SULFUR DYNAMICS AND ACTIVITIES OF SULFUR-TRANSFORMING ENZYMES IN PRAIRIE SOILS UNDER DIFFERENT MANAGEMENT PRACTICES

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FORMAT OF THESIS

This thesis is presented in a combination of formats required for publishing in Soil Biology and Biochemistry or Applied Soil Ecology, and formats outlined by the Oklahoma State University graduate college style manual. This allows the independent chapters to be suitable for submission to scientific journals. Each main chapter is complete with an abstract, an introduction, materials and methods, results, discussion, and a reference section.

CHAPTER I

INTRODUCTION

Conservation of soil productivity and quality is crucial for continuing sufficient food production to meet the needs of increasing population and for maintaining environmental quality and sustainability for future generations (Schloter et al., 2006). It is long been recognized that cultivation and soil management practices could impact soil quality and productivity. History has taught us unforgettable lessons. In the Great Plains, cultivation for about 70 years followed by severe drought conditions led to the “Dust Bowl” era in 1930s. Consequently, several soil and ecosystem conservation programs, such as Conservation Reserve Program (CRP), were established to reduce soil erosion and conserve ecosystem health and function. For land enrolled in CRP, the producers receive an annual rental payment for the term of a multi-year contract to establish vegetative cover in the effort of conserving and restoring ecosystem health and function. As a result, much of the Great Plains was converted back to rangeland. One half of the 42 million acres available land in Oklahoma is currently rangeland. Ten million acres of these rangelands were once cropland, with only 1.02 million acres of these rangelands enrolled in CRP.

Although half of the once cultivated cropland is now abandoned from cultivation (these are also termed restored rangeland), much of this land is still not enrolled in any conservation programs.

Although extensive research has been conducted to evaluate effect of management practices on soil ecosystem health and function, it is still not clear what the best practices are in continuing agricultural production while maintaining ecosystem function. Moreover, most research conducted has focused on the above-ground plant community with little known about the below-ground soil system. Understanding effects of different management practices on the below-ground soil properties and function may assist the development of management strategies that maintain soil quality and conserve soil productivity for the future generations.

Research data suggested that different management practices affect soil quality and functions differently. Management practice such as grazing, cropping systems, tillage, and fertilization can affect microbiological activity, biochemical properties, and nutrient cycling of soils (Dodor and Tabatabai, 2003; Green et al., 2007; Klose et al., 1999, Mestelan., 2008). Minimum tillage not only reduced soil erosion and water evaporation and promoted storage of water and nutrient, but also increased the activities of enzymes involved in phosphorous (P) and sulfur (S) cycling in soils (Deng and Tabatabai, 1997).

Grazing disturbs surface soils, affects biological soil crust, and impacts nutrient cycling. Su and coworkers (2005) showed that continuous grazing decreased ground cover which increased soil erosion leading to loss of soil

organic nitrogen (N) and carbon (C) when compared with exclusion of grazing. Grazing has been shown to increase the loss of soil nutrients to wind and water erosion. Historical grazing through the 20th century led to a reduction of organic matter content (SOM) and 60-70% decline of surface C and N contents when compared with sites that were never grazed (Neff et al., 2005). On the other hand long-term exclusion from grazing (more than 50 years) resulted in significant reductions in microbial biomass and activity in the surface soil (Bardgett et al., 1997). These findings imply that grazing could have a long lasting effect on the soil fertility and quality. Based on evaluation of soil microbial activity, high intensity and short duration grazing had less effect than continuous grazing (Southorn, 2002).

Similarly, many studies have demonstrated that long-term repeated cultivation reduced soil structural stability and decreased SOM (McArthur et al., 2001, Caldwell et al., 1999; Warlrop et al., 2000). Cultivation led to reduction of extractable P, total C, organic C, total N, and microbial biomass C (Malo et al., 2005; Parton et al., 2005; Acosta- Martinez et al., 2004; Saviozzi et al., 2001; Holt and Mayer, 1998). Extended cultivation led to progressive soil degradation and decreased soil fertility and nutrients availability (Jaiyeoba, 2003).

In general, microorganisms respond more rapidly than chemical and physical parameters to changes in land use (Burns et al., 2006). Enzymes are the driving force in nutrient cycling and have been suggested as indicators in detecting changes of the soil ecosystem (Naseby and Lynch, 2002). Amidase, arysulfatase, deaminase, invertase, cellulase, and urease activities were lower in

cultivated fields compared to grassland fields (Bandick and Dick, 1999). Arylsulfatase activity was lower in cotton fields than in uncultivated native grassland (Acosta-Martinez et al., 2003).

Nutrient cycling is a key ecosystem function and essential for the conversion of nutrients to plant available forms. Cultivation and grazing affect C, N, P, and S cycling in soils differently (Green et al., 2007; Dodor and Tabatabai, 2003; Doran and Parkin, 1994).

Of the top four macronutrients required by plants, much of research effort have been devoted to examine the cycling of C, N, and P while relatively little attention has been paid to S (Barbosa et al., 1998). Sulfur deficiency in soils has been detected in different parts of the world. Availability of organic and inorganic soil S to plants and microbes can both be controlled through enzyme activities. Arylsulfatase and rhodanase are two commonly detected S-transforming enzymes in soil. Arylsulfatase catalyzes the mineralization of organic sulfur, which leads to the release of plant available inorganic S. Rhodanase is an enzyme that catalyzes the oxidation of inorganic S to SO_4^{2-}. These two enzymes play crucial roles in S cycling in soil.

Therefore, the objectives of this study were to assess the impact of different management systems on microbial properties related to nutrient cycling in prairie soils. The specific objectives were (1) to evaluate impacts of grazing and cultivation on sulfur transformation and enzyme activities involved in sulfur cycling; and (2) to reveal drivers from the interrelationship between soil C, N, P and S in different ecosystems using multivariate analysis.

Sulfur transformation and activity of enzymes involved in S cycling under different management practices is discussed in chapter III. The regulation and relationship between C, N, P, and S cycling under different management practices is presented in chapter IV.

References

Acosta-Martinez, V., Klose, S., Zobeck, T.M., and Kennedy, A.C. 2003. Enzyme activities in semiarid soils under conservation reserve program, native grassland, and cropland. Journal of Plant Nutrition and Soil Science 166:699-707.

Acosta-Martinez, V., Zobeck, T.M., and Allen, V. 2004. Soil microbial, chemical and physical properties in continuous cotton and integrated crop-livestock systems. Soil Science Society of America Journal 68:1875-1884.

Bandick, A.K., and Dick, R.P. 1999. Field management effects on soil enzyme activities. Soil Biology & Biochemistry 31:1471-1479.

Barbosa, V., Zhao, F., and McGrath, S. 1998. Thiosulphate and etrathionate Oxidation in arable soils. Soil Biology & Biochemistry 30:553-559.

Bardgett, R.D., Leemans, D.K., Cook, R., and Hobbs, P.J. 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. Soil Biology & Biochemistry 29:1285-1294.

Burns, R.G., Nannipieri, P., Benedetti, A., and Hopkins, D.W. 2006. Defining Soil Quality, pp. 15-22, *In* J. Bloem et al., (eds.) Microbiological methods for Assessing Soil Quality. CAB international, Oxfordshire.

Caldwell, B.A., Griffiths, R.P., and Sollins, P. 1999. Soil enzyme response to vegetation disturbance in two lowland Costa Rican soils. Soil Biology & Biochemistry 31:1603-1608.

Deng, S., and Tabatabai, M. 1997. Effect of tillage and residue management on enzyme activities in soils: III phosphatases and arylsulfatase. Biology and Fertility of Soils 42:141-146.

Dodor, D., and Tabatabai, M. 2003. Amidohydrolases in soils as affected by cropping systems. Applied Soil Ecology 24:73-90.

Doran, J.W., and T.B. Parkin. 1994. Deffining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A., (Eds.) Defining soil quality for a 14 sustainable environment. SSSA Special Publication Number 35. SSSA and ASA, Madison WI, pp. 3-22

Green, V., Stott, D., Cruz, J., and Curi, N. 2007. Tillage impacts on soil biological activity and aggregation in Brazilian creeado oxisol. Soil & Tillage Research 92:114-121.

Holt, J.A., and Mayer, R.J. 1998. Changes in microbial biomass and protease activities of soil associated with long-term sugar cane monoculture. Biology and Fertility of Soils 27:127-131.

Jaiyeoba, I.A. 2003. Changes in soil properties due to continuous cultivation in Nigerian semiarid Savannah. Soil & Tillage Research 70:91-98.

Klose, S., Moore J., and M., T. 1999. Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biology and Fertility of Soils 29:46-59.

Malo, D.D., Schumacher, T.E., and Doolittle, J.J. 2005. Long-term cultivation impacts on selected soil properties in the northern Great Plains. Soil & Tillage Research 81:277-291.

McArthur, D.F.E., Huang, P.M., and Kozak, L.M. 2001. Impact of long-term cultivation on the status of organic matter and cadmium in soil. Canadian Journal of Soil Science 81:349-355.

Mestelan, S.A. 2008. Impact of long- term no till and plow till on soil porperties and soil nutrient cycling, PhD Dissertation, The Ohio State University, Columbus, OH, USA.

Naseby, D.C., and Lynch, J.M. 2002. Enzymes and microorganisms in rhizosphere., pp. 109-123, *In* R. G. Burns. and R. P. Dick., (eds.) Enzymes in Soil Environment. Activity, Ecology and Application, Vol. pp. Marcel Dekker, New York.

Neff, J.C., Reynolds, R.L., Belnap, J., and Lamothe, P. 2005. Multi- decadal impacts of grazing on soil physical and biogeochemical properties in southeast Utah. Ecological Applications 15:87-95.

Parton, W.J., Gutmann, M.P., Williams, S.A., Easter, M., and Ojima, D. 2005. Ecological impact of historical land -use patterns in the Great plains: A methodological assessment. Ecological Applications 15:1915-1928.

Saviozzi, A., Levi-Minzi, R., Cardelli, R., and Riffaldi, R. 2001. A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant and Soil 233:251-259.

Schloter, M., Tittarelli, F., and Miunch, J.C. 2006. Managing Soil Quality, pp. 50-62, *In* J. Bloem et al., (eds.) Microbiological Methods for Assessing Soil Quality. CAB International, Oxfordshire.

Southorn, N.J. 2002. The soil structure component of soil quality under alternate grazing management strategies. Advances in Geoecology 35:163-170.

Su, Y.Z., Li, Y.L., Cui, J.Y., and Zhao, W.Z. 2005. Influences of continuous grazing and livestock exclusion on soil properties in degraded sandy grassland, Inner Mongolia, northern China. Catena 59:267-278.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., and Polasky, S. 2002. Agricultural sustainability and intensive production practices. Nature 418:671-677.

Waldrop, M.P., Balser, T.C., and Firestone, M.K. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32:1837-1846.

CHAPTER II

REVIEW OF LITERATURE

Soil Health and quality

Soil health refers to the biological, chemical, and physical features of soil that are essential to long-term, sustainable agricultural productivity with minimal environmental impact. Thus, soil health provides an overall picture of soil functionality. Although it cannot be measured directly, soil health can be inferred by measuring specific soil properties (e.g. organic matter content) and by observing soil status (e.g. fertility) (Enriqueta Arias et al., 2005).

Soil quality influences agricultural sustainability, environmental quality and consequently plant, animal and human health. Historically, chemical and physical properties have been used as a crude measure of soil productivity. The evaluation of soil quality is quite complex and requires the consideration of physical, chemical and biological variables (Monokrousos et al., 2006).

Soil organic matter (SOM) was used as an indicator of soil health and quality. Measurements of certain characteristics of the active fraction of SOM and soil metabolic activities have been used to indicate changes in soil quality (Ajwa et al., 1998). However, SOM change very slow and therefore many years may be required to measure changes resulting from perturbations (Dick, 1994).

Soil microbiological and soil biochemical parameters for example pH, inorganic and organic P, N, C, S pools, microbial biomass, and enzyme activities;

now are used as indicators of soil quality. Soil microbial characteristics are attractive as potential soil quality indicators. They are considered as sensors and integrators for variety of stresses in soil as they respond to changes in land use, environmental conditions, and contaminations more rapidly than chemical and physical parameters (Doran and Parkin, 1994).

Soil microbial activities lead to the liberation of nutrients available for plants, and are of crucial importance in biogeochemical cycling. Microorganisms can degrade pollutants and have an important role in stabilizing soil structure and conserving organic matter for sustainable agriculture and environmental quality (Dilly, 2005).

Soil chemical, physical, and biological characteristics changed under different types of management, and this can affect the availability of nutrients from inorganic fertilizers (Navida et al., 2007). Dick specified that enzyme activities are good early indicators of changes in soil properties because of their relationship to soil microflora, and their rapid response to changes in soil management (Dick, 1994). In the last two decades, much has been published indicating the significance of soil microbial properties for agriculture ecosystem function and overall soil fertility in different intensive agriculture management systems. Soil microbial biomass was decreased by intensive grazing in semiarid grassland soils (Holt and Mayer, 1998), while long term exclusion of grazing from grassland resulted in significant reduction in microbial biomass and activity in the surface soil (Bardgett et al. 1997).

Another study investigated the interrelationships between cropping, microbial biomass S, and S uptake by plant showed increases in the biomass S in the soil amended with cattle manure compost (CMC), while the addition of saw dust compost or rice husk compost resulted in sever sulfur deficiency in soil. This limitated plant growth due to a net loss of plant available S through microbial immobilization and transformation reactions (Chowdhury et al., 2000).

SOIL ENZYMES

A living system controls its activity through enzymes. Enzymes are proteins that serve as catalysts for biological reactions; the basic function of enzymes is to increase the rate of a reaction by combining with their specific substrate. Most cellular reactions occur about a million times faster than they would in the absence of an enzyme. Much of the information about enzymes has been made possible because they can be isolated from cells and made to work in a test tube environment. Since enzymes are extremely selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell (Tabatabai and Singh1979).

One of the most difficult problems facing soil biologist and biochemists is the separation of the extracellular enzymatic activities (were enzymes are found and remain in an active state outside the living organism) from intercellular enzymes (those associated with the living organisms) (Tabatabai, 1994).

