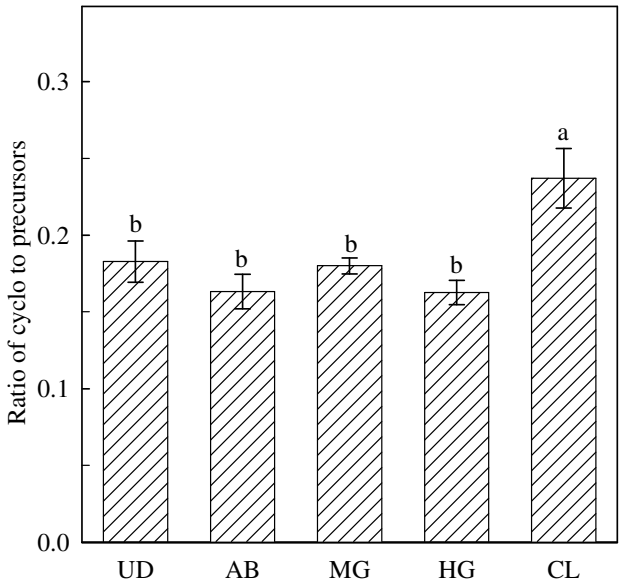

Fig. 5. Effect of management practices on the ratio of cyclopropyl fatty acids to their precursors [(*cy*17+*cy*19)/(16:1ω7*c*+18:1ω7*c*)]. UD: Undisturbed, AB: Abandoned from cultivation, MG: Moderately grazed, HG: Highly grazed, CL: Winter wheat. Columns are means ± standard errors. Different letters indicate significantly different means according to least significant difference test ($n = 9$, $P \leq 0.05$).

3.2. Principal component analysis of fatty acids

The impacts of management practices on the relative abundance of evaluated microbial groups and the integrated interactions between microbial groups and with their habitats were further assessed by PCA. The microbial communities in the CL systems were significantly different from those of uncultivated ones, and dominated by bacteria, especially Gram(-) bacteria (Fig. 6). The microbial communities in the UD soils were clustered together, and dominated by Gram(+) and actinomycetes. Microbial communities in the AB, MG, and HG were clustered according to management systems, but the separation between systems was not significant. These systems had high fungi to bacterial ratios, and showed relatively higher abundance of fungi and protozoa.

Overall, the first two principal components, PC1 and PC2, accounted for 83% of the variance among the phenotypic microbial groups (Fig. 6). The microbial groups that had most substantial loading on PC1 were, in order, bacteria > ratio fungi to bacteria > Gram(-) bacteria, and on PC2, Gram(+) bacteria > actinomycetes > fungi. PC1 uniquely differentiated the CL treatment from all the others, and the grazing systems according to their intensities. The PC2 uniquely differentiated the UD treatment from the grazed systems.

3.3. Linking phenotypic microbial groups to soil functional capacity and properties

The number and total abundance of fatty acids were positively correlated with C_{org}, N_t, and P_{inorg}) and microbial biomass and activity (C_{mic}, N_{mic}, P_{mic}, DH) (Table 1).

