BIOREMEDIATION OF BRINE AND HYDROCARBON CONTAMINATED SOILS AND DIRECT GRADIENT ANALYSIS OF A TALLGRASS PRAIRIE

By

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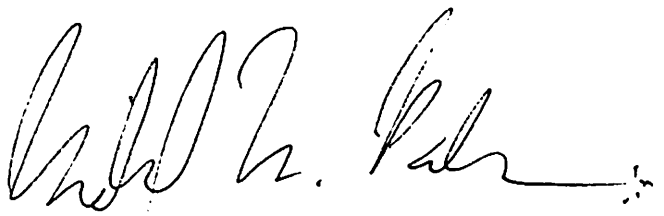

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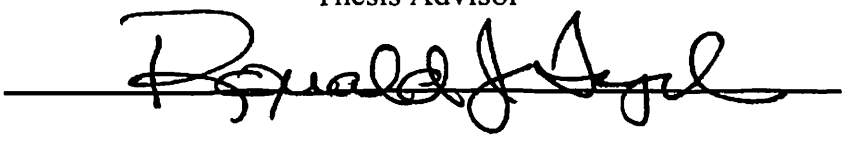

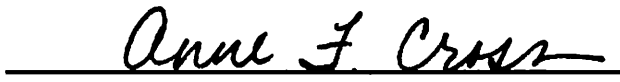

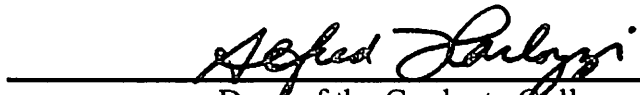

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CHAPTER I

PLANT COMMUNITY CHARACTERISTICS AND SOIL NUTRIENT DYNAMICS IN HYDROCARBON-CONTAMINATED SOILS OF A TALLGRASS PRAIRIE

Abstract

In 1999 a study was begun to compare the effects of conventional bioremediation methods on ecosystem recovery in soils with differing degrees of crude oil and produced brine contamination. Two contaminated sites at the Tallgrass Prairie Preserve in Osage County, Oklahoma were divided and tilled with hay or hay plus fertilizer. A control section of uncontaminated prairie also was tilled with hay. Total petroleum hydrocarbon levels were monitored through spring 2002 and tilling was discontinued in fall 2001. I analyzed soils collected from fall 2001 to fall 2003 and estimated percent cover of plant species to determine whether differences in nitrogen and phosphorus pools and plant growth persisted in the contaminated sites due to the residual contamination and/or bioremediation methods. I collected soil samples within the preserve from 20 grassland sites in 2002 and 40 grassland sites in 2003 to provide a replicated control. I found that soils collected from the tilled and contaminated areas continued to exhibit significant differences in nitrogen and phosphorus pools by fall 2003. Plant growth and species composition varied as a function of residual salinity and petroleum hydrocarbons. Residual hydrocarbons appear to limit plant growth and perturb nutrient cycles due to changes in soil permeability and water holding capacity.

Introduction

The biogeochemistry of tallgrass prairie soils has become well studied in recent decades (Risser and Parton, 1982, Seastedt, 1988, Hayes and Seastedt, 1989, Groffman *et al.*, 1993, Chaneton *et al.*, 1996, Turner, *et al.*, 1997, Baer *et al.*, 2002). The creation of nature preserves dedicated to conservation has facilitated such research. However, conservation is potentially threatened by petroleum exploration, and mineral oil hydrocarbons are the most frequently occurring environmental contaminants (Margesin *et al.*, 2000). Although crude oil is among the Applied Biotreatment Association's list of contaminants considered successfully bioremediated in the field (Skladany and Metting, 1993), little is known about the effects of contamination and subsequent remediation processes associated with oil spills on the nutrient dynamics and plant communities of natural grasslands.

Grassland ecosystems can be significantly perturbed through the release of petroleum and extracted brine. Crude oil inhibits plant germination and growth (Chaîneau *et al.*, 2003). Although toxins may play a biological role in the distribution of elements in the soil, microbial competition for nutrients and changes in the physical properties of soils resulting from petroleum hydrocarbon contamination could produce a greater effect on nutrient dynamics and community structure (Xu and Johnson, 1997). Because the passive biodegradation of hydrocarbons in soils is often relatively slow, it is desirable to decontaminate locations that have had high cumulative loading of petroleum (Morgan and Watkinson, 1989).