In addition, enzymes can be classified based on their ecological function into six functional classes by the international Union of biochemists (I.U.B); oxidases or dehydrogenases (enzymes that catalyze oxidation-reduction reactions), transferases (enzymes that catalyze transfer of molecular substituents among molecules), hydrolysis, lyases, isomerases, and ligases.(Webb, 1992)

Soil enzymes (intracellular and extracellular) are important mediators and catalysts for biochemical processes such as mineralization and nutrient cycling (C, N, and S). Enzymes play key roles in the biochemical functioning of soils, including organic matter formation and degradation, and decomposition of xenobiotics. Knowledge of enzyme activities can be used to describe changes in soil quality due to land use management (Acosta- Martines et al., 2007), and have been suggested as indicators in detecting changes of the soil ecosystem (Naseby and Lynch., 2002). Enzyme activities in the soil can be affected by different management. Deng and Tabatabai studied enzyme activity affected by trace element, including β-glucosidase, urase, phosphatase, arylsulfatase and nitrate reductase. The trace elements were added to soils as fertilizers. The result showed that most of the trace elements studied inhibited cellulase activity. Among the trace elements (Ag) was the most effective inhibitor of cellulase activity (Deng and Tabatabai, 1995). A study to investigate the activity of different enzymes involve in C, N, P, and S cycling as affected by soil order and land use within a watershed in north central Puerto Rico, showed significant effects of soil order and land use on the soil enzyme activities in the tropical watershed studies. (Acosta- Martines et al., 2007). Different studies assessed the impact of

tillage on soil enzymes activities. Deng and Tabatabai (1996) studied effect of three tillage system and four residue placements on the activities of four amidohydrolases (amidase, L-asparaginase, L-glutaminase,and urease), phosphotases (acid phosphatase, alkaline phosphatase, phosphodiesterase, and inorganic pyrophosphatase) and arylsulfatase in soils. The results showed that the effect of mulching on enzyme activity was more significant than the effect of tillage management with normal placement of plant residue. The activities of phosphatase and arylsulfatase decreased markedly with increasing depth of tillage. The highest arylsulfatast activity was found in no-till/double mulch, these results suggest that minimum tillage not only reduces soil erosion and water evaporation, and promotes storage of water and nutrient, but also increased the activities of phosphatase and arylsulfatase, thereby increasing the P and S cycling in soils (Deng and Tabatabai, 1997). While another study to assess the impact of tillage on soil enzymes activities showed that no till system increases up to 46% for acid phosphatase 68% for amylase, 90% for cellulose, 219% for arylsulfatase. These result showed that soil enzyme activity is a sensitive indicator of soil quality by management (Balota et al., 2003). A study to assess the effect of cultivation on the activity and kinetic of arylsulfatase reported that cultivation of the native grassland and forest soils decreased natural enzymatic activity. Clearly the decease in arylsulfatase activity reflects the reduction in organic matter content and microbial biomass and activity with the soil associated with land management (Farrell et al., 1994). The activity of α-and ß-glucosidase, α- and ß-galactosidase, amidase, arylsulfatase, deminase,

invertase, cellulose and urease were generally higher in continuous grass fields than in cultivated fields (Bandick and Dick, 1999).

Long term cultivation can provide important information about the effect of soil management practices on soil enzyme activities. Cultivation for 1500 years in Colca Vally of Peru has maintained similar or higher organic matter, nitrogen, phosphorous and enzyme activities than the uncultivated /native soils. These results suggested that cultivation of soil for 1500 years did not deplete soil fertility or soil biological activity (Dick et al., 1994). Long term grazing could have a long lasting effect on the soil fertility and quality, as it reduces the organic matter content compared to sites that never grazed (Neff et al., 2005). These findings imply that grazing based on evaluation of soil microbial activity, high intensity and short duration grazing had less effect than continuous grazing (Southorn, 2002).

Sulfur cycling

Sulfur is an essential element needed for plant growth and all biological systems. Together with nitrogen it is necessary for the synthesis of amino acids, proteins and various other components. Under normal conditions sulfate is taken up by the roots and transported to the shoot where it has to be reduced before it can be incorporated into various essential organic sulfur compounds. Reduced inorganic S compounds that are found or produced in the biosphere include sulfides (S^{2-}) elemental S (S^{0}), thiosulfate ($S_2O_3^{2-}$) and sulfite. Biotic and a biotic oxidation of these compounds are important in S cycling (Deng and Dick, 1990).

Chemical and spectroscopic studies have shown that in agriculture soils most of the soil S is present as sulfate eaters or as carbon-bonded sulfur (amino acid sulfur), rather than inorganic sulfate (Kertesz and Mirleau, 2004). Many studies showed that most (95%) of the total soil S in intensively managed grazed grassland present as organic S and the reminder is in readily soluble and adsorbed S. Soil organic S that has been accumulated with time in grazed grassland receiving regular S fertilizers application can provide a significant amount of S for plant S nutrition. The storage of sulfur in the various compartment of earth and its biosphere, and the transfer's processes occurring among them, is referred to as the sulfur cycle.

Grazing animals have a major effect on the amount and rate of S cycling as most (87-90 %) of S ingested by grazing animals is returned to soils as excrete (Nguyen and Goh, 1994). The amount of inorganic sulfate (SO_4^{2-}) and organic S held within soil microorganisms at any time is referred to as soil microbial biomass S. This normally accounts for only .04-2.6% of total soil S in intensively managed grassland and is influenced by the seasonal variation, temperature, and soil moisture (Saggar et al., 1981).

The increase in S deficiency in soils of several parts of the world has led to the use of fertilizer S to enhance production and quality of crops. Previous studies showed large differences in S availability among applied organic residues. For example, cruciferous crop like rape provides a large and rapid release of available S, but cereal residues release only minimal amounts of available S (Chowdhury et al., 2000). In the past few decades, continuous use of

the primary fertilizer nutrient, NPK has resulted in depletion of secondary and micro nutrients. In many instances fertilizers have boosted crop yield to a point where S is now the limiting factor to future crop production (Jaggi et al., 1999). Therefore, S mineralization rates and potentials are essential parameters in predicting plant nutritional needs and the amount of S fertilizer needed for optimum crop yields.

Knowledge of the S mineralization rates also important for modeling the cycling of this element in the environment (Pierla and Tabatabai, 1988). Gypsum and elemental S are both effective and efficient sources of S. Gypsum resulted in a greater yield than did the elemental S for winter wheat in Oklahoma (Girma et al., 2005).Gypsum contains 12-16% of plant available S so it is often not economical to use (Navida et al., 2007). Plants are not able to directly use elemental sulfur. Instead, they rely on the ability of certain types of bacteria to convert elemental sulfur to another form.

There are two categories of sulfur bacteria; sulfur oxidizer and sulfur reducer. Sulfur oxidizer bacteria can convert sulfide into sulfate, producing a dark slime that can clog plumbing. Sulfate may be oxidized to elemental sulfur aerobically by species of *Thiothrix* and *Beggiatoa* (morphologically conspicuous sulfur oxidizers), and anaerobically by the purple sulfur bacteria. In the soil the predominant microbes involved in the oxidation of sulfide to element sulfur belong to the genus *Thiobacillus.* Several genera of soil bacteria oxidize sulfides (H_2S or metal sulfides), S^0, or $S_2O_3^{2-}$. Complete oxidation of these substrates

yields SO_4^{2-}, the form of S that is most commonly used as a nutrient by plants and soil microbes. (Konopka et al.,1986).

S reducing bacteria are the more common such as *Desulfovibrio* and *Desulfomicrobium* (Michel et al., 2001). They live in an oxygen-deficient environment; break down S compounds, producing H_2S gas in the process. Bacteria can participate in the reduction of S, in which S compounds are acting as electron receptors, or in the oxidation of S in which an electron is removed from S compounds. S Reducing Bacteria perform dissimilatory reduction of S compounds such as SO_4^{2-}, SO_3, $S_2O_3^{2-}$ and S itself to sulfide. *D.acetotoxidans*, a true sulfur bacterium, is strictly anaerobic, gram negative, flagellated, and rod shaped. It acquires its energy from sulfur respiration and completely oxidizes acetate with sulfur to carbon dioxide via the citric acid cycle.

Cycling of S in the soil environment is often governed by activities of microorganisms and S-transforming enzymes, including arylsulfatase and rhodanese which are two commonly detected S-transforming enzymes in soil. Arylsulfatase, also known as arylsulfate sulfohydrolase EC (3. 1. 6. 1) is the enzyme that catalyzes the hydrolysis of an arylsulfate anion by fission of the O-S bond. It, therefore, participates in the soil processes where organic sulfur is mineralized and plant available inorganic S is released. The reaction involved is as follows:

$$R.O.SO_3^- + H_2O \rightarrow R.OH + H^+ + SO_4^{2-}$$

Arylsulfatase activities of soils have been assayed by quantifying the rates of hydrolysis of *p*-nitrophenyl sulfate to release *p*-nitrophenol and sulfate in buffered soil suspensions in the presences of toluene (Tabatabai and Bremner, 1970). Many studies have investigated the impact of different management practices such as tillage, crop rotations, or fertilization on the activity of this enzyme (Deng and Tabatabai, 1997; Farrell et al., 1994; Klose and Tabatabai, 1999).These Studies showed that cultivation of native North America grassland (Bradwell) and forest (Loon Lake; Waitville) soils decreased soil enzymatic activity including arylsulfatase activity (Farrell et al., 1994). Tillage and residue management also have significant effect on arylsulfatase activity in soils (Deng and Tabatabai, 1997). Although increasing levels of applied S reduced soil arylsulfatase activities, the highest level of applied S in fact stimulated higher enzyme activities (Baligar et al., 2005). Rhodanese, EC (2. 8. 1. 1) Is another S-transforming enzyme that has been commonly detected in soil (Tabatabai and Singh, 1976) and its possible importance in the S cycle was demonstrated by numerous studies. Rhodanese, also known as thiosulfate-cyanide sulfurtransferase, is the enzyme that catalyses the formation of thiocyanate (SCN^-) from $S_2O_3^{2-}$ and cyanide.

$$S_2O_3^{2-} + CN^- \rightarrow SCN^- + SO_3^{2-}$$

Sulfite is an intermediate S compound produced during oxidation of S^o in soil (Nor and Tabatabai, 1999). Activity of rhodanse is correlated with organic C

in soil and is affected by inorganic salts and trace elements (Tabatabai and Singh, 1979). The relationship between Sº oxidation and rhodanese activity was not consistent among soils tested (Deng and Dick, 1990). Rhodanse activity was related to S-oxidation in aerobic soils, but not in a simulated oxidized surface layer in a flooded soil (Ramesh et al., 1985). Singh and Tabatabai (1978) showed that storage at -20° or 5º did not affect rhodanese activity significantly, but air-drying of field moist soils resulted in a marked decrease of rhodanese activity. They also found that preincubation of six soils for 24 h and 48 h with glucose resulted in an increase of average rhodanese activity by 9-23%. When the buffer was made to contain 1 mM with respect to inorganic compounds tested, $NaNO_2$, NaN_3, NaCl, NaF, and Na_2SO_4 activated rhodanese activity in soils, while $NaNO_3$, Na_2SO_3, Na_2S, KH_2PO_4 and $NaHCO_3$ inhibited its activity (Singh and Tabatabai, 1978).

Given the fact that environmental conditions are constantly changes and ecosystem functions and soil processes are regulated by multiple variables with considerable temporal variation measuring S content and the activity of S-transforming enzymes in this study will enhance our understanding to S cycling in prairie soil as affected by different management practices. Multivariate analysis of selected variables involved in the N, C, P, and S, cycling will provide information on the drivers of soil processes and functions in different management practices which may help developed management practices that can maintain soil sustainability.

References

Acosta-Martinez, V., Cruz, L., Sotomayor-Ramı´rez, D., and Pe´rez-Alegrı´a, L. 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. Applied Soil Ecology 35: 35- 45.

Ajwa, H., Rice, C.W., and Sotomajor, D. 1998. Carbon and nitrogen mineralization in tallgrass prairie and agricultural soil profiles. Soil Science Society of America journal 62:942-951.

Baligar, V.C., Wright, R.J., and Hern, v. 2005. Enzyme Activities in Soil Influenced by Levels of Applied Sulfur and Phosphorus. Communications in soil science and plant analysis 36:1727-1735.

Balota, E., Arnaldo, C., Diva, S., and Dick, R. 2003. Microbial biomass in soils under different tillage and crop rotation systems. Biology and Fertility Soils 38:15-20.

Bandick, A.K., and Dick, R.P. 1999. Field management effects on soil enzyme activities. Soil biology and biochemistry 31:1471-1479.

Bardgett, R.D., and Leeman, D.K. 1995. The short-term effects of cessation of fertiliser applicatios, liming, and grazing on microbial biomass and activity in a reseeded upland grassland. Biology and Fertility of Soils 19:148-159.

Chowdhury, A.H., Kouno, K., Tadao, A., and Nagaoka, T. 2000. Microbial biomass, S mineralization and S uptake by African millet from soil amended with various composts. Soil Biology and Biochemistry 32:845-852.

Deng, S., and Dick, R. 1990. Sulfur oxidation and rhodanese activity in soils. Soil Science Society of America Journal 190:552-560.

Deng, S., and Tabatabai, M. 1996. Effect of tillage and residue management on enzyme activities in soils: I Amidohydrolases. Biology and Fertility of Soils 22:202-207.

Deng, S., and Tabatabai, M. 1997. Effect of tillage and residue management on enzyme activities in soils: III phosphatases and arylsulfatase. Biology and Fertility of Soils 42:141-146.

Deng, S.P., and Tabatabai, M.A. 1995. cellulase activity of soils: effect of trace elements. Soil Biology & Biochemistry 27:977-979.

Dick, R. 1994. Soil enzyme activities as indicator of soil quality. Soil Science Society of America Special Publication 35:107-124.

Dick, R.P., Sandor, J.A., and Eash, N.S. 1994. Soil enzyme activities after 1500 years of terrace agriculture in the Colca Vally, Peru. Agriculture, ecosystems & environment 50:123-131.

Dilly, Oliver. 2005. Estimating soil microbial activity, pp 114-116. In: Bloem et al., (eds.) Microbiological methods for Assessing Soil Quality. CAB international, Oxfordshire.

Doran, J.W., and T.B. Parkin. 1994. Deffining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A., (Eds.) Defining soil quality for a 14 sustainable environment. SSSA Special Publication Number 35. SSSA and ASA, Madison WI, pp. 3-22.

Enriqueta Arias, M., González-Pérez, J.A., González-Vila, F.J., and Ball, A.S. 2005. Soil health- a new challenge for microbiologists and chemists. International Microbiology 8:13-21.

Farrell, R.E., Gupta, S.R., and Germida, J.J. 1994. Effect of cultivation on the activity and kinetics of arylsulfatase in Saskatchewan. Soil Biology & Biochemistry 26:1033-1040.

Girma, K., Mosali, J., Freeman, K., Raun, W., Martin, K.L., and Thomason, W.E. 2005. Forage and grain yield response to applied sulfur in winter wheat as influenced by source and rate. Journal of Plant Nutrition 28:1541-1553.

Holt, J.A., and Mayer, R.J. 1998. Changes in microbial biomass and protease activities of soil associated with long-term sugar cane monoculture. Biology and Fertility of Soils 27:127-131.

Jaggi, R., Aulkha, M., and Sharma, R. 1999. Temperature effects on soil organic sulphur mineralization and elemental sulphur oxidation in subtropical soils of varying pH. Nutrient Cycling in Agroecosystems 54:175-182.

Kertesz, M., and Mirleau, P. 2004. The role of soil microbes in plant sulphur nutrition. Journal of experimental botany 55:1939-1945.

Klose, S., Moore J., and M., T. 1999. Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biology and Fertility of Soils 29:46-59.