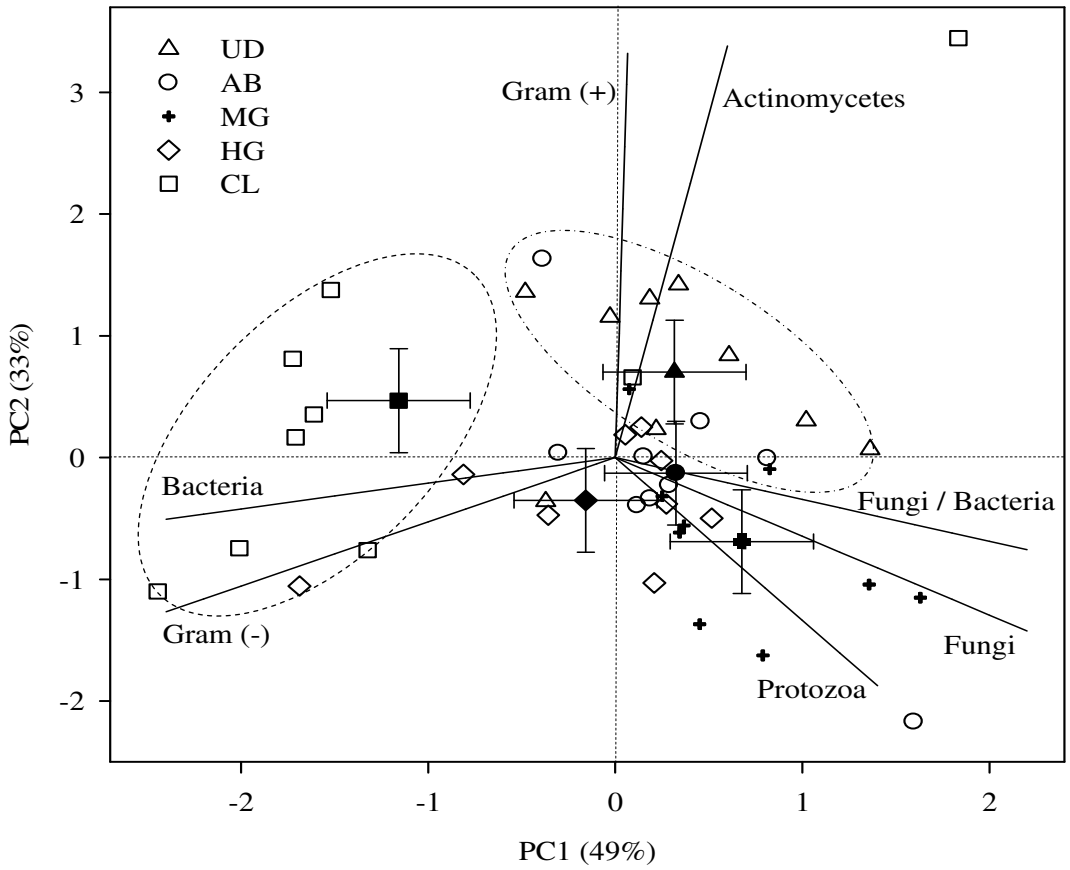

Fig. 6. Gabriel biplot of microbial phenotypic groups detected by FAME analysis against the first two principal components (PC1 and PC2). Rays that have small angle with a PC axis contribute more to that PC. Filled symbols represent treatment means (n = 9). Horizontal and vertical error bars are based on 95% confidence intervals for the mean PC1 and PC2, respectively. Overlapping bars indicate that two means are not significantly different at the 95% confidence level. Undisturbed, AB: Abandoned from cultivation, MG: Moderately grazed, HG: Highly grazed, CL: Winter wheat.

Table 1. Correlation coefficients (r) of the number of detected fatty acids, total area, and phenotypic groups of microorganisms revealed by FAME analysis with selected soil properties (n = 45).

Soil property	Detected fatty acids	Total area	Bacteria			Actino-mycetes	Fungi	Mycor-rhiza	Protozoa
			Total	Gram(+)	Gram(-)				
Organic C	0.63 ***	0.63 ***	0.56 ***	0.75 ***	0.48 ***	0.69 ***	0.48 ***	0.46 **	0.71 ***
Total N	0.59 ***	0.63 ***	0.58 ***	0.73 ***	0.52 ***	0.71 ***	0.46 **	0.44 **	0.70 ***
Inorganic P	0.39 **	0.39 **	-0.14	-0.12	-0.09	0.20	0.06	0.05	0.27
Organic P	-0.32 *	-0.25	-0.10	-0.20	-0.07	-0.16	-0.34 *	-0.40 **	-0.29
Microbial C	0.63 ***	0.73 ***	0.66 ***	0.77 ***	0.60 ***	0.73 ***	0.61 ***	0.61 ***	0.79 ***
Microbial N	0.57 ***	0.67 ***	0.61 ***	0.72 ***	0.55 ***	0.70 ***	0.53 ***	0.54 ***	0.73 ***
Microbial P	0.68 ***	0.72 ***	0.69 ***	0.79 ***	0.63 ***	0.74 ***	0.56 ***	0.56 ***	0.78 ***
Dehydrogenase activity	0.33 *	0.49 ***	0.44 **	0.56 ***	0.39 **	0.55 ***	0.36 *	0.39 **	0.54 ***