Brine should also have a considerable impact on soil nutrients and plant community processes in contaminated sites. Salinity may affect the growth of soil

organisms through changes in osmotic potentials (Brady and Weil, 1999). Sodium salts disperse clay particles, causing disruption of the soil structure (Brady and Weil, 1999). This effect may cause the loss of topsoil through erosion and inhibit nutrient and moisture uptake by plants and microorganisms.

Bioremediation

Bioremediation of chemically contaminated soils involves the transformation of complex or simple chemical compounds into nonhazardous forms (Skladany and Metting, 1993). A common method for remediating petroleum spill sites involves adding nutrients and oxygen to the subsurface soil to stimulate the resident bacteria and fungi, which use hydrocarbons as a substrate (Skladany and Metting, 1993, Walworth and Reynolds, 1995, Balba *et al.* 1998, Gogoi *et al.*, 2003). Factors affecting bioremediation include microbial toxins, the composition of the microbial populations present, aeration, moisture content, temperature, pH, and nutrient levels. Nitrogen and phosphorus are the soil nutrients most often found to limit biodegradation (Walworth and Reynolds, 1995). C:N and C:P ratios are considered to be especially important for bioremediation (Zhou and Crawford, 1995), but optimum ratios are unclear (Line *et al.*, 1996), and the ratios can be made both too low and too high for optimum biodegradation rates through fertilizing (Dibble and Bartha, 1979). In addition, the forms of N and P must be considered when adjusting nutrient ratios due to their differing bioavailability and reactivity. Overall, the supply of oxygen is considered to be the major problem of bioremediation and is most often increased by repeated tilling of the spill site (Morgan and Watkinson, 1989).

Direct physical impacts of bioremediation could also influence nutrient dynamics and plant communities (Xu and Johnson, 1997). Because the supply of oxygen is essential for the efficient mineralization of organic carbon, tilling is often considered an irreplaceable component of remediation. However, tilling is an extreme form of soil disturbance that produces almost immediate changes in the community's chemical cycles and may have long-lasting effects on the dynamics of plant production (Haddad *et al.*, 2002). As it is designed to do in bioremediation, tilling generally decreases organic carbon in the soil by increasing respiration (Tiessen *et al.*, 1982, Elliot, 1986). Tilling also substantially reduces soil nitrogen and labile forms of phosphorus because much of the nitrogen and biologically active phosphorus in soils is found in organic compounds (Tiessen *et al.*, 1983, Jug *et al.*, 1999).

In addition to the increased mineralization of organic compounds, tilling may affect soil chemistry by increasing erosion and leaching (O'Halloran *et al.*, 1987a, Mehdi and Madramootoo, 1999). Erosion can lead to changes in phosphorus fractions (O'Halloran *et al.*, 1987a, O'Halloran *et al.*, 1987b). Organic phosphorus and nitrate-nitrogen are especially susceptible to leaching following removal of plants through tilling (Hedley *et al.*, 1982, Frossard *et al.*, 1989).

Tilling and fertilizing are expected to alter plant species composition by increasing resource availability thus permitting coexistence of ruderal species. Fertilizer is commonly applied during bioremediation in order to increase microbial degradation of petroleum hydrocarbons (Alvarez *et al.*, 1998). Changes in N-mineralization rates caused by fertilizing could persist beyond the time of remediation through positive feedback with plant species and/or herbivores (Vinton and Burke, 1995, Janssens *et al.*, 1998, Ritchie *et*

al., 1998, Evans *et al.*, 2001, Booth *et al.*, 2003; but see Knops *et al.*, 2002). Added phosphorus could enter the more biologically active side of the phosphorus cycle as organic matter with the potential to affect the plant community (Janssens *et al.*, 1998), or it could become bound in more recalcitrant forms in the geochemical side of the cycle (O'Halloran *et al.*, 1987b, Cross and Schlesinger, 1995). Changes in the type or degree of spatial heterogeneity in the soil caused by both of these remediation practices may also significantly affect community structure during early succession (Robertson *et al.*, 1988).

Community restoration

This study provides a starting point for research aimed at understanding the relationship between the short-term results of bioremediation and long-term ecosystem processes and plant community dynamics. Although bioremediation of crude oil spills has met the criteria of success status in previous field studies (Skladany and Metting, 1993), maintaining or restoring a native species composition was not among the criteria of such research.