Konopka, A.E., Miller, R.H., and Sommeres, L.E. 1986. Microbiology of the sulfur cycle, p. 23-55, *In* M. Tabatabai, ed. Sulfur in agriculture, Vol. 27.

American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI.

Ladd, J. N., 1978. Origin and range of enzymes in soil. In: Burns, R. G. (Ed), Soil Enzymes. Academic Press INC, New York, 51-96.

Masciandaro, G., Ceccanti, B., and Gallardo-Lancho, J.F. 1998. Organic matter properties in cultivated versus set-aside arable soils. Agriculture, Ecosystems and Environment 67:267-274.

Michel, C., Brugna, M., Aubert, C., Bernadac, A., and Bruschi, M. 2001. Enzmatic reduction of chromate:comparative studies using sulfate reducing bacteria. Applied microbiology and biotechnology 55:95-100.

Monokrousos, N., Papatheodorou, E., Diamantopoulos, J., and Stamou, G. 2006. Soil quality variables in organically and conventionally cultivated field sites. Soil Biology & Biochemistry 38:1282-12891.

Naseby, D.C., and Lynch., J.M. 2002. Enzymes and microorganisms in rhizosphere., pp. 109-123, *In* R. G. Burns. and R. P. Dick., (eds.) Enzymes in Soil Environment. Activity, Ecology and Application.

Navida, Y., Graeme, B., and Till, R. 2007. Effect of elemental sulfur, gypsum, and elemental sulfur coated fertilizers, on the availability of sulfur to rice. Journal of Plant Nutrition 30:79-91.

Neff, J.C., Reynolds, R.L., Belnap, J., and Lamothe, P. 2005. Multi- decadal impacts of grazing on soil physical and biogeochemical properties in southeast Utah. Ecological Applications. 15:87-95.

Nguyen, M.L., and Goh, K.M. 1994. Sulphur cycling and its implication on sulphur fertilizer requirements of grazed grassland ecosystems. Agriculture, Ecosystems and Environment 49:173-206.

Nor, Y.M., and Tabatabai, M.A. 1999. Arylsulfatase activity of microbial biomass in soils. Soil Science Society of America journal 63:569-574.

Pierla, H.J., and Tabatabai, M. 1988. Sulfur minerlization rates and ptentials of soils. Biology and Fertility of Soils 6:26-32.

Saggar, S., Bettany, J., and Stewart , J. 1981. Measurement of microbial sulfur in soil. Soil Biology &. Biochemistry 13:493-498.

Singh, B.B., and Tabatabai, M.A. 1978. Factors affecting rhodanese activity in soils. Soil Science Society of America journal 6:337-342.

Southorn, N.J. 2002. The soil structure component of soil quality under alternate grazing management strategies. Advances in Geoecology 35:163-170.

Tabatabai, M. A., 1994. Soil enzymes. In: Weaver, R.W., Angel, J. S., Bottomley, P. S. (Eds.), Methods of Soil Analysis. Part II: Microbiological and Biochemical Properties. Soil Science Society of America, Madison, pp. 775-833.

Tabatabai, M., and Singh, B.B. 1979. Kinetic parameters reaction of the rhodanese in soils. Soil Biology & Biochemistry 11:9-12.

Tabatabai, M.A., and Bremner, J.M. 1970. Arylsulfatase activity of soils. Soil Science Society of America Proceedings 34:225-229.

Tabatabai, M.A., and Singh, B.B. 1976. Rhodanese activity of soils. Soil Science Society of America journal 40:381-385.

Webb, E. C. 1992. Enzyme nomenclature 1992: recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the nomenclature and classification of enzymes. Academic Press, Inc. San Diego, CA, USA.

CHAPTER III

SULFUR AND ACTIVITIES OF ITS TRANSFORMING ENZYEMES IN PRAIRIE SOIL ECOSYSTEMS

Abstract

Understanding sulfur (S) dynamics in the soil environment is important in assessing ecosystem functions related to land use and management practices. The main objective was to evaluate the impacts of grazing and cultivation on sulfur pools and enzyme activities involved in sulfur cycling. Soils from five different management systems, including undisturbed, set-aside from cultivation (was cultivated but returned to grassland >30 yr ago), moderately grazed, heavily grazed, and cultivated with continuous winter wheat (*Triticum aestivum* L.) were evaluated. Total S, soluble S, and activities of arylsulfatase, rhodanese were determined. Principle component analysis of the tested variables revealed that organic carbon, total nitrogen, microbial biomass carbon and nitrogen and arylsulfatase activity were drivers in the soil ecosystems. Of the two S transforming processes evaluated, mineralization of organic S contributed more to S cycling than S oxidation processes. Total and soluble S contents were affected significantly by cultivation and to certain degree by grazing.

When compared to the undisturbed systems, total sulfur was significantly lower and the soluble S was significantly higher in the cultivated soils. Grazing promote or maintained S pools and the capacity of the soils to transform S to a degree similar to those in the undisturbed soils. Systems set aside from cultivation for more than 30 years allowed the soil to regain its capacity to cycle S and to evolve towards native system. However, activity of S transforming enzymes showed that 30 years of conservation did not completely erase the impact of cultivation.

1. Introduction

Sulfur is a highly reactive element for which an elaborate biogeochemical cycle has evolved with intermediate exchanges between atmospheric, aquatic and terrestrial phases of the environment. As a major macronutrient essential in all biotic components for the formation of amino acids, enzymes, vitamins and other biomolecules, sulfur plays a vital role in functions of terrestrial ecosystems (Wang et al., 2006). It has been suggested that mineralization of soil organic S involves two distinct biological pathways: oxidative or hydrolytical processes, both lead to the ultimate release of sulfate (Kinghts et al., 2001). Two enzymes; arylsulfatase and rhodanese, play crucial roles in the two respective S mineralization processes. Arylsulfatase catalyzes the hydrolysis of an anion by fission of the O-S bond and release plant available inorganic S as (SO_4^{2-}). Rhodanese catalyses the formation of thiocyanate (SCN^-) from thiosulfate and

cyanide that eventually also lead to the release of SO_4 (Tabatabai and Singh, 1976). Conversion of organic S to inorganic S is important for plant and microbial growth in a given environment because about 95% of the total soil sulfur in intensively managed grazed grassland presents as organic S (Pierla and Tabatabai, 1988).

Arylsulfatase activities are affected by different management practices such as tillage, crop rotations (Deng and Tabatabai, 1997; Klose et al., 1999), cultivation (Farrell et al., 1994), and grazing (Acosta-Martinez et al., 2003). The levels of applied sulfur fertilizers (Baligar et al., 2005), and trace elements (Al-Khafaji and Tabatabai, 1979) also affected arylsulfase activities in soil. Activities of both arylsulfatase and rhodanese were affected by continuous cropping systems (Szajdak, 1996), moisture content, and pre-incubation with glucose (Singh and Tabatabai, 1978).

Converting native grassland to long-term cropping systems reduced organic S in the soil (Wang et al., 2006).The availability and the adsorption of inorganic S compounds are affected mainly by organic mineralization rate which affected by enzyme activities (Appiah and Ahenkorah, 1989) and the concentration of metal ions (Ajwa and Tabatabai, 1995). Limited studies conducted in the evaluation of S cycling were focused on crop production soil with one of these two processes.

Thus, the objectives of this study were to evaluate the impacts of grazing and cultivation on S pools, and the activities of Arylsulfatase and Rhodanese in semiarid prairie ecosystems.

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. The treatments that were sampled for this study were: Undisturbed (UD), no grazing or cultivation for more than 50 years; abandoned (AB), set aside from cultivation for at least 30 years and grazed since 1996; moderate grazing (MG), 25 animal unit days per hectare (AUD ha^{-1}); heavy grazing (HG), 50 AUD ha^{-1} ; and cultivated with continuous winter wheat (*Triticum aestivum* L.)(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 the treatments many of the herbaceous plants were in bloom.

2.2. Sampling and analysis

Soil sampling procedure was reported by Katsalirou (2006). Briefly, nine plots (71x71m, 0.5 ha) were randomly selected for each treatment to serve as field replications. Within each plot, a composite sample (35 to 45 cores, 0 to 10 cm depth) was taken along the diagonal of the plot. Fresh soils were sieved (2 mm sieve) and mixed thoroughly. A portion of the soil was air-dried for chemical analysis. Another portion of the field-moist soil was stored in sealed plastic bags at 4°C for biochemical and microbiological analysis. Soil moisture content was determined gravimetrically after drying at 105°C for 48 h. All analysis were conducted in duplicate and results are expressed on moisture-free basis.

Activities of arylsulfatase was measured by the method of Tabatabai and Bremner (1970a), which is based on colorimetric determination of *p*-nitrophenol released by arylsulfatase activity when 1 g of soil was incubated with potassium *p*-nitrophenyl sulfate in acetate buffer at pH 5.8 and toluene for one hour at 37ºC. After incubation, NaOH was added to stop the reaction and $CaCl_2$ was added to prevent dispersion of clay and extraction of soil organic matter during the extraction for p-nitrophenol released. The released *p*-nitophenol was quantified by measuring the yellow color intensity at 415 nm using a spectrophotometer. The control was performed by the same procedure as for arylsulfatase activity, but the substrate, *p*-nitrophenyl sulfate solution, was added after the enzymatic reaction was stopped by the addition of NaOH.

Rhodanese activity was measured by the method of Tabatabai and Singh, (1976), which involves the incubation of 4 g soil with THAM buffer, toluene, $Na_2S_2O_3$ and KCN at 37ºC for 1 hour. After incubation, a $CaSO_4$–formaldehyde

solution was added to stop the reaction. Following filtration of the suspension, a ferric nitrate reagent was added and the reddish brown color developed was measured at 460 nm with a spectrophotometer.

Available S, water soluble and adsorbed SO_4^{2-} was measured using a method of Dick & Tabatabai (1979). Briefly, soil was extracted using 500 ppm P as $Ca(H_2PO_4)_2$ by shaking 10 g soil with 50 ml of extractant for 1 h and filtered with Whatman no.42 filter paper. The available S in the filtrates was quantified by inductively coupled plasma analyses (ICP). For total S, soil was digested with nitric acid and hydrogen peroxide, S in the digested solutions was quantified with ICP (Dick and Tabatabai, 1979).

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), and total N (N_t) with Kjeldahl digestion (Bremner and Mulvaney, 1982). Soil pH was determined using a combination glass electrode (soil to 0.01 M CaCl2 ratio = 1:2.5).

Soil microbial biomass carbon and nitrogen were both determined using the 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) as reported by Katsalirou (2006).

2.3. Statistical methods

Significance 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, $P \leq 0.05$) by using the general linear model procedure of the Statistical Analysis System (SAS, 1999). Correlations between soil chemical and biological properties as well as enzyme activities were calculated using Pearson correlation coefficient. The ratios between enzymes activity to microbial biomass carbon (C_{mic}) was calculated to assess the impact of treatments on metabolic activity of microbial community. The S_t to S_{sol} ratio was calculated to assess the relationship between S pools.

Multivariate analysis can be used in an environmental study involving multiple variables to reveal the drivers of the ecosystem therefore, principle component analysis (PCA) was applied to reduce the dimensionality of different chemical and microbiological variable using JMP® start statistics software (Sall et al., 2005). 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.

The obtained PC scores were plotted by Sigmaplot 9.0 (2004, Systat Software, Inc. Point Richmond, CA, USA).

3. Results

3.1. Soil properties

The soil texture varied from loam to silt loam. The pH ranged from neutral to alkaline for all treatments (Table 1). UD and MG soils had the highest C_{org} and N_t; the UD and AB soils had the highest S_t, while CL was the lowest for all total C, N, and S contents. However, cultivation affected total C and N contents differently. Contents of C_{org} and N_t in the cultivated soils were 45% and 55% of those in the UD soils, respectively. The grazed and abandoned soil ecosystems were not significantly different from the UD system in total sulfur content. The CL system had the least total sulfur content, showing 29% lower than those of the UD system (Table 1). However, opposite trends of total C, N, and S were observed across the treatments for the soluble C, N, and S pools. Soluble S accounted for 4-6% of the total sulfur in the grazed, UD and AB soils, while it accounted for 9% in the CL system. Soluble S contents in the HG soils and UD soils were not significantly different from each other, but both were significantly lower than those in the MG soils (Fig.1).

3.2. Enzymes activities

Rhodanese activities showed mixed responses to grazing and were sensitive to grazing intensity (Fig. 2). When compared to the UD soils, rhodanese activity was higher in the MG soils and lower in the HG soils. Of the soils tested, rhodanese activity was highest in the MG soils, while the lowest rhodanese activity was found in the AB soil. Activities of this enzyme in the cultivated soils were not significantly different from those of UD, HG, and AB soils.

The trends of arylsulfatase activities in soils under different management were somewhat different from those detected for rhodanese activities (Fig. 2). When compared to the UD system, arylsulfatase activity was significantly lower in cultivated and grazed soils. Cultivated soils had the lowest arylsulfase activities, which were about 25% of those found in the UD soils. Arylsulfase activities in the cultivated soil were significantly lower not only than those in the UD soils but also in the AB, MG and HG soils (Fig 2).

Table 1. Effect of different management practices on soil properties.

Soil Property	Treatments					LSD P≤0.05
	UD	MG	HG	AB	CL	
pH	7.2	7.5	7.4	7.6	7.5	0.2
Sand (%)	33	27	39	30	32	7
Silt (%)	49	51	44	51	49	6
Clay (%)	18	22	17	19	19	3
C_{org}(g C kg^{-1} soil)	21.5	20.9	17.7	15.8	9.7	3.3
N_t (g N kg^{-1} soil)	2.2	2.1	1.9	1.6	1.2	0.3
S_t (g S kg^{-1}soil)	0.60	0.52	0.53	0.60	0.43	0.14
DOC (mg C kg^{-1}soil)	96	92	106	81	117	20
N_{sol} (mg N kg^{-1} soil)	23	24	17	17	25	1
S_{sol} (mg SO_4-S kg^{-1}soil)	21	29	20	35	35	7.4
P_t (mg P g^{-1}soil)	760	703	627	594	521	134.4
P_{org} (mg P g^{-1}soil)	150	159	166	92	310	65.2

[1] UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: highly grazed; CL; Cultivated with winter wheat. C_{org}: Organic carbon; N_t: Total nitrogen; S_t: total sulfur; S_{sol}: soluble sulfur; DOC: Dissolve organic carbon.