Phenotypic groups of microorganisms estimated as the sum of proportions (%) of fatty acid biomarkers multiplied by the total area determined from the chromatograph of each sample. Bacteria were estimated as the sum of Gram(+) and Gram(-) biomarkers; Gram(+) biomarkers were *i*14:0, *i*15:0, *a*15:0, *i*16:0, 17:0, *i*17:0, *a*17:0, *i*19:0, and *i*20:0; Gram(-) biomarkers were 14:1ω5*c*, 15:1ω6*c*, 16:1ω9*c*, 16:1ω5*c*, 17:1ω8*c*, 17:1ω6c, *cy*17:0, 18:1ω7*c*, 18:1ω9c, and *cy*19:0; Actinomycetal biomarkers were 10*me*16:0, 10*me*17:0, and 10*me*18:0; Fungal biomarkers were 18:1ω9*c*, 18:2ω6*c*, 18:3ω6*c*, 20:1ω9*c*, and 20:2ω6*c*; Mycorrhizal biomarkers were 18:1ω9*c* and 18:3ω6*c*; and, the protozoan biomarker was 20:4ω6*c*. $^{*}P<0.1$; $^{**}P<0.05$; $^{***}P<0.001$.

The phenotypic groups of microorganisms, including bacteria, actinomycetes, fungi, protozoa, and mycorrhiza, were correlated with all soil chemical and microbial properties evaluated except organic and inorganic P. The ratio of fungi to bacteria from the pooled data of all soils tested was not correlated with soil organic C ($r = 0.20$, $P \leq 0.05$, data not shown). When data were analyzed separately, these ratios and organic C were negatively correlated for the uncultivated treatments, and positively correlated for the CL treatment (Fig. 7).

Relationships between enzyme activities and phenotypic groups of microorganisms may provide information about the origin of soil enzymes, and therefore the contribution of specific microbial groups to the cycling of different nutrients. Significant correlations were detected between microbial groups and activities of several hydrolytic enzymes involved in the cycling of organic C, N, and P (Table 2). Activities of β-glucosaminidase, L-glutaminase, and phosphodiesterase correlated with all phenotypic groups. Gram(+) bacteria and actinomycetes correlated to galactosidases, β-glucosaminidase, amidohydrolases (urease, L-asparaginase and L-glutaminase), phosphomonoesterases, and phosphodiesterase. Gram(-) bacteria, fungi, and mycorrhizae correlated to activities of invertase, β-glucosaminidase, L-glutaminase, and phosphodiesterase. Fungi and mycorrhizae correlated with more enzymes involved in N and P cycling rather than those involved in C cycling. Specifically, these two microbial groups correlated to amidohydrolases (urease, L-asparaginase and L-glutaminase), acid phosphomonoesterase, and phosphodiesterase. Protozoa correlated with all enzyme activities except for glucosidases, protease, nitrate reductase, and inorganic pyrophosphatase.

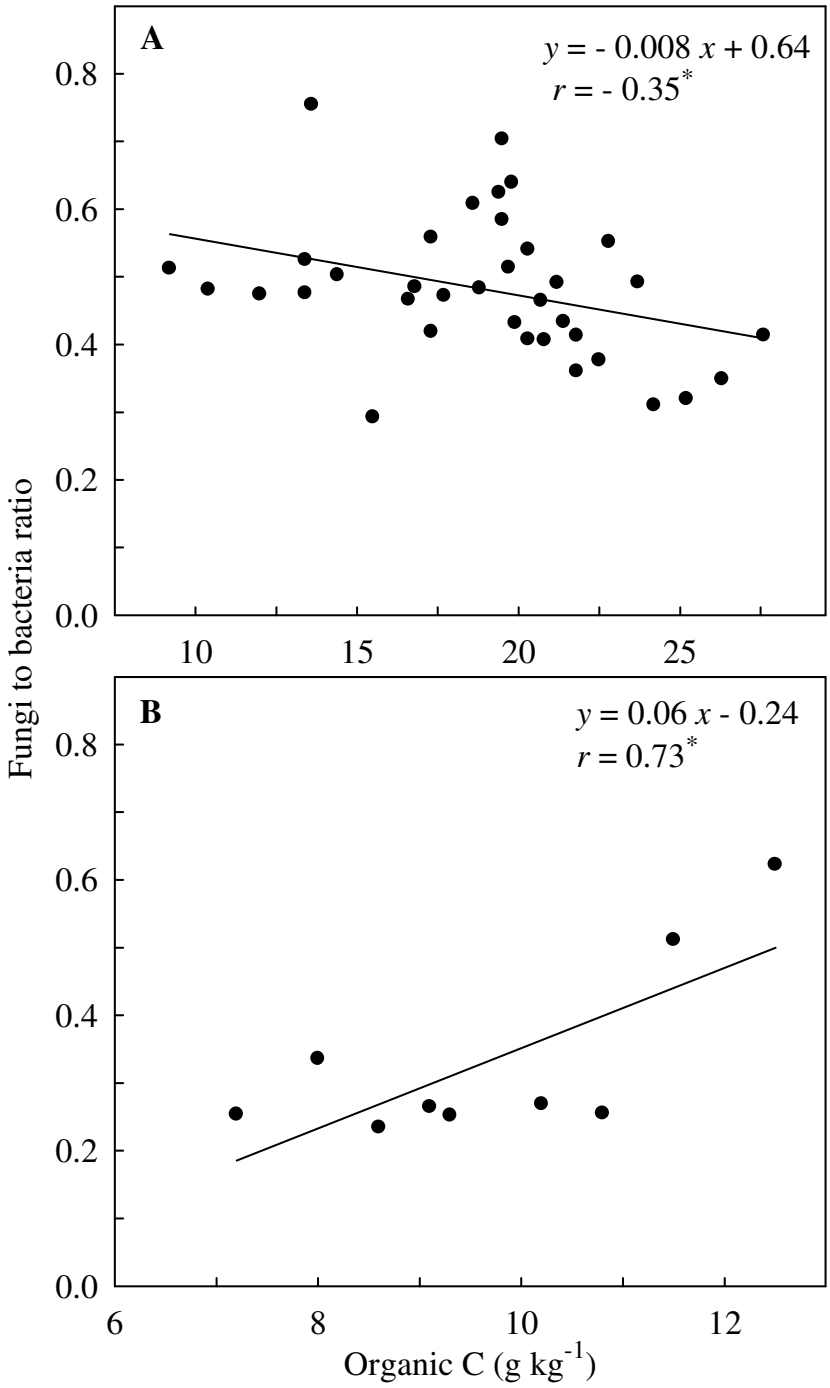

Fig. 7. Relationship between soil organic C content and the ratio of fungi to bacteria for (A) uncultivated soils (n=36) , and (B) cultivated soils (n = 9), $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Table 2. Relationships between phenotypic groups of microorganisms revealed by FAME analysis and enzyme activities (n = 45).

Enzyme activities	Bacteria		Actino-mycetes	Fungi	Mycor-rhizae	Protozoa
	Gram (+)	Gram (-)				
α-glucosidase	0.15	0.01	0.17	0.09	0.10	0.21
β-glucosidase	0.29	-0.04	0.29	0.16	0.18	0.28
α-galactosidase	0.36 *	0.06	0.35 *	0.29	0.29	0.42 **
β-galactosidase	0.35 *	0.09	0.36 *	0.29	0.29	0.39 **
Cellulase	0.27	-0.01	0.26	0.17	0.20	0.30 *
Invertase	0.25	0.49 ***	0.23	0.44 **	0.39 **	0.33 *
β-glucosaminidase	0.48 ***	0.39 **	0.44 **	0.59 ***	0.58 ***	0.62 ***
Urease	0.41 **	0.17	0.41 **	0.34 *	0.33 *	0.46 **
L-asparaginase	0.36 *	0.19	0.32 *	0.29	0.30 *	0.38 **
L-glutaminase	0.58 ***	0.50 ***	0.51 ***	0.60 ***	0.61 ***	0.64 ***
Protease	0.09	0.17	0.04	-0.01	-0.03	0.01
Nitrare Reductase	-0.02	-0.18	-0.01	-0.06	-0.04	-0.05
Acid phosphomonoesterase	0.35 *	0.14	0.30 *	0.34 *	0.34 *	0.37 *
Alkaline phosphomonoesterase	0.33 *	0.12	0.32 *	0.24	0.26	0.40 **
Phosphodiesterase	0.47 **	0.37 *	0.44 **	0.45 **	0.46 **	0.60 ***
Inorganic pyrophosphatase	0.23	0.16	0.16	0.27	0.25	0.25