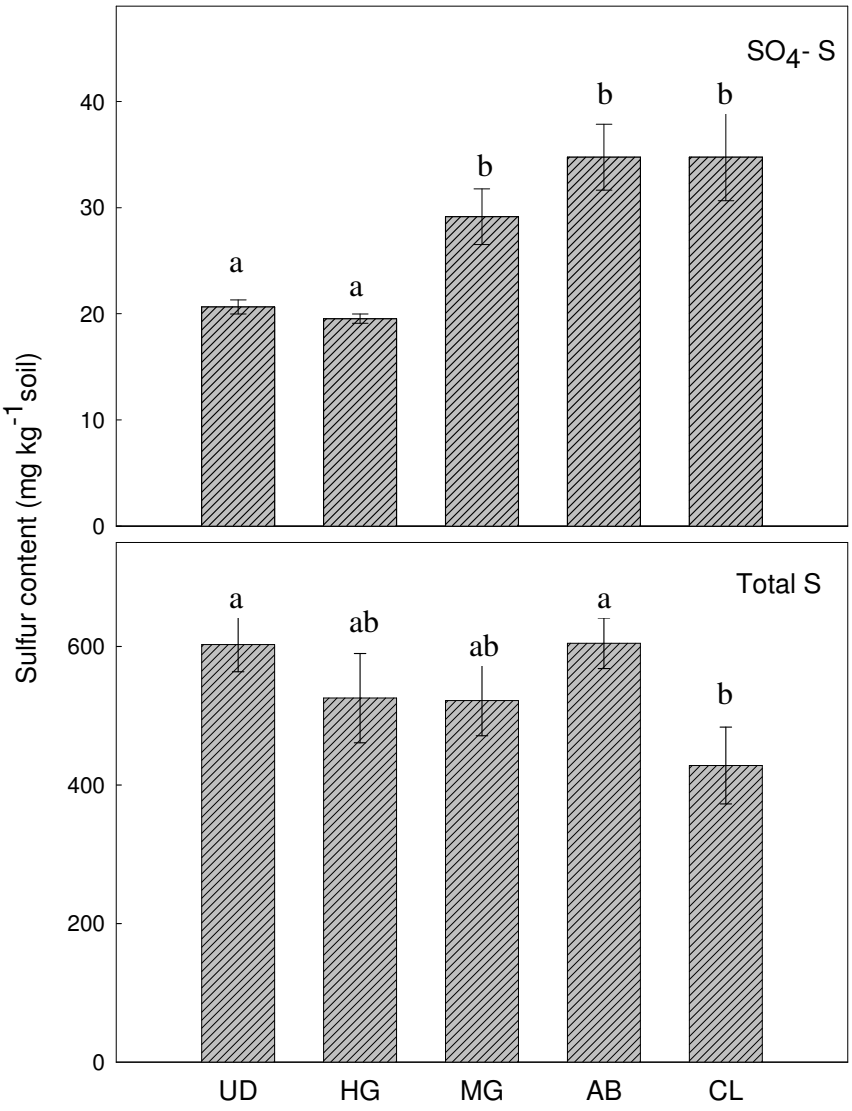

Fig. 1. Effect of land use and management practices on the size of soil sulfur pools. UD: Undisturbed; HG: Highly grazed; MG: Moderately grazed; AB: Abandoned from cultivation; CL: Cultivated with winter wheat. Columns are means ± standard error. Different letters indicate significantly different means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

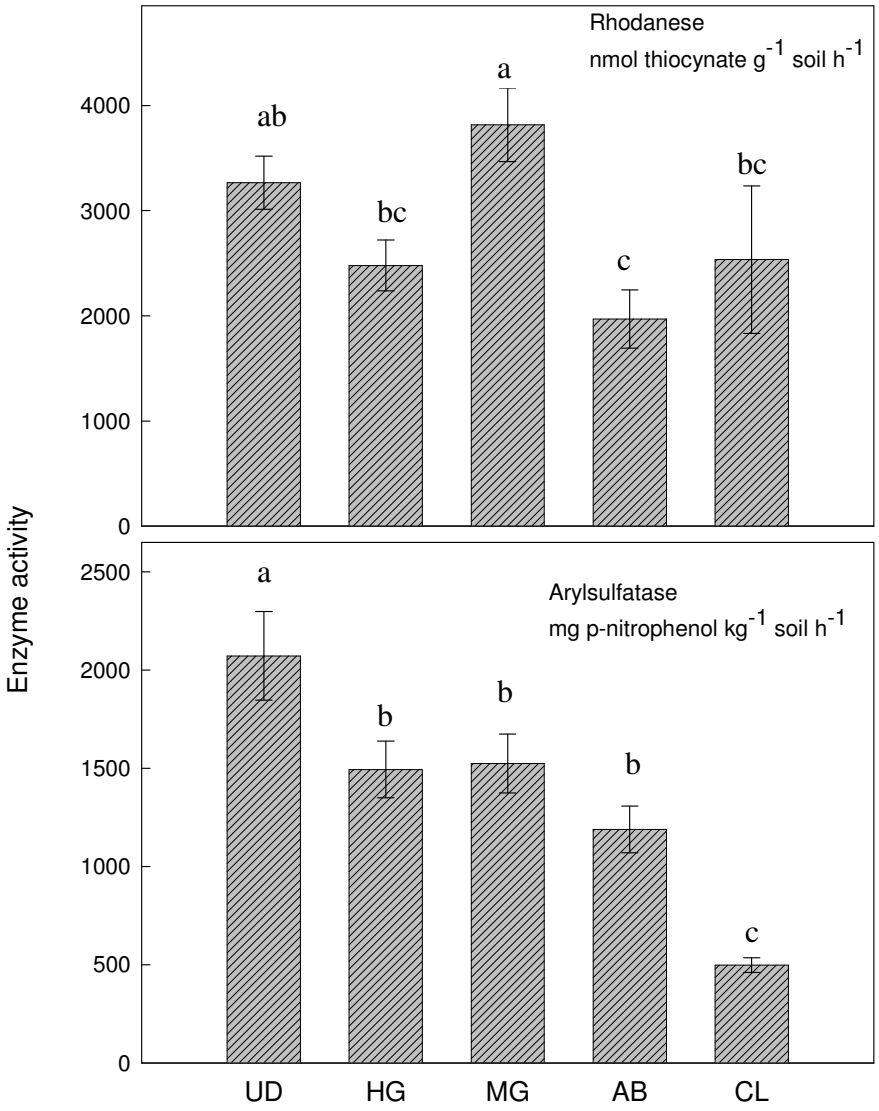

Fig. 2. Effect of land use and management practices on activities of sulfur transforming enzymes. UD: Undisturbed; HG: Highly grazed; MG: Moderately grazed; AB: Abandoned from cultivation; CL: Cultivated with winter wheat. Columns are means ± standard error. Different letters indicate significantly different means according to least significant difference test (n=9, $P \leq 0.05$).

3.3. Relationship between soil chemical and microbiological parameters

In a soil system, the measured soil parameters interwove, exhibit complex relationships. Ratios of two measured parameters, Pearson correlation coefficients between two variables, and principle component analysis of multiple variables were employed to reveal insights into their relationships.

Results from this study showed that soil C to N ratio among all systems was the lowest in the cultivated soils and highest in the HG soils. Soil C to S ratio was lowest in the AB soils and highest in the MG soils. The ratios of soluble S content to soil organic carbon in the CL soils was 0.004, which was four times of the ratio in the UD soil. The higher soluble S content and lower total S in the CL soils led to a significantly higher S_{sol} to S_t and $S_{so}l$ to C_{org} ratios for these soils compared to all other soils tested (Table 2).

The ratio of arylsulfatase to rhodanese activity ranged from 0.49 to 11.2, and was highest in the UD soils and lowest in the CL soils. When compared to UD system, cultivation led to significant increase of the rhodanese activity per unit of C_{mic}, while opposite trend for the ratio of arylsulfatase activity per unit of C_{mic} was observed where the lowest ratio was found in the cultivated soils (Table.2).

Pearson correlation coefficients showed that the activity of S- transforming enzymes was positively correlated with each other. Both enzymes activities were significantly and positively correlated with C_{org} and N_t. Rhodanese activity showed little correlation with microbial biomass, while Arylsulfatase was

significantly and positively correlated with microbial biomass (C_{mic} and N_{mic}). With respect to total and soluble S, these two enzymes showed opposite trends. Rhodanese activity showed little correlation with soluble S but significantly and negatively correlated with total S content. Arylsulfatase was significantly and negatively correlated with S_{sol}, but showed little correlation to S_t (Table 3).

Results from multivariate PCA for different variables evaluated showed that factor I accounted for 59% of the total variance and was loaded by organic C, total N, microbial biomass C, microbial biomass N content, and arylsulfatse activity while factor II accounted for 23% of the total variance and was loaded with total sulfur content and the activity of rhodanese (Table.4). When principal scores of soil variables tested were plotted against different management systems, grazing and cultivation altered factor I loadings significantly, but not factor II loadings (Fig.3). In the UD system, PC1 values averaged around a positive 1.99. As grazing intensity increased, the positive PC values shifted toward zero. The PC1 values were negative for AB and CL systems, averaging around negative 2.95 for the CL soils.

Table 2. Elemental and eco-physiological ratio between selected soil properties, enzyme activity ratio and ratio of enzyme activities to microbial biomass carbon

Ratio[1]	Treatments[2]					LSD P≤0.05
	UD	MG	HG	AB	CL	
C_{org}:N_t	9.7	9.6	9.8	9.3	7.9	0.9
C_{org}: S_t	37.9	43.5	37.3	28.3	29.4	14.9
S_{sol}:C_{org}	0.001	0.001	0.001	0.002	0.004	0.0006
S_{sol}:S_t	0.04	0.06	0.05	0.06	0.11	0.05
Aryl:Rhod	11.2	0.75	1.16	1.10	0.49	1.60
Rhod:C_{mic}	0.3	0.3	0.3	0.2	1	0.4
Aryl:C_{mic}	3.4	0.2	0.3	0.3	0.3	0.3

[1] C_{org}; Organic C; N_t: Total N; S_t: Total S; S_{sol}: soluble S. C_{mic}; Microbial biomass C; Aryl: Arylsulfatase; Rhod: Rhodanese. All ratios was calculated based in the same unit as shown in table 1 except for the ratios that included enzymes activity were mg of substrate per kg of soil unit was used.[2] UD: Undisturbed; AB: Abandoned from cultivation; MG: Moderately grazed; HG: Highly grazed; CL: Cultivated with winter wheat.

Table 3. Correlation coefficient (*r*) of the liner relationship between S-transforming enzyme activities and soil properties (n=45)*

Variable	C_{org}	N_t	DOC	S_t	N_{sol}	S_{sol}	C_{mic}	N_{mic}	Rhod
Rhodanese	.42**	.32*	-0.16	-.55**	0.21	0.23	0.21	0.15	
Arylsulfatase	.50**	.51**	-0.73	0.19	0.17	-.35*	.34*	.41**	0.2

*C_{org}: Organic C; N_t: total N; S_t: Total S; S_{sol}: Soluble S; P_t: Total P; N_{sol}: soluble N; C_{mic}; Microbial biomass C; N_{mic}: Microbial biomass N; DOC: Dissolve organic C; Aryl: Arylsulfatase; Rhod: Rhodanese. *P≤0.05, **P≤0.01.

Table 4. Factor loadings of soil parameters tested and enzymes activity involved in S- transformation.

Variables	Factor I	Factor II
Arylsulfatase	**0.57**	0.04
Rhodanese	0.33	**0.89**
Organic C	**0.97**	0.12
Total N	**0.98**	0.02
Total S	0.34	**-0.87**
Microbial biomass C	**0.92**	-0.06
Microbial biomass N	**0.93**	-0.15
Eigenvalue	4.15	1.59
Explained Variance (%)	59%	23%

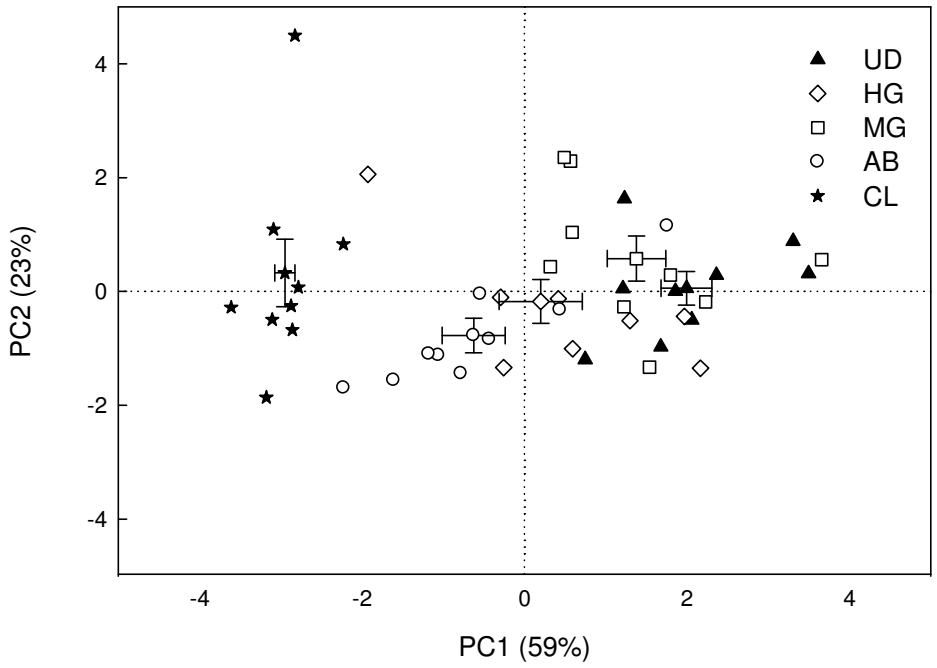

Fig. 3. Factor scores of soil chemical, microbiological properties and S-transforming enzyme activities against different management practices. UD: Undisturbed; HG: Highly grazed; MG: Moderately grazed; AB: Abandoned from cultivation; CL: Cultivated with winter wheat.

4. Discussion

4.1. Soil chemical and microbial properties

It has long been recognized that long -term repeated cultivation reduce soil structural stability and changes the distribution of soil organic matter (SOM) (Parton et al., 2005; Malo et al., 2005). Cultivation resulted in significant reduction in soil organic C, total N, and total S (Caldwell et al.; 1999; Warlrop et al.; 2000; Haynes and Goh, 1980; Masciandaro et al.; 1998). In this study, this reduction was 55% for organic carbon, 45% for total nitrogen, but only 29% for total sulfur. Reducing the residue and disturbing soil C equilibrium through cultivations may led to changes in availability of C and other nutrients to the microbial community, which may suppress and cause changes in the microbial community structure and growth.

Although long-term cultivation led to significant reduction of soil organic C, total N, and total S, it increased concentrations of dissolved organic C, soluble N, and soluble S significantly. This led to increase the percentage of soluble C to total C, soluble N to total N, and soluble S to total S. Tabatabai and Bremner (1972) found that soluble sulfur (sulfate sulfur) was about 1.3% to 15% of the total S in 37 Iowan soils. Soluble S in soils tested in this study accounted for 4 to 11% of the total S, which is in the range of Iowan soils reported. In this study, soils were sampled in late spring, which may explain the high soluble nutrients

content in the cultivated soil. According to Haynes (2005), the soluble form of different nutrients increase in winter cereal cultivated soils from spring to summer, while in rangeland it is higher in late summer and early fall.