Phenotypic groups of microorganisms estimated as the sum of proportions (%) of fatty acid biomarkers multiplied by the total area determined from the chromatograph of each sample. Bacteria were estimated as the sum of Gram(+) and Gram(-) biomarkers; Gram(+) biomarkers were *i*14:0, *i*15:0, *a*15:0, *i*16:0, 17:0, *i*17:0, *a*17:0, *i*19:0, and *i*20:0; Gram(-) biomarkers were 14:1ω5*c*, 15:1ω6*c*, 16:1ω9*c*, 16:1ω5*c*, 17:1ω8*c*, 17:1ω6c, *cy*17:0, 18:1ω7*c*, 18:1ω9c, and *cy*19:0; Actinomycetal biomarkers were 10*me*16:0, 10*me*17:0, and 10*me*18:0; Fungal biomarkers were 18:1ω9*c*, 18:2ω6*c*, 18:3ω6*c*, 20:1ω9*c*, and 20:2ω6*c*; Mycorrhizal biomarkers were 18:1ω9*c* and 18:3ω6*c*; and, the protozoan biomarker was 20:4ω6*c*. Enzyme activities were expressed as mg product per g dry soil per incubation time. $^{*}P<0.1$; $^{**}P<0.05$; $^{***}P<0.001$.

4. Discussion

Although microbes in the undisturbed system were not as abundant as in the moderately grazed soils, the microbial communities in this system were richer and more diverse than all other systems tested. This was attributed to its high spatial variability, as revealed in Chapter I, that allowed diverse habitats and micro-niches that harbor species of different growth requirements to co-exist (Wardle, 2002). Within these communities, Gram(+) organisms contributed proportionally more to the microbial biomass. McKinley et al. (2005) also found that in virgin prairie the relative concentration of Gram(+) markers was higher than either the cultivated or successional systems. Changes in the ratio of Gram(-) to Gram(+) markers may signify different types of plant residues and organic inputs in the soil system (Zelles, 1999). The long-term stability of the undisturbed system and the presence of more complex (recalcitrant) organic materials (Bardgett et al., 1998) promoted relatively slow growers such as fungi, actinomycetes, and Gram(+) organisms. The significantly higher fungal to bacterial biomass in the non-cultivated systems was consistent with the higher microbial biomass C to N ratios compared to the cultivated ones. Significantly higher fungal to bacterial ratios in less intensively managed farming systems have been reported by others (Acosta-Martinez et al., 2004; Grayston et al., 2004; Zhang et al., 2005).

4.1. Effect of cultivation

It has long been recognized that cultivation leads to reduction in microbial abundance. The significant correlations between microbial biomass and the total abundance of fatty acids further suggested that fatty acids provided a measure of microbial biomass, which is consistent with findings reported by Frostegård and Bååth (1996).

Although cultivation led to significant reduction in microbial abundance, it promoted dominance of bacteria, in particular Gram(-) bacteria, while reducing the biomass of fungi and actinomycetes. Fast growing bacteria, such as many Gram(-) bacteria, are characterized as r-strategists that compete for simple substrates (Singh et al., 2006). In intensively managed ecosystems, r-strategists grow fast by taking advantage of new resources, adapt quickly, and dominate in the changing environments (Andrews and Harris, 1986). Contrary to the concept of r/K strategists in which competition and natural selection should favor the K-selection, greater nutritional stress detected in the cultivated system did not promote slow growers, such as fungi and actinomycetes. Possibly this was due to physical disruption in these soils. Studies showed that conventional agricultural management promoted growth of bacteria but disrupted growth of fungi (Moore, 1994). The reduction in fungal and actinomycetal biomass by cultivation may be caused by physical disturbance that breaks up their filamentous hyphae (Kaur et al., 2005; Zhang et al., 2005). Studies also showed that inorganic fertilizer applications reduced the proportion of fungi (Smith et al., 2003).

Cultivation reduced habitat heterogeneity (Zhang et al., 2005) and led to significant reduction in microbial diversity. Although there is no direct evidence that plant species diversity affects belowground diversity (Wardle, 2002), the annual input of wheat straw and residues for over 50 years in the cultivated system did not provide much variability in substrates to favor the growth of a diverse community. Soil disturbance, in general, adversely affects colonization and establishment of certain groups of microbes, in particular slow growing bacteria, fungi, and actinomycetes that are shown to be sensitive to disturbance (Kaur et al., 2005).