Grazing can enhance or promote plant growth by returning nutrients back to the soil as organic waste (Haynes, 1993). In this study, soil S levels in grazed systems were similar to those in the undisturbed systems, indicating that removal of S from the system by animals and plants was accompanied with addition of materials that contain higher S content to the soil. These result showed interrelationships between different processes that may controlled S concentration in soils. The higher the grazing intensity (more animal unit /ha/day) the more S that is consumed by animals that feed on plants and the more S content that can be accumulating in the soil as organic form. Total S in AB soils was not significantly different form those in grazed and undisturbed soil. Removing soils from cultivation and introducing it to grazing since 1996 enhanced the ability of the soils to evolve and restore its total nutrients content. However, grazing at high intensity reduced S_{sol} pool significantly when compared to the moderately grazed system and that could be related to the slow mineralization of S in the dung and possibly leaching of released SO_4 (Mathews, 1994). Set aside from cultivation for 30 years resulted in higher soil microbial and chemical property values when compared to the cultivated soil. This could be an indication that soils under intensive cultivation can recover and evolve toward native soil (Masciandaro, 1998). Set aside from cultivation promoted organic

matter build up and led to increase in microbial biomass content (Wali et al., 1999., Zak et al., 1990., sparling et al., 1994).

4.2. Enzyme activities

In general, the activities of sulfur transforming enzymes in the undisturbed and moderately grazed soils were higher than those in AB and CL systems. However, management practices affected rhodanese and arylsulfatase activity differently. The lower enzyme activities in the cultivated soil were accompanied by lower contents of total S, organic C, and total N. Deng and Tabatabai (1997) reported that different soil properties could work interdependent. Relatively low nutrient levels in the cultivated soils supported lower levels of microbial life, which led to lower rhodanese activity when compared with UD and MG soils.

Rhodanese activity did not show a clear trend in the grazed soils. Rhodanese is involved in the oxidization process of inorganic S to release available S to plant and micros and that was affected by grazing processes.

Of ecosystems evaluated, moderately grazed system had the highest rhodanese activity. However, effect of grazing on activity of this enzyme was not consistent. Rhodanese activity in the HG soils was not only significantly lower than those in the MG soils, but also lower than the UD soils. Studies showed that continuous grazing reduced vegetation cover, which led to increased erosion and loss of organic C and N contents as well as microbial activity (Fuhlendorf et al., 2002; Su et al., 2005; Zhao et al., 2007). Grazing at high intensity decreased root

biomass and increased mineralization rates that would also lead to the decrease in organic C contents (Holland and Delting, 1990; Mawdsley and Bardgett, 1997). The low rhodanese activity in the AB soils indicated that it takes a long time to restore biological activity following abandonment from cultivation. Fuhlendorf et al. (2002) reported that restoration of native prairie soils required decades to restore organic matter and nutrient contents. Suggesting that 30 years abandoned from cultivation is not enough to restore biological activity in prairie soils.

Of the soils tested, the significantly lower arylsulfatase and relatively low rhodanese activities in the CL soils could be attributed to lack of plant residue cover during winter and spring period (Acosta-Martines et al., 2003; Acosta-Martines et al 2007; Bandick and Dick 1999; Gupta and Germida 1988), Lack of S fertilizers affect enzymes activity. As reported by Baligar 2005; high levels of applied S stimulated higher arylsulfatase activity. Arylsulfatase reduction in the cultivated soils was accompanied by reductions in organic C, total N, total S, and microbial biomass C and N. Deng and Tabatabai (1997) suggested that soil organic matter plays an important role in maintaining soil enzymes activity. Although cultivation reduced arylsulfatase activity, a higher level of available S was found in the cultivated soils. Because the Aryl:C_{mic} ratios were similar in the cultivated and grazed soils, the results suggested that sulfur consumption in the cultivated soils was slow.

Arylsulfatase activity in AB soils was not significantly different from those in the grazed soils, suggesting that conservation helped restoring nutrients and the ability of soil to support microbial life.

The reduction of total S was accompanied with the reduction of arylsulfatase activity and with an increase in available S content. On the other hand, rhodanese activity was significantly and negatively correlated with total S content. However the increase of rhodanese activity was accompanied by an increase of available S content. These results suggested that rhodanese reactions are regulated by the substrate availability, while arylsulfatase reactions are regulated by the end product concentrations (feedback inhibition).

4.3. Relationship between soil chemical and microbiological parameters

Cultivation led to lower ratios of C_{org} to N_t and C_{org} to S_t, indicating that the loss of C was greater than the loss of N and S upon cultivation. On the other hand, grazing, especially at moderate intensity, led to high C: S ratios. Compared to C, S was more slowly but restored more rapidly in the soil ecosystems. The relatively stability of S compared C could suggest that C is more limiting in the soil ecosystems, especially under cultivation. This is further evidenced by organic C being one of main loading factors for PC1.

Ratios between enzymes may indicate substrate composition in soil (Caldwell et al., 2005). Higher arylsulfatase to rhodanese ratio in the UD soils indicated higher organic sulfur to inorganic form in the system. This is further

supported by data showing that UD soils had the highest total S but lowest soluble S in soil systems evaluated. Soluble S in soil is dominated by sulfate-S (Tabatabai, 1982).

Enzyme activity per unit of microbial biomass provides an indication of microbial metabolic activity. In this study, the arylsulfatase to microbial biomass C ratio in the UD soils was 10 fold of those found in all other soils tested. This suggests that production of arylsulfatase by microbial biomass was enhanced by its relatively high substrate (organic S) content. On the other hand, the synthesis of rhodanese activity was enhanced by the presence of high inorganic S in the cultivated soils, resulting ratios of this enzyme to microbial biomass C in the CL soils 3-5 folds of those found in other soils tested.

Both rhodanese and arylsulfatase activities were correlated with organic carbon and total N contents, as reported in previous studies (Singh and Tabatabai, 1978; Deng, 1990; Tabatabai and Bremner 1970; Deng and Tabatabai 1997; Klose and Tabatabai 1999; Chang et al., 2007). Soil organic matter plays an important role in maintaining soil enzymes activity (Deng and Tabatabi, 1997). The positive correlations between arylsulfatase activity and total S content, and the significantly negative correlations between this enzyme and soluble S form were consistent with other studies as well (Tabatabai and Bremner 1970a; Baligar et al., 2005). The observed phenomenon could indicate that synthesis of arylsulfatase by microorganisms was inhibited by the end product concentrations of high soluble S content. Rhodanese and arylsulfatse

activities are involved in different S transforming processes. It is, therefore, not surprising that activities of these two enzymes were not correlated.

According to PCA of all variables, grazing and cultivation significantly altered factor 1 loading variables, including C_{org}, N_t, C_{mic}, N_{mic} and arylsulfatase activity, but not factor 2 loading variables of S_t content and rhodanese activity. The shift of factor I variables toward the negative PC1 values in CL soils suggested limitations of organic carbon, total nitrogen, and microbial biomass contents. The limited contribution of rhodanese activity to total variance and little impact of management practices on its PC2 values suggested that arylsulfatase activity contributed more to sulfur cycling in soil than rhodanese did. As cultivation led to limitation of arylsulfatase activity, this would result in limited functional capacity for cultivated soils to cycle S.

4.3. Conclusion

Principle component analysis of the tested variables revealed that organic carbon, total nitrogen, microbial biomass carbon and nitrogen and arylsulfatase activity were drivers in the soil ecosystems. Of the two S transforming processes evaluated, mineralization of organic S contributed more to S cycling than did the S oxidation processes. Total and soluble S contents as important nutrient pools or reservoir were affected significantly by cultivation and to certain degree by grazing. When compared to the undisturbed systems, total sulfur was significantly lower and the soluble S was significantly higher in the cultivated

soils. Grazing promote or maintained S pools and the capacity of the soils to transform S to a degree similar to those in the undisturbed soils. Systems set aside from cultivation for more than 30 years allowed the soil to regain its capacity to cycle S and to evolve towards the native system. However, activity of S transforming enzymes showed that 30 years of conservation did not completely erase the impact of cultivation.

References

Acosta-Martinez, V., and Daren Harmel, R. 2006. Soil microbial communities and enzymes activities under various poultry litter application rates. Journal of Environmental Quality 35:1309-1318.

Acosta-Martinez, V., Klose, S., Zobeck, T.M., and Kennedy, A.C. 2003. Enzyme activities in semiarid soils under conservation reserve program, native grassland, and cropland. Journal of Plant Nutrition and Soil Science 166:699-707.

Acosta-Martinez, V., Cruz, L., Sotomayor-Ramı´rez, D., and Pe´rez-Alegrı´a, L. 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. Applied Soil Ecology 35:35–45.

Ajwa, H.A., and Tabatabai, M.A. 1995. Metal – induced sulfate adsorption by soils.II: Effect of metal type, valence, and concentration. Soil Science 160:281-290.

Al- Khafaji, A.A., and Tabatabai, M.A. 1979. Effect of trace elements on arylsulfatase activity in soils. Soil Science 127:129-133.

Appiah, M.R., and Ahenkorah, Y. 1989. Determination of available sulfate in some soils of Ghana using five different extraction methods. Biology and Fertility of Soils 8:80-89.

Baligar, V.C., Wright, R.J., and Hern, v. 2005. Enzyme Activities in Soil Influenced by Levels of Applied Sulfur and Phosphorus. Communications in Soil Science and Plant Analysis 36:1727-1735.

Bandick, A.K., and Dick, R.P. 1999. Field management effects on soil enzyme activities. Soil Biology & Biochemistry 31:1471-1479.

Bremner, J.M., Mulvaney, C.S., 1982. Total Nitrogen. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed. Agronomy Monograph No. 9. ASA and SSSA, Madison, WI, pp. 595-624.

Brookes, P.C., Landman, A., Pruden, G., and Jenkinson, D.S. 1985b. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17:837-842.

Caldwell, B.A. 2005. Enzyme activities as a component of soil biodiversity: A review. Pedobiologia 49:637-644.

Caldwell, B.A., Griffiths, R.P., and Sollins, P. 1999. Soil enzyme response to vegetation disturbance in two lowland Costa Rican soils. Soil Biology & Biochemistry 31:1603-1608.

Chang, E.-H., Chung, R.-S., and Tsai, Y.H. 2007. Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. Soil Science and Plant Nutrition 53:132-140.

Deng, S. 1990. Sulfur oxidation and rhodanese activity in soils. M.S. Thesis, Oregon State University, Corvallis, OR, USA.

Deng, S., and Tabatabai, M. 1997. Effect of tillage and residue management on enzyme activities in soils: III phosphatases and arylsulfatase. Biology and Fertility of Soils 42:141-146.

Dick, W.A., and Tabatabai, M.A. 1979. Ion chromatographic determination of sulfate and nitrate in soils. Soil Science Society of America Journal 43:889-904.

Farrell, R.E., Gupta, S.R., and Germida, J.J. 1994. Effect of cultivation on the activity and kinetics of arylsulfatase in Saskatchewan. . Soil Biology & Biochemistry 26:1033-1040.

Fuhlendorf, S.D., Zhang, H., Tunnell, T.R., Engle, D.M., and Cross, A.F. 2002. Effects of grazing on restoration of southern mixed prairie soils. Restoration Ecology 10:401-407.

Gupta, V.V.V.R., and Germia. 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biology &Biochemistry 20:777-786.

Gee, G.W., Or, D., 2002. Particle-size analysis. In: Dane, J.H., Topp, G.C. (Eds.) Methods of Soil Analysis. Part 4. Physical Methods. Soil Science Society of America, Madison, WI, pp. 255-293.

Haynes, R.J. 2005. Labile organic matter fractions as central components of the quality of agricultural soils: An overview. Advances in Agronomy 85:221-268.

Haynes, R.J., and Goh, K.M. 1980. Seasonal levels of available nutrients under grassed-down, cultivated and zero tilled Orchard management practices. Australian Journal of Soil Research 18:363-373.

Haynes, R.J., and Williams, P.H. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49:119-199.

Holland, E.A., and Detling, J.K. 1990. Plant response to herbivory and belowground nitrogen cycling. Ecology 71:1040-1049.

Katsalirou, E. 2006. Microbial community and enzyme activities in paririe soil ecosystems under different management. Ph.D. Dissertation, Oklahoma State University, Stillwater. OK, USA.

Klose, S., Moore J., and Tabatabai, M., 1999. Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biology and Fertility of Soils 29:46-59.

Knights, J.S., Zhao, F.J., McGrath, S.P., and Magan, N. 2001. Long-term effects of land use and fertilizer treatments on sulphur transformations in soils from Broadbalk experiment. Soil Biology & Biochemistry 33:1797-1804.

Szajdak,L. 1996. Impact of crop rotation and phenological periods on rhodanese activity and free sulfuric amino acids concentration in soils under continuous rye cropping and crop rotation. Environment International 22(5):563-569.

Malo, D.D., Schumacher, T.E., and Doolittle, J.J. 2005. Long-term cultivation impacts on selected soil properties in the northern Great Plains. Soil & Tillage Research 81:277-291.

Masciandaro, G., Ceccanti, B., and Gallardo-Lancho, J.F. 1998. Organic matter properties in cultivated versus set-aside arable soils. Agriculture, Ecosystems and Environment 67:267–274.

Mathews, B.W., Sollenberger, L.E., Nair, D.V., and Staples, C.R. 1994. Impact of grazing management on soil nitrogen, phosphours, potassium and sulfur distribuation. Journal of Environmental Quality 23:1006-1013.

Mawdsley, J.L., and Bardgett, R.D. 1997. Continuous defoliation of perennial ryegrass (Lolium perrene) and white clover (Trifolium repens) and associated changes in the composition and activity of the microbial population of an upland grassland soil. Biology and Fertility of Soils 24:52-58.

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed. Agronomy Monograph No. 9. ASAand SSSA, Madison, WI, pp. 539-579.

Pierla, H.J., and Tabatabai, M. 1988. Sulfur minerlization rates and ptentials of soils. Biology and Fertility of Soils 6:26-32.

Sall, J., L. Creighton, and A. Lehman. 2005. JMP® start statistics, 3rd ed. Thompson/Brooks/Cole, Belmont, CA.

Singh, B.B., and Tabatabai, M.A. 1978. Factors affecting rhodanese activity in soils. Soil Science Society of America Journal 6:337-342.

Sparling , G.P., Brandenburg A. S., and Zhu C. 1994. Microbial C and N in revegetated wheatbelt soils in Western Australia: Estimation in soil, humus and leaf-litter using the ninhydrin method. Soil Biology & Biochemistry 26:1179-1184.

Su, Y.Z., Li, Y.L., Cui, J.Y., and Zhao, W.Z. 2005. Influnces of continous grazing and livestock exclusion on soil properties in a degraded sandy grassland, Inner Mongolia, northern China. Catena 59:267-278.

Tabatabai, M.A. 1982. Sulfur. *In* A.L. Page et al. (ed) Methods of soil analysis, Part 2. 2nd ed. Agronomy 9:501-538.

Tabatabai, M.A., and Bremner, J.M. 1970. Arylsulfatase activity of soils. Soil Science Society of America Proceedings 34:225-229.