4.2. Effects of grazing

Grazing at moderate intensity increased microbial abundance and the proportion of fungi and actinomycetes in the community while intensive grazing led to decrease in fungal biomass and increase in Gram(-) bacteria in the microbial community. This finding is in agreement with results obtained by Bardgett et al., (1996) and support the idea that light grazing supports 'slow cycles' dominated by more resistant substrates and fungi, while heavy grazing and hence frequent and more severe plant defoliation favors 'fast cycles' dominated by labile substrates and bacteria, (Bardgett et al., 1998). Grazing activity altered substrate availability and increased the competitive advantage of fast-growing bacteria. High grazing pressure increases nutrient cycling and availability through enhanced animal excreta and root exudation (Bardgett et al., 1998; Ruess and McNaughton, 1987). In this study site, increased grazing intensity also shifted plant species composition toward fewer tallgrasses and more midgrasses, annuals and forbs

(Fuhlendorf et al., 2002), which altered litter quality and may have contributed to the reduction in fungal abundance. Wilson and Hartnett (1998) found that such shifts in the plant community composition reduced mycorrhizal fungi colonization, attributing the changes to plant physiology (C_3 vs. C_4 metabolism) and root morphology.

The significantly lower Shannon indices in the grazed systems than the undisturbed ones do not support the "intermediate disturbance hypothesis" that was developed for evaluation of plant communities (Grime, 1973). Based on this hypothesis, an undisturbed system would support a few dominating and competitive species, while a system of intermediate disturbance may have higher diversity. In this study, grazed systems can be considered as intermediate in disturbance between the cultivated and the undisturbed soil. Plants in response to stimulated growth and defoliation by grazing allocated more resources belowground, and increased exudation of organic materials into the rhizosphere (Bardgett et al., 1998). Consequently, this may have promoted competitive exclusion of some microbial species and led to some reduction in microbial diversity.

4.3. Microbial competition, interaction, and function

Organisms inhabiting the environment are constantly competing for available space and resources. Management practices changed the habitats and resource availability, which lead to change in resource partitioning and community abundance, structure and activity. Therefore, microbial phenotypic groups responded to key changes brought by different management practices as revealed by PCA. Given the similarity in

microbial biomass C, N, and P contents among moderately grazed, highly grazed, and undisturbed sites (Chapters III and V), the separation of the moderately grazed system suggested that PCA is a powerful tool in discriminating effects of integrated interactions in complex soil environments.

Predation plays a key role in microbial competition that governs community structure and function. Bacterial populations in soils are in part controlled by predators such as protozoa. The higher relative abundance of protozoa in the non-cultivated systems was partially responsible for the relatively lower bacterial abundance. In the cultivated system, protozoa growth was limited in part by lower soil water storage (data not shown) because of tillage, crop uptake, and reduced litter cover. In drier soils, water is retained in smaller pores, where the bacteria are protected from predators such as protozoa (Hassink et al., 1993).

Organic matter provides resources as well as stable soil structure and diverse habitats for microbial life. Fungal to bacterial biomass was negatively correlated to organic C, whereas in the cultivated soils it was positively correlated to organic C, a finding that was also reported by Allison et al. (2005). In general, minimum physical disruption in the uncultivated systems allowed filamentous microbes, such as fungi and actinomycetes, to colonize, establish, and compete for resources with other microbes (Andrews and Harris, 1986). Many of these microorganisms are also slow growers that are capable of utilizing complex organics with high molecular weight and high C to nutrient ratios (Bardgett et al., 1998). Growth of slow growers was promoted by increased litter cover in a no-tillage agroecosystem (Frey et al., 1999). During

succession, such as the set-aside from cultivation system, microbial communities shifted the proportion of bacteria to fungi (Ohtonen et al., 1999).

It has been proposed that the increased proportion of fungi in soils may be a mechanism for long-term soil C storage, because: (i) decomposer fungi are capable of using spatially separated nutrient resources to translocate litter-derived C into the soil where it becomes stabilized as soil organic matter (Frey et al., 2003), (ii) fungi, in general, use available substrate more efficiently than bacteria (Holland and Coleman, 1987; Ohtonen et al., 1999), and (iii) fungal biomass is composed of more complex and recalcitrant compounds (Guggenberger et al., 1999). Moreover, fungi contribute to organic C sequestration through the creation of protection mechanisms associated with the formation of a stable soil structure and the buildup of recalcitrant residues, rather than through litter decomposition (Allison et al., 2005). This may explain the negative correlation between fungal to bacterial biomass and organic C in the non-cultivated soils of this study. The cultivated soil ecosystem depends on fungi for the decomposition of the more recalcitrant wheat litter, so that increases in fungi relative to bacteria are positively related to increases in organic C.

Correlations between enzymes and phenotypic groups of microorganisms suggest how shifts in microbial community composition could affect soil functions. Reduction in the relative abundance of Gram(+) bacteria over Gram(-) bacteria affected the activities of the galactosidases more than the activities of the glucosidases. That some enzymes tested correlated with several phenotypic groups implied functional redundancy in soil microbial communities, and suggested resilience of the microbial community in performing ecosystem functions. Often, more than one phenotype can fulfill the same

function (Giller et al., 1997). In this study, except for invertase and inorganic pyrophosphatase, the correlations of enzyme activities with microbial biomass were stronger than with any phenotypic group of microorganisms, so that the functional capacity of the soil ecosystem depended more on the size of the microbial community rather than on its structure. Some enzyme activities were unrelated to phenotypic groups, possibly because extracellular enzymes persist in soil in protected forms that are not directly associated to living microbial cells (Burns, 1982). Functional capacity as measured by enzyme activities integrates the long term effect of soil processes, while FAME analysis largely reflects the current state of the microbial community.

4.4. Conclusions

Quantifying fatty acids revealed differences in microbial community composition across management systems. Bacteria were the dominant decomposers in all evaluated systems. High variability in the uncultivated soil favored development of diverse habitats to harbor a wide-range of microbial groups with relatively high proportion of Gram(+) organisms in the community. Changes in the ratio of Gram(-) to Gram(+) signify different types of plant residues and organic inputs in the soil systems evaluated. The long-term limited disturbance of the uncultivated systems and the presence of more complex (recalcitrant) organic materials promoted fungi, actinomycetes, and Gram(+) organisms, resulting in significantly higher fungal to bacterial biomass ratios.

Cultivation, physical disruption, and monoculture cropping system led to significant reduction in microbial abundance and diversity, promoted dominance of

bacteria, in particular Gram(-) bacteria, while reducing the biomass of fungi and actinomycetes. Grazing, especially at moderate intensity, increased microbial abundance and the proportion of fungi and actinomycetes in the community, but did not significantly affect the relative abundance of protozoa. During successional development, microbial diversity increased, accompanied by increasing proportion of fungal and slow grower microbial biomass.

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CHAPTER VII

SUMMARY AND CONCLUSIONS

Contents of microbial biomass C and N, dissolved organic C and soluble N were the most variable of the evaluated properties. The high spatial variability of microbial biomass C and N indicated that the factors that control these parameters operate at a micro-scale. The high spatial dependence of microbial biomass, soluble C and N in the cultivated systems and their similar ranges of autocorrelation and similar shapes of variograms in the uncultivated soils suggested that these soil parameters were more sensitive to perturbations than less active parameters such as organic C and processes with similar effects acted over closely related spatial scales.

When compared with the undisturbed soils, long-term cultivation decreased organic C, total N, and inorganic P, but did not alter the size of total soil P pool. It led to significant increases in organic P contents, and in the soluble forms of C, N, and P. Cultivation also decreased microbial biomass C, N, and P. Soil microbial communities in the cultivated soils had reduced microbial biomass per unit of organic C and enhanced metabolic activity. Activities of C- and N- and P-transforming enzymes were lower in the cultivated soils, suggesting a lower capacity to cycle nutrients. Fertilizer application suppressed activities of most of the tested amidohydrolases and phosphatases. Grazing, especially at moderate intensity, did not lessen the soil capacity to support microbial life

and cycle C, N, or P, and sustained microbial communities with relatively high microbial biomass per unit of organic C. However, due to increased mineralization and reduced belowground biomass accumulation, grazing at high intensity reduced the soil organic C and total N pools.

The intermediate status of the soil chemical, microbiological, and biochemical properties in the set-aside form cultivation system suggested that through secondary succession the soil ecosystem was restoring its capacity to sequester C, recycle C, N and P, support microbial life, and was evolving towards the functional capacity of the undisturbed soil ecosystem. However, the impact of cultivation to the soil ecosystem was detectable following more than 30 years of restoration.