Tabatabai, M.A., and Bremner, J.M. 1972. Forms of sulfur and carbon, nitrogen and sulfur relationships, in Iowa soils. Soil Science 114:380-386.

Tabatabai, M.A., and Singh, B.B. 1976. Rhodanese activity of soils. Soil Science Society of America Journal 40:381-385.

Vance, E.D., Brookes, P.C., and Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19:703-707.

Waldrop, M.P., Balser, T.C., and Firestone, M.K. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32:1837-1846.

Wali, K.M. 1999. Ecological succession and the rehabilitation of disturbed terrestrial ecosystems. Plant and Soil 213:195-220.

Wang, J., Solomon, D., Lehmann, J., Zhang, X., and Amelung, W. 2006. Soil organic sulfur forms and dynamic in the Great Plains of North America as influenced by long term cultivation and climate. Geoderma 133:160-172.

Zak, D.R., Grigal, D.F., Glesson, S., and Tilman, D. 1990. Carbon and nitrogen cycling during old field succession: constrains on plant and microbial biomass. Biogeochemistry 11:111-129.

Zhao, Y., Peth, S., Kru¨ mmelbein, J., Horn, R., Wang, Z., Steffens, M., Hoffmann, C., and Peng, X. 2007. Spatial variability of soil properties affected by grazing intensity in Inner Mongolia grassland. Ecological Modeling 205:241–254

CHAPTER IV

INTERACTIONS OF CARBON, NITROGEN, PHOSPHORUS, AND SULFUR POOLS AND ENZYME ACTIVITIES INVOLVED IN THEIR TRANSFORMATION

Abstract

Understanding the interaction between different soil nutrients and their cycling processes under different management practices could assist the evaluation and development of management systems that sustain and enhance ecosystems functions. The objective of this study was to use multivariate analysis to reveal the interrelationships of carbon (C), nitrogen (N), phosphorous (P), and sulfur (S) cycles by evaluating 11 key enzyme activities involved in transforming these nutrients under different management practices. Forty-five soils were taken from five long-term (more than 30 years) treatments, including undisturbed, set-aside from cultivation, moderately grazed, heavily grazed, and winter wheat (*Triticum aestivum* L.). Nutrient pools evaluated include total, soluble, and microbial forms. Of micronutrients tested, B and Mg were most limiting in these systems. Of macronutrients evaluated, C_{org}, C_{mic}, N_t, and N_{mic} were more limiting in the cultivated than in the uncultivated soils. Although there

is some indication that mineralization of organic S is a key process that governs ecosystem functions, there was generally no clear trend that one nutrient transforming enzyme or process was more dominating than other enzyme activities or processes in nutrient transformation. However, the capacity of soil enzymes to release simple sugar and inorganic nutrients appeared to be key factors regulating nutrient cycling, suggesting microbial biomass was the driver of C, N, P, and S transformation processes of all variables evaluated.

1. Introduction

Nutrient cycling in soils is a continual process of biological-geological-chemical transformation that constantly changes nutrients from one form to another. Microbiological and biochemical activities are often the driving force for nutrient cycling, and thus control the size of nutrient pools in soil. Soil enzyme activities are the driving force in nutrient cycling therefore; have also been suggested as indicators in detecting changes or disturbances of the soil ecosystem (Naseby and Lynch, 2002).

Management practices such as cultivation, fertilization, and grazing affect C, N, P, and S cycling in soils differently, as it causes different degrees of soil disturbance and different effects on soil ecosystem functions (Dodor and Tabatabai, 2003; Green et al., 2007; Klose et al., 1999; Doran and Parkin, 1994). Long term cultivation and continuous grazing decrease soil organic matter content, total N, and available phosphorus (Su et al., 2005; Malo et al., 2005;

Parton et al., 2005; Neff et al., 2005; Jaiyeoba, 2003; Davinson and Ackerman, 1993). Continuous cultivation reduced enzymes activity such as arylsulfatase, β-glucosaminidase and β-glucosidase in semiarid agricultural soils compared to conserved fields and integrated crop-livestock systems (Acosta-Martinez et al., 2003; Acosta-Martinez et al., 2004). Low organic matter content in the semiarid environments can led to progressive degradation of their quality and productivity (Caravaca et al., 2002). Leaving crop residue on the soil surface improves nutrient cycling and, ultimately, soil quality that will increase and sustain soil productivity (Al-Kaisi et al., 2005). Incorporation of canola residue into nutrient poor sandy soil did not affect C and S cycling, but altered N cycling (Singh et al., 2006). Cultivation and intensive grazing reduced microbial biomass C (Holt and Mayer, 1998; Saviozzi et al., 2001; Sankaran and Augustine., 2004). On the other hand, grazing in mixed-grass rangelands led to increased levels of soil C and N through enhanced incorporation and decomposition of the litter and standing dead plant material (Schuman et al., 1999), and exclusion from grazing in grassland reduced microbial biomass C in the soil (Bardgett et al., 1997).

Past research effort has been focused on individual nutrient cycling, while little is known about the relative importance and interactions among nutrients and their transformation processes. Understanding the drivers and interactions of an ecosystem would assist in developing management practices that enhance the functioning capacity of a soil ecosystem. Therefore, the objective of this study was to reveal the drivers of soil ecosystems and to understand the interactions

and relationships of four major nutrient cycles by evaluating their key enzyme activities using multivariate analysis.

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. The treatments that were sampled for this study were: Undisturbed (UD), no grazing or cultivation for more than 50 years; abandoned (AB), set aside from cultivation for at least 30 years and grazed since 1996; moderate grazing (MG), 25 animal unit days per hectare (AUD ha^{-1}); heavy grazing (HG), 50 AUD ha^{-1}; and cultivated with continuous winter wheat (*Triticum aestivum* L.)(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 the treatments many of the herbaceous plants were in bloom.

2.2. Sampling and analysis

Soil sampling procedure was reported by Katsalirou (2006). Briefly, nine plots (71x71m, 0.5 ha) were randomly selected for each treatment to serve as field replications. Within each plot, a composite sample (35 to 45 cores, 0 to 10 cm depth) was taken along the diagonal of the plot. Fresh soils were sieved (2 mm sieve) and mixed thoroughly. A portion of the soil was air-dried for chemical analysis. Another portion of the field-moist soil was stored in sealed plastic bags at 4°C for biochemical and microbiological analysis. Soil moisture content was determined gravimetrically after drying at 105°C for 48 h. All analysis was conducted in duplicate and results are expressed on moisture-free basis.

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), and total N (N_t) with Kjeldahl digestion (Bremner and Mulvaney, 1982). Soil pH was determined using a combination glass electrode (soil to 0.01 M CaCl2 ratio = 1:2.5). Soil microbial biomass carbon and nitrogen were both determined using the 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) as reported by Katsalirou (2006).

Many trace elements are inhibitors or cofactors of enzymatic functions. Therefore, soil was extracted using 500 ppm P as $Ca(H_2PO_4)_2$ by shaking 10 g

soil with 50 ml of extractant for 1 h and filtered with Whatman no.42 filter paper. Extractable elemental contents, including Boron (B), magnesium (Mg), Iron (Fe), Copper (Cu), and Zinc (Zn) were determined using inductively coupled plasma (ICP). Activities of key enzymes involved in C, N, P, cycling were determined using methods reported by Katsalirou (Katsalirou, 2006). Activities of enzymes involved in S cycling were reported in chapter III.

2.3. Statistical analysis

Significant differences among treatments were determined using one-way analysis of variance. The ratio between each enzyme activity to microbial biomass carbon (C_{mic}) was calculated to assess the impact of treatments on metabolic activity of microbial community.

Principle component analysis (PCA) was applied to reduce the dimensionality of different chemical and microbiological variables. Principle component analysis was conducted using JMP® start statistics software (Sall et al., 2005). The correlation rather than covariance matrix was used because the tested soil variables were expressed in different units (Johnson, 1998). Analysis of variance for the principal component scores of the first two principal axes (PC1 and PC2) was performed to test the significance of separation between the management systems. The obtained PC scores were plotted by Sigma plot 9.0 (2004, Systat Software, Inc. Point Richmond, CA, USA).

3. Results

3.1. Nutrient pools, microbial biomass, and their interactions

As discussed in Chapter III, soil organic C, and total N, P and S contents were all highest in the UD soils and lowest in the CL soils. These nutrients contents in the grazed systems were not significantly lower than the UD soil. However, the opposite trends were observed for soluble nutrient contents, which were always the highest in CL soils compared to UD soils.

Similarly, the extractable K contents were significantly higher in the cultivated and AB systems when compared with uncultivated ones (Fig. 1). Trends for micronutrients in these systems varied depending on the nutrient element. Grazing at high intensity led to reduced availability of Mg and B, while moderate grazing actually enhanced Mg extractability. Cultivation did not show any detectable impact on the extractability of these two micronutrients, but significantly enhanced extractable Fe contents, and to certain degree, Cu contents as well. Grazing and cultivation both led to lower extracted Zn concentrations when compared with the UD system. Of the micronutrients tested, B and Mg were most discriminating among soil systems and loaded factor I, which accounted 40% of the total variance (Table 1). Factor II was loaded with Cu and Zn, and accounted for 29% of the total variance. Iron was the primary loading for factor III, which accounted for 16% of the total variance.

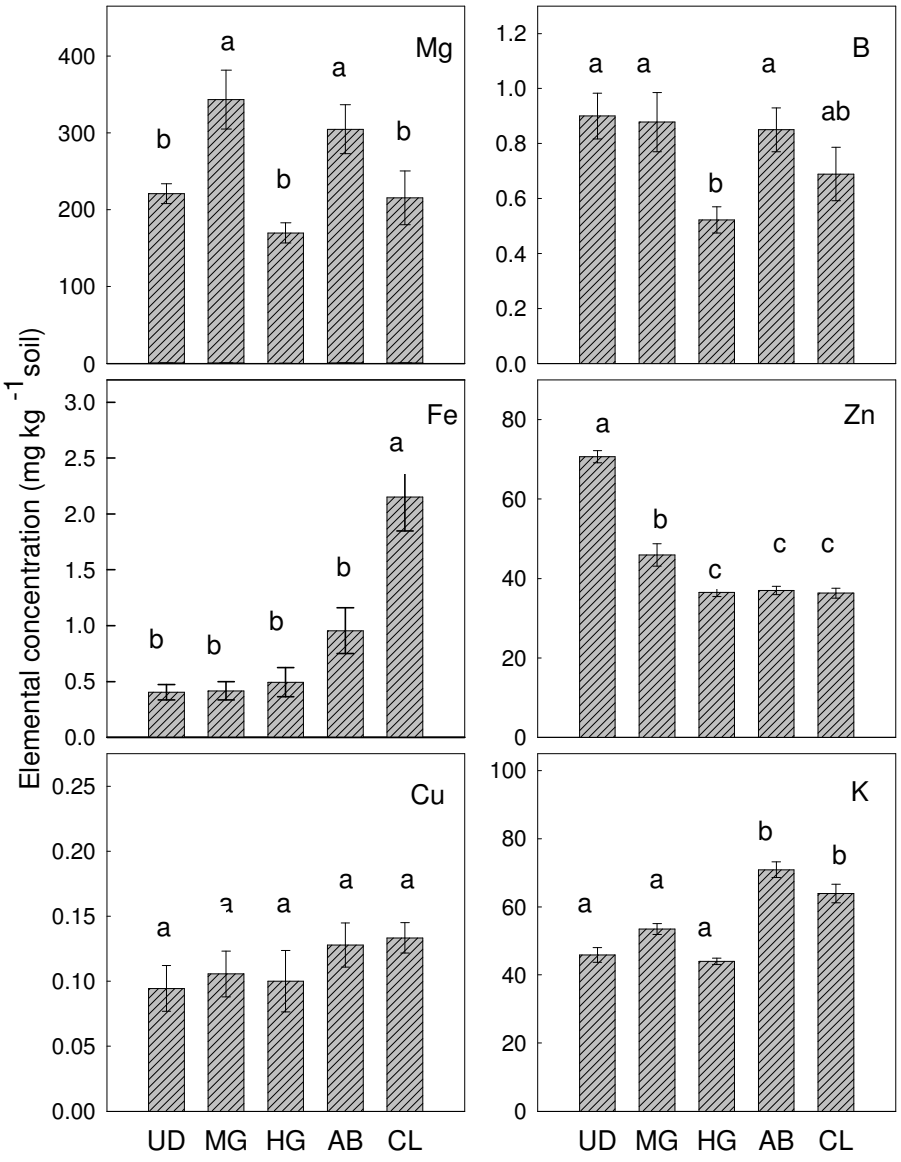

Fig 1. Treatments effect in soil elemental content. UD: Undisturbed; HG: Highly grazed; MG: Moderately grazed; AB: Abandoned from cultivation; CL: Cultivated with winter wheat. Columns are means ± standard error. Different letters indicate significantly different means according to least significant difference test (n=9, P ≤ 0.05).

Table1. Factor loadings of soil micronutrient contents.

Variable	Factor I	Factor II	Factor III
Boron	0.85	-0.35	0.04
magnesium	0.91	0.18	0.21
Iron	0.16	-0.04	0.98
Copper	0.25	-0.79	0.12
Zinc	-0.12	-0.90	-0.06
Eigenvalue	2.01	1.46	0.82
Explained Variance (%)	40%	29%	16%

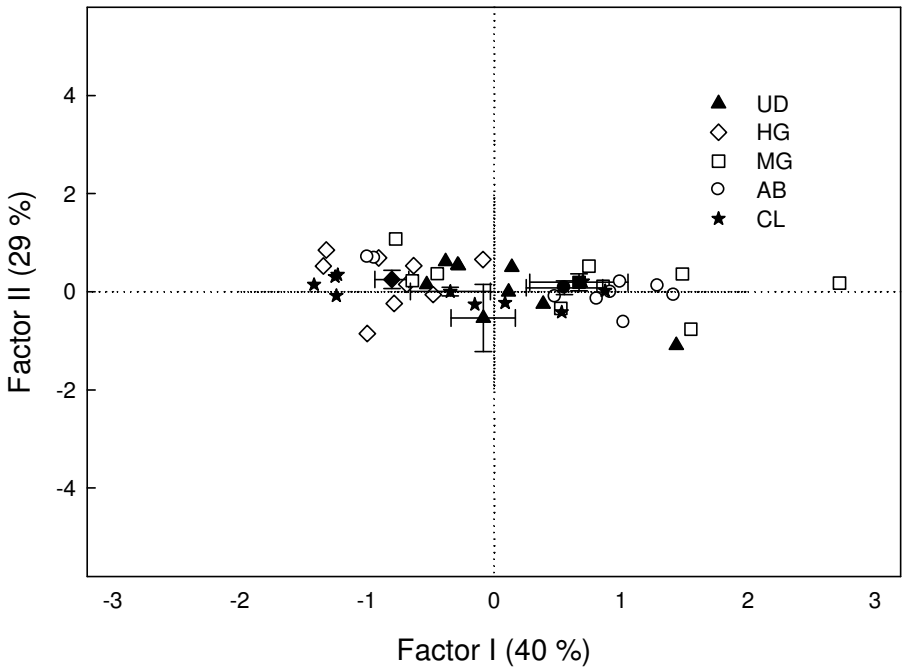

Fig 2. Factor scores of soil elemental content against different management practices . UD: Undisturbed; HG: Highly grazed; MG: Moderately grazed; AB: Abandoned from cultivation; CL: Cultivated with winter wheat.

The management practices tested affected factor I loadings, but little on factor II and factor III loadings (Fig. 2). In other words, all the systems showed close relationships to factor II and to factor III, but their relationships to factor I were shifted by management practices. Of the systems evaluated, UD and MG systems were most closely related to factor I. AB and HG systems were not only less related to factor I, but also showed opposite relationships with AB being positively and HG being negatively related to factor I.

The relative importance of major macronutrients in their total and soluble forms as well as microbial biomass was examined by multivariate analysis. Microbial biomass was included because microbes are key players in nutrient transformations and because previous studies have shown that microbial biomass was the lowest in the cultivated systems (Katsalirou, 2006). According to PCA factorial analysis, factors I, II, and III together accounted for 77 % of the total variance (Table. 2). Factor I, explained 41% of the total variance, and was loaded by organic C, C_{mic}, total N and N_{mic}. Factor II explained 24% of the total variance and was loaded by DOC, N_{sol}, and P_{org}. Factor III accounts for 12% of the total variance and was loaded by P_{inorg} and S_t. When factor scores were plotted against different management practices, AB soils were mostly related to factor I, while HG systems were closely related to factor II as well as factor III (Fig 3). Both UD and MG systems were closely related to factor II and both CL and UD systems were closely related to factor III.

Table 2. Factor loadings of soil parameters tested.

Variable	Factor I	Factor II	Factor III
Organic C	0.96	0.05	-0.07
DOC	-0.18	-0.54	-0.26
Microbial biomass C	0.93	0.17	-0.12
Total N	0.96	-0.01	-0.20
N_{sol}	0.03	-0.81	0.17
Microbial biomass N	0.90	0.15	-0.26
Total P	0.16	-0.66	-0.67
P_{inorg}	0.35	-0.21	-0.83
P_{org}	-0.22	-0.88	0.01
Total S	0.16	0.14	-0.91
S_{sol}	-0.32	-0.04	0.27
Eigenvalue	4.52	2.6	1.29
Explained Variance	41%	24%	12%

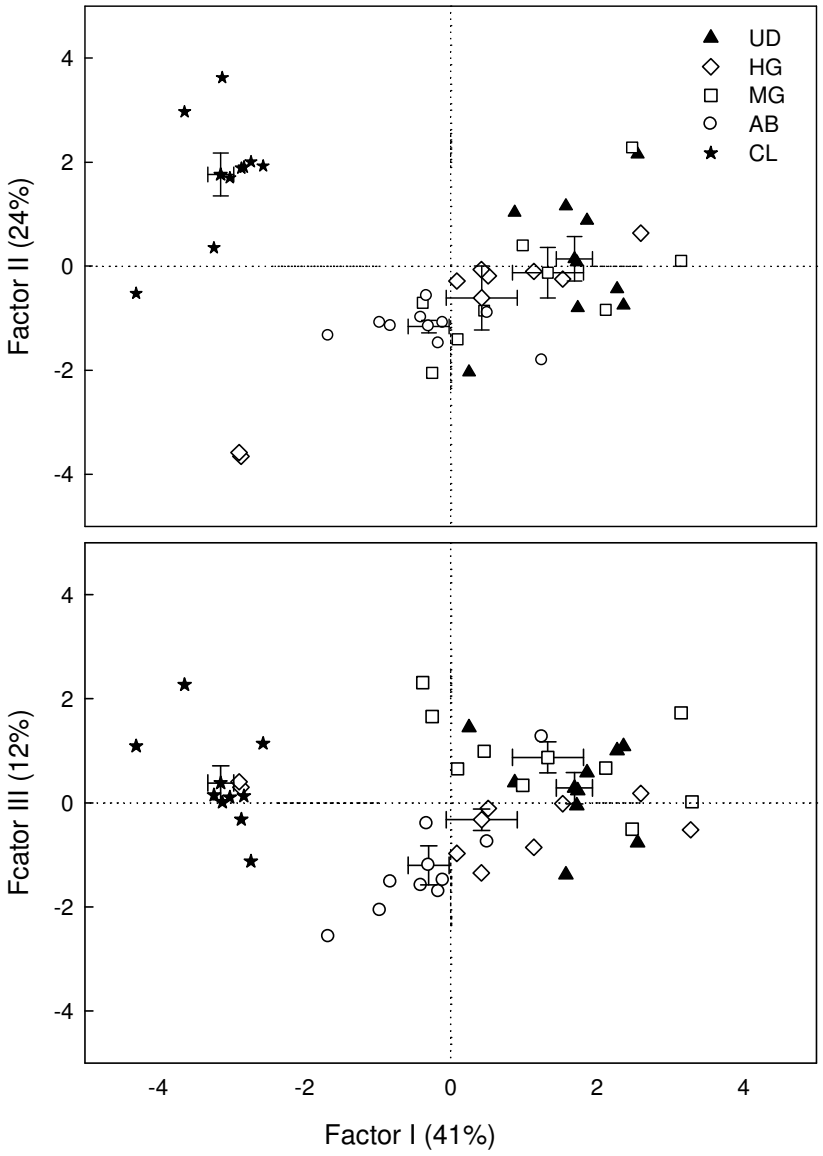

Fig 3. Factor scores of soil chemical and microbiological parameters against management practices systems. UD: Undisturbed; HG: Highly grazed; MG: Moderately grazed; AB: Abandoned from cultivation; CL: Cultivated with winter wheat.

3.2. Relationships of carbon-, nitrogen-, phosphorus-, and sulfur- transforming enzymes

Principal component analysis of 11 key enzymes involved in C-, N-, P-, and S-transformation showed that factor I explained 63% of the total variance, and factor II explained 15% of the total variance (Table 3). Factor I was loaded by activities of β-glucosidase, cellulase, urease, L-asparaginase, alkaline phosphatase, and arylsulfatase, while factor II was loaded with β-glucosaminidase, acid phosphatase, and rhodanese activity (Table 3). Management practices affected the relative importance and relationships among factor I loadings (Fig 4). There were considerable variations within the UD, HG, or MG samples, but all AB and CL soils were closely related to PC2. Although none of the systems tested showed a close relationship to PC1, the UD and grazed systems were generally on the positive side of PC1 while AB and CL were on the negative side of the PC1.

PCA factorial analysis of these enzymes within each management system was conducted to reveal whether management practices changed the relative importance and relationships among these enzymes (Table 4). Factor I and factor II within each system accounted for 74-94% of the total variance within the system. When compared with the UD system, the relative importance for some of the variables was not significantly affected by grazing or cultivation, including α-galactosidase and urease, which generally loaded factor I in all systems. On the other hand, the relative importance of many enzymes,

Table 3. Factor loadings of selected enzyme activities.

Variable	PC1	PC2
β-glucosidase	0.84	0.44
α-galactosidase	0.75	0.60
Cellulase	0.83	0.31
Urease	0.83	0.37
L-asparaginase	0.77	0.22
β-glucosaminidase	0.26	0.89
Acid phosphomonoesterase	0.23	0.88
Phosphodiesterase	0.65	0.60
Alkaline phosphatase	0.94	0.20
Arylsulfatase	0.70	-0.12
Rhodanese	0.06	0.77
Eigenvalue	6.92	1.16
Explained Variance (%)	63	15

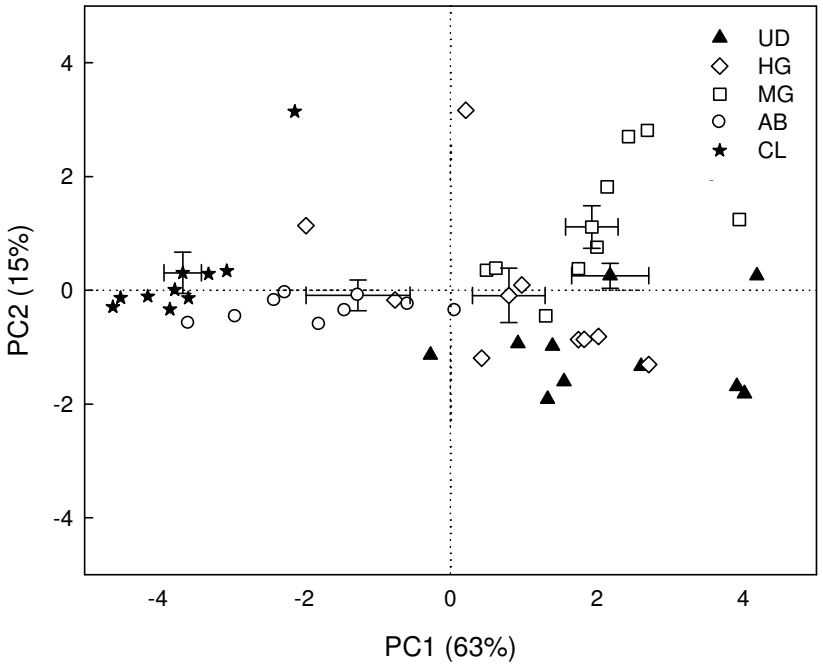

Fig 4. Factor scores of the carbon, nitrogen, phosphorous, and sulfur transforming enzyme activities in soil under different management systems. UD: Undisturbed; HG: Highly grazed; MG: Moderately grazed; AB: Abandoned from cultivation; CL: Cultivated with winter wheat.

Table 4. Factor loadings of enzyme activity analyzed by management systems

Variables	UD		HG		MG		AB		CL	
	Factor I	Factor II	Factor I	Factor II	Factor I	Factor II	Factor I	Factor II	Factor I	Factor II
Cellulase	-0.02	0.88	0.80	-0.47	0.47	0.67	0.38	0.79	0.43	0.67
β-glucosidase	0.73	0.61	0.91	-0.18	0.86	0.32	0.87	0.48	0.97	0.11
α- galactosidase	0.83	0.27	0.97	0.03	0.41	0.88	0.90	0.41	0.97	-0.13
Urease	0.92	-0.23	0.75	-0.43	0.72	0.47	0.97	0.08	0.80	-0.17
β-glucosaminadase	0.75	0.56	0.01	0.88	0.05	0.96	0.85	0.47	0.93	0.29
L- asparaginase	0.29	0.84	0.24	-0.62	-0.79	-0.44	0.25	0.93	-0.09	0.11
Phosphodiesterase	0.92	0.25	0.69	0.05	0.94	0.20	0.08	0.05	-0.10	0.87
Acid-phosphatase	0.75	0.25	0.05	0.93	0.09	0.91	0.94	0.25	0.88	-0.45
Alkaline phosphatase	0.80	0.44	0.69	-0.69	0.96	-0.15	0.55	0.81	-0.62	0.71
Arylsulfatase	0.55	0.54	0.71	-0.62	0.90	-0.05	0.20	0.95	-0.58	0.23
Rhodanese	0.30	0.80	-0.31	0.73	-0.15	0.89	0.82	0.55	0.86	-0.45
Eigenvalue	6.92	1.76	6.25	2.15	5.49	3.78	8.78	1.51	6.11	2.01
Explained Variance (%)	63%	16%	57%	20%	50%	34%	80%	14%	56%	18%

including cellulase, β-glucosidase, , acid-phosphatase, and arylsulfatase, were altered by grazing. Cultivation shifted the relative importance of phosphodiesterase and rhodanese

3.3. Interrelationships between microbial communities and their contributions to soil enzyme activities

Activity and growth of the microbial community are often regulated by the resource availability in soil, while enzyme syntheses are closely associated with microbial activities. On the other hand, soil enzyme activities could regulate labile C pools, and thus regulate microbial growth. The complex interrelationships are often evaluated by ratios between multiple microbial and C related soil variables. Principle component analysis of these ratios may reveal microbial community structures, processes or coupled relationships that regulate soil ecosystem functions. PCA factor analysis showed that three factors explained 79 % of the total variation (Table 4). Factor I was loaded by ratios of activity of α-galactosidase/ $C_{mic,}$ β-glucosidase/C_{mic}, β-glucosaminadase/ C_{mic} , acid-phosphatase to C_{mic}, and rhodanese to C_{mic}, while factor II was loaded by ratio of C_{mic} to N_{mic} , C_{mic} to C_{org} and the ratio of Phosphodiesterase/C_{mic} and alkaline phosphatase/ C_{mic}. Ratio of arylsulfatase to C_{mic} loaded factor III. Based on factor scores, management practices did not have detectable effect on PC1 variables, but they affected the PC2, and especially the PC3 variables (Fig. 5). All grazed

systems, including HG, MG, and AB, were closely related to PC3, while UD and CL systems clustered close to each other, but away from PC3.