Carbon-, N-, and P-transforming enzymes were sensitive in discriminating soil ecosystems under various land uses and can be used as indicators for detecting differences in the capacity of soil to cycle C, N, and P among a wide range of management practices and among systems at various stages of ecological succession. Of the 16 soil enzymes evaluated, the most sensitive in discriminating soil ecosystems were the activities of β-glucosidase, α-galactosidase, cellulase, urease, L-asparaginase, β-glucosaminidase, acid phosphomonoesterase, and phosphodiesterase.

Bacteria were the dominant decomposers in all evaluated systems. High variability in the uncultivated soils favored development of diverse habitats that harbor a wide-range of microbial groups with relatively high proportion of Gram(+) organisms in the community. Changes in the ratio of Gram(-) to Gram(+) signify different types of plant residues and organic inputs in the soil systems evaluated. The long-term stability of the uncultivated systems and the presence of more complex (recalcitrant) organic

materials promoted fungi, actinomycetes, and Gram(+) organisms, resulting in significantly higher fungal to bacterial biomass ratios.

Cultivation, physical disruption, and monoculture cropping system created a stressed environment for microbial communities and led to significant reduction in microbial abundance and diversity, promoted dominance of bacteria (mostly Gram(-)), while reducing the biomass of fungi and actinomycetes. Grazing increased microbial abundance and the proportion of fungi and actinomycetes in the community, but did not significantly affect the relative abundance of protozoa. During successional development, microbial diversity increased, accompanied by increasing proportion of fungal and slow grower microbial biomass.

Overall, this study reveals that grazing at moderate intensities is a sustainable alternative management practice for the semiarid prairie soil ecosystems to sustain organic C, support diverse microbial communities, and preserve the soil functional capacity. Intensive management practices such as long-term cultivation under monoculture reduce the functional capacity and diversity of the soil ecosystem, disturb soil C equilibrium, affect nutrient availability to the microbial community, and lead to less diverse habitats and microbial communities. Removal of marginal lands from cultivation in the long term may restore their functional capacity and regain their ability to support diverse microbial communities, and serve as C sink that contributes to regulation of the global C cycle.

VITA

Eirini Katsalirou

Candidate for the Degree of

Doctor of Philosophy

Dissertation: MICROBIAL COMMUNITY AND ENZYME ACTIVITIES IN PRAIRIE SOIL ECOSYSTEMS UNDER DIFFERENT MANAGEMENT

Major Field: Soil Science

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Scope and Method of Study: The aim of this study was to evaluate the effects of long-term management practices on microbial properties and biochemical processes related to C, N, and P cycling of semiarid prairie soil ecosystems. The management systems included undisturbed, abandoned from cultivation, moderately grazed, heavily grazed and cultivated soils. Soil chemical and microbial properties, enzyme activities involved in C, N and P cycling, microbial diversity and community structure were tested in surface soil samples taken from nine randomly selected plots for each treatment. The spatial variability and dependency of organic C and microbial biomass and activity were also evaluated.

Findings and Conclusions: The moderately grazed system had similar chemical properties, and microbial biomass and activity to the undisturbed soil ecosystem. More intensive grazing reduced organic C and total N. Grazed systems showed similar activities for most enzyme activities tested to the undisturbed system. Long-term cultivation decreased organic C, total N, total P, and microbial biomass and activity, while increasing the concentrations of labile nutrients. Enzyme activities were the least in the cultivated system, indicating its lower potential for C, N and P transformations, and resulted in accumulation of organic P. The abandoned from cultivation system had intermediate organic matter, microbial biomass, and enzyme activities between the cultivated and the undisturbed and grazed systems. The undisturbed soils had the most diverse microbial communities with relatively higher proportions of k-strategists, while the cultivated had the least diverse microbial communities with high proportions of r-strategists. Microbial biomass C and N were the most spatially variable, especially in cultivated soils. The spatial structure of microbial biomass in cultivated soils revealed a periodicity caused by cultivation operations. Overall, through secondary succession the abandoned soil ecosystem indicated slow recovery from cultivation, and that it can regain its capacity to sequester C and to recycle nutrients. Grazing did not degrade soil chemical and microbial properties, sustains the biochemical capacity of the soil ecosystem for nutrient cycling, and in particular moderate grazing can be a sustainable management alternative for the semiarid soil ecosystems of the Southern Great Plains.

ADVISER'S APPROVAL: Shiping Deng