Table 5. Factor loadings of different ratio of chemical and microbiological properties

Variable	Factor I	Factor II	Factor III
C_{mic}: C_{org}	-0.57	-0.66	-0.22
C_{mic}:N_{mic}	0.08	0.88	0.06
Cellulase/C_{mic}	0.59	0.62	0.09
B-glucosidase/C_{mic}	0.82	0.47	0.15
A-galctosidase/C_{mic}	0.92	0.31	0.15
Urease/C_{mic}	0.48	0.23	0.53
B-glucosaminidase/C_{mic}	0.83	0.14	-0.29
L-asparaginase/C_{mic}	0.20	0.48	0.36
Phosphodiesterase/C_{mic}	0.52	0.70	-0.27
Acid phosphatase/C_{mic}	0.92	-0.11	-0.01
Alkaline phosphatase/C_{mic}	-0.02	0.92	0.24
Arylsulfatase/C_{mic}	-0.12	0.02	0.88
Rhodanese/C_{mic}	0.93	0.01	0.11
Eigenvalue	6.51	2.37	1.37
Explained Variance (%)	50%	18%	11%

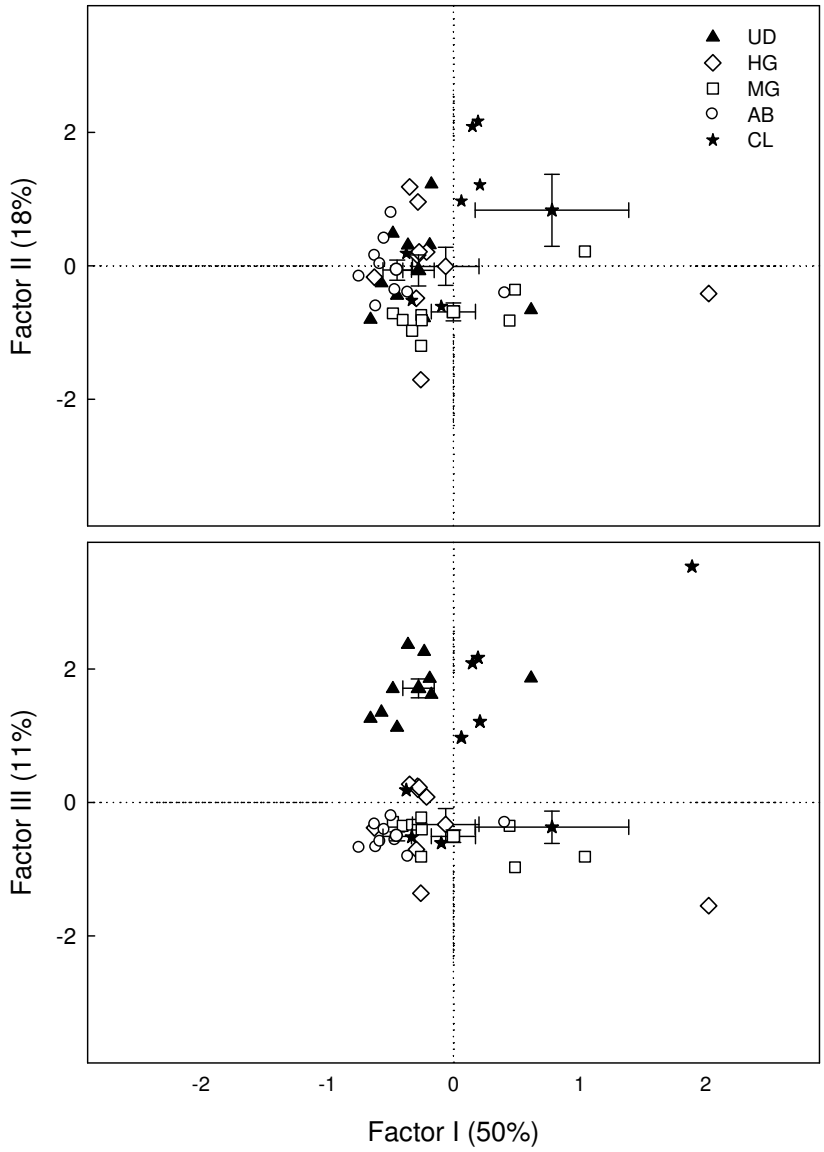

Fig 5. Factor scores of the ratio of basic soil properties and specific enzyme activities against management practices systems. UD: Undisturbed; HG: Highly grazed; MG: Moderately grazed; AB: Abandoned from cultivation; CL: Cultivated with winter wheat

4. Discussion

Grazing at moderate degree altered Mg and B concentrations. When compared to UD soils, Fe content in cultivated soils increased significantly. Both AB and HG management practices affected the relative importance of Mg and B in the trace elements evaluated, led to shifting their PC1 scores to positive and negative values, respectively. This indicated that B and Mg were limiting in those systems.

Of macronutrients evaluated, C_{org}, C_{mic}, N_t, and N_{mic} were most limiting in the cultivated soils, while these nutrients were not significantly affected by grazing. Studies have shown that cultivation of prairie soils led to reduction in organic matter content and microbial biomass (Saviozzi et al., 2001). On the other hand, grazing may lead to increase in C_{org} content by influencing the storage of the above and below ground biomass and stimulating plant growth and nutrient flow (Schuman et al., 1999). The close relationship between UD and grazed systems with soil P content (P_t, P_{inorg} and P_{org}), total S and soluble N suggest that these PC2 and PC3 variables were also important in ecosystem processes and function. The AB system was closely related to PC1, indicate that C_{org}, N_t, C_{mic}, N_{mic} were still important drivers of ecosystem function in these systems and that 30 years of conservation were not sufficient in erasing affect of cultivation on soil nutrient reservoir and its capacity to support microbial life.

Management systems were separated by the activities of enzymes involving in the C, N, P, and S cycling, indicating that enzyme activities are sensitive indicators of ecosystems disturbance. When eleven different enzymes involved in the C, N, P, and S cycling were plotted using factor scores from PCA, factor I variables separated managements practices with UD, MG and HG positively correlated with factor I loadings. The impacts of long term cultivation in the AB soils were still detectable after 30 years of conservation, indicating that building healthy soils by improving its organic matter content and its ability to cycle nutrient could take long time. The CL and AB systems were negatively correlated with factor I loadings. Annual applications of inorganic fertilizer in the cultivated system increased the concentration of nutrients in the soil solution which suppressed enzyme activities and that reduced the potential for C, N, and P cycling in these systems (Kandeler et al., 1999). As shown by Katsalirou (2006), the activities of these enzymes were lowest in the CL and AB soils and highest in the UD MG and HG soils, suggesting that the presence of natural vegetation and the widespread root system of perennial grasses in the uncultivated sites enhanced the rhizosphere effect and promoted microbial community activity compared to cultivated soils (Bandick and Dick, 1999). The obtained results suggested that enzymes involved in C cycling (β-glucosidase and cellulase), N cycling (Urease), P cycling (alkaline phosphatase), and S cycling (arylsulfatase) were most limiting factors that controlled the transformation processes of these major biological nutrients. The capacity of soil enzymes to release simple sugars, ammonia, orthophosphate, and sulfate to

support microbial activity in these soils predominantly regulated nutrient cycling and transformation processes in these soil systems. This is evidenced by the important PC1 loading factors such as α-galactosidase for C cycling to release simple sugar, urease for N cycling to release ammonia, alkaline phosphatase for P cycling to release inorganic P, and arylsulfatase in S transformation to release inorganic S.

All management practices were separated based on factor III loadings variables. The UD and CL were affected by arylsulfatase/C_{mic} ratio which indicated that the ability of the microbial community to release soluble S was the limiting factors in these soils.

Data analysis from this study suggested that it is challenging to pin point a single driver that regulates processes and function in an ecosystem. The fact that all management practices were separated based on ratios of arylsulfatase/C_{mic} suggested that organic S as an often neglected nutrient could be a key factor driving ecosystem functions. Moreover, this study also confirmed our long-term hypothesis that microbial community is the driver of all nutrients cycling process, labile nutrients regulate microbial growth, and enzyme activities that release labile nutrients govern microbial growth.

4.2. Conclusion

Of micronutrients tested, B and Mg were most limiting in these systems. Of macronutrients evaluated, C_{org}, C_{mic}, N_t, and N_{mic} were more limiting in the

cultivated than the uncultivated soils. Although there is some indication that mineralization of organic S is a key process that governs ecosystem functions, there was generally no clear trend that one nutrient transforming enzyme or process was more dominating than another enzyme activities or processes in nutrient transformation. However, the capacity of soil enzymes to release simple sugar and inorganic nutrients appeared to be key factors regulating nutrient cycling, suggesting microbial biomass as the drivers of C, N, P, and S transformation processes of all soil variables evaluated. In addition, PCA factorial analysis of enzymes evaluated revealed that management practices changed the relative importance and relationships among these enzymes within an ecosystem.

References

Acosta-Martinez, V., Zobeck, T.M., and Allen, V. 2004. Soil microbial, chemical and physical properties in continuous cotton and integrated crop-livestock systems. Soil Science Society of America Journal 68:1875-1884.

Acosta-Martinez, V., Klose, S., Zobeck, T.M., and Kennedy, A.C. 2003. Enzyme activities in semiarid soils under conservation reserve program, native grassland, and cropland. Journal of Plant Nutrition and Soil Science 166:699-707.

Al-Kaisi, M.M., Yin, M., and Licht, L.A. 2005. Soil carbon and nitrogen changes as affected by tillage system and crop biomass in a corn–soybean rotation. Applied Soil Ecology 30:174-191.

Bandick, A.K., and Dick, R.P. 1999. Field management effects on soil enzyme activities. Soil Biology & Biochemistry 31:1471-1479.

Bardgett, R.D., and Leeman, D.K. 1995. The short-term effects of cessation of fertiliser applicatios, liming, and grazing on microbial biomass and activity in a reseeded upland grassland. Biology and Fertility of Soils 19:148-159

Bremner, J.M., Mulvaney, C.S., 1982. Total Nitrogen. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed. Agronomy Monograph No. 9. ASA and SSSA, Madison, WI, pp. 595-624.

Brookes, P.C., Landman, A., Pruden, G., and Jenkinson, D.S. 1985b. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17:837-842.

Caravaca, F., Masciandaro, G., and Ceccanti, B. 2002. Land use in relation to soil chemical and biochemical properties in a semiarid Mediterranean environment. Soil & Tillage Research 68:23-30.

Davinson, E.T., and Ackerman, I.L. 1993. Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochemistry 20:161-171.

Dodor, D., and Tabatabai, M. 2003. Amidohydrolases in soils as affected by cropping systems. Applied Soil Ecology 24:73-90.

Doran, J.W., and T.B. Parkin. 1994. Deffining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A., (Eds.) Defining soil quality for a 14 sustainable environment. Soil Science Society of America Special Publication Number 35. SSSA and ASA, Madison WI, pp. 3-22.

Gee, G.W., Or, D., 2002. Particle-size analysis. In: Dane, J.H., Topp, G.C. (Eds.) Methods of Soil Analysis. Part 4. Physical Methods. Soil Science Society of America, Madison, WI, pp. 255-293.

Green, V., Stott, D., Cruz, J., and Curi, N. 2007. Tillage impacts on soil biological activity and aggregation in Brazilian creeado oxisol. Soil & Tillage Research 92:114-121.

Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: An overview. Advances in Agronomy 85, 221-268.

Holt, J.A., and Mayer, R.J. 1998. Changes in microbial biomass and protease activities of soil associated withb long-term sugar cane monoculture. Biology and Fertility of Soils 27:127-131.

Jaiyeoba, I.A. 2003. Changes in soil properties due to continuous cultivation in Nigerian semiarid Savannah. Soil & Tillage Research 70:91-98.

Johnson, D.E. 1998. Applied multivariate methods for data analysts Duxbury Press, Pacific Grove, CA.

Kandeler, E., Stemmer, M., Klimanek, E.-M., 1999. Response of microbial biomass, urease and xylanase within particle size fractions to long-term soil management. Soil Biology & Biochemistry 31: 261-273.

Katsalirou, E. 2006. Microbial community and enzyme activities in paririe soil ecosystems under different management., Oklahoma State University, Stillwater.

Klose, S., and Tabatabai, M.A. 1999. Arylsulfatase activity of microbial biomass in soils. Soil Science Society of America Journal 63:569-574.

Klose, S., Moore J., and M., T. 1999. Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biology and Fertility of Soils 29:46-59.

Malo, D.D., Schumacher, T.E., and Doolittle, J.J. 2005. Long-term cultivation impacts on selected soil properties in the northern Great Plains. Soil & Tillage Research 81:277-291.

Naseby, D.C., and Lynch., J.M. 2002. Enzymes and microorganisms in rhizosphere., pp. 109-123, *In* R. G. Burns. and R. P. Dick., (eds.) Enzymes in Soil Environment. Activity, Ecology and Application, Vol. pp. Marcel Dekker, New York.

Neff, J.C., Reynolds, R.L., Belnap, J., and Lamothe, P. 2005. Multi- decadal impacts of grazing on soil physical and biogeochemical properties in southeast Utah. Ecological Applications 15:87-95.

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil

Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed. Agronomy Monograph No. 9. ASAand SSSA, Madison, WI, pp. 539-579

Parton, W.J., Gutmann, M.P., Williams, S.A., Easter, M., and Ojima, D. 2005. Ecological impact of historical land -use patterens in the Great plains:A methodological assessment. Ecological Applications 15:1915-1928.

Sall, J., L. Creighton, and A. Lehman. 2005. JMP® start statistics, 3rd ed. Thompson/Brooks/Cole, Belmont, CA.

Sankaran, M., and Augustine, D.J. 2004. Large herbivores suppress Decomposer abundance in a semiarid grazing ecosystem. Ecology 85:1052-1061.

Saviozzi, A., Levi-Minzi, R., Cardelli, R., and Riffaldi, R. 2001. A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant and Soil 233:251–259.

Schuman, G.E., Reeder, J.D., Manley, J.T., Hart, R.H., and Manley, W.A. 1999. Impact of grazing management on the carbon and nitorgen balance of a mixed-grass rangeland. Ecological Applications 9:65-71.

Singh, B., Rengela, Z., and Bowden, J.W. 2006. Carbon, nitrogen and sulphur cycling following incorporation of canola residue of different sizes into a nutrient-poor sandy soil. Soil Biology & Biochemistry 38:32-42.

SigmaPlot. 2004. SigmaPlot 9.0 user's guide. Systat softerware, Inc. CA.

Su, Y.Z., Li, Y.L., Cui, J.Y., and Zhao, W.Z. 2005. Influences of continuous grazing and livestock exclusion on soil properties in a degraded sandy grassland, Inner Mongolia, northern China. Catena 59:267-278.

Vance, E.D., Brookes, P.C., and Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19:703-707.

CHAPTER V

SUMMARY AND CONCLUSIONS

Sulfur dynamic in soil is governed more by mineralization of organic S than inorganic S oxidation processes. When compared to the undisturbed systems, total sulfur was significantly lower and soluble S was significantly higher in the cultivated soils. Grazing promote or maintained S pools and the capacity of the soils to transform S to a degree similar to the undisturbed soils. Systems that set aside from cultivation for more than 30 years allowed the soil to regain its capacity to cycle S and are evolving towards the undisturbed systems. However, analysis based on S transforming enzymes showed that 30 years of conservation did not completely erase the impact of cultivation.

Of the five micronutrients tested, B and Mg were most limiting in soils. Contents of C_{org}, C_{mic}, N_t, and N_{mic} were more limiting in the cultivated than the uncultivated soils. However, there were no clear general trends that one nutrient transforming enzyme or process was more dominating than enzyme activities or processes involved in the transformation of another nutrient. The capacity of soil enzymes to release simple sugar and inorganic nutrients appeared to be key factors regulating nutrient cycling, suggesting microbial biomass as the drivers of C, N, P, and S transformation processes of all soil variables evaluated.

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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 sulfur cycling of semiarid prairie soil ecosystems, and to reveal the drivers of nutrients cycling under different management practices. The management systems included undisturbed, set-aside from cultivation, moderately grazed, heavily grazed and cultivated. Surface soil samples taken from nine plots of each system were evaluated based on chemical and microbial properties as well as enzyme activities involved in carbon, nitrogen, phosphorus, and sulfur transformations.

Findings and Conclusions: Sulfur dynamic in soil is governed more by mineralization of organic S than inorganic S oxidation processes. Of systems evaluated, total sulfur was significantly lower and soluble S was significantly higher in the cultivated ones. Grazing promote or maintained S pools and the capacity of the soils to transform S to a degree similar to the undisturbed soils. Of the five micronutrients tested, B and Mg were most limiting in soils. Of all nutrients and pools evaluated, contents of organic carbon, total nitrogen, and microbial biomass carbon and nitrogen were most impacted soil variables by management practices, and are more limiting in the cultivated than the uncultivated soils. Management practices also changed the relative importance and relationships among these enzymes within an ecosystem. The capacity of soil enzymes to release simple sugar and inorganic nutrients appeared to be key factors regulating nutrient cycling, suggesting microbial biomass as the drivers of C, N, P, and S transformation processes of all soil variables evaluated.

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