Residual soil fertility through positive feedbacks may cause long-term loss of diversity or persistence of exotic species without continuous disturbance or nutrient additions (Olff *et al.*, 1994, Mountford *et al.*, 1996, Willems and Van Nieuwstadt, 1996).

Tilling clears space and increases resource availability for invasive species. According to Davis *et al.* (2000), fluctuation in resource availability is the key factor controlling the susceptibility of an environment to invasion by non-resident species. For instance, nitrogen application has been observed to cause the replacement of warm season grasses by non-native cool season grasses (Wedin and Tilman, 1996).

In addition, fertilizer application should increase short-term nutrient availability. It is generally agreed that nutrient availability in grassland communities can affect species diversity (Rajaniemi, 2002). Diversity may decrease in response to increases in both nitrogen and phosphorus availability (Janssens *et al.*, 1998), and nitrogen has been observed to decrease functional group diversity of microorganisms (Sarathchandra *et al.*, 2001). Efforts to reduce nutrients such as increasing carbon amendments have met only short-term success (Morghan and Seastedt, 1999).

These observations of short-term nutrient dynamics and plant succession will begin to provide the necessary data with which the results of conventional bioremediation methods may be compared to the long-term success criteria of nature preserves. Although community responses observed in the first year of revegetation might not be indicative of later trends (Carson and Barrett, 1988), these nutrient and vegetation analyses should be valuable to future studies of the long-term effects of bioremediation on prairie restoration.

The objectives of this study are to: 1) document the short-term biogeochemical processes and community succession during bioremediation in a restored prairie ecosystem; 2) compare *ex situ* results and hypotheses from previous studies to results of *in situ* remediation in a natural setting; and 3) document biogeochemical characteristics of native Oklahoma tallgrass prairie soils.

Methods

Study area

The Nature Conservancy's (TNC) 15410 hectare Tallgrass Prairie Preserve (TGPP) is located in northeastern Oklahoma, Osage County, between 36.73° and 36.90° N

latitude, and 96.32° and 96.49° W longitude. With a rolling topography ranging between 253m and 366m in elevation, approximately 90% of the TGPP consists of grasslands dominated by switchgrass (*Panicum virgatum*), little bluestem (*Schizachyrium scoparium*), big bluestem (*Andropogon gerardii*), tall dropseed (*Sporobolus compositus*), and Indiangrass (*Sorghastrum nutans*). Mean annual temperature is 15.2° C with a 17.3° July mean and a 2.7° January mean. The annual precipitation is 84 cm with 75% falling during the 205-day growing season (Risser and Parton, 1982, Palmer *et al.*, 2003). There are over 100 oil producing wells on the Preserve extracting an average of 15-20 barrels of crude oil per day and as much as 10 times that volume of brine (K. Sublette, personal communication). The TGPP is managed using a randomized burning regime and gradual replacement of cattle grazing with bison grazing (Hamilton, 1996, Palmer *et al.*, 2003).

Remediation treatments

On the northwestern edge of the TGPP, two spill sites were bioremediated to help assess the feasibility and costs of restoring the natural system (Figure 1). The first site (J6) consisted of two contaminated areas separated by about 100 m, which received approximately 10 m^3 of dewatered crude oil from a spill on January 6, 1999. An area of about 900 m^2 adjacent to the pipeline on a 5% east-facing slope (J6 north) was contaminated with an average level of total petroleum hydrocarbons (TPH) of 35,000 mg kg^{-1}. At the bottom of an adjacent gully, the second area contained about 450 m^2 of prairie (J6 south) on a 7.5% south-facing slope, and the contamination resulted in an initial TPH level of 8,000 mg kg^{-1}. In May of 1999, prairie hay was applied to both areas at a rate of 1.0 - 1.3 Kg m^{-2} with tilling to a depth of 20 cm. Each area was divided perpendicular to the slope into roughly equal parts with corrugated plastic sheeting, and

the down slope sections were fertilized with NH_4NO_3, P_2O_5, and K_2O using 20% of the U. S. Environmental Protection Agency (EPA, 1993) recommended application rates of 100:10:5 (C:N:P) by weight. A control area of 450 m^2 was also created east of J6 north on a 0-1% west facing slope and tilled with the same rate of prairie hay addition but with no fertilizer. Fertilizer and hay additions were repeated during November 1999, July 2000, and April 2001. The sites were tilled again without nutrient additions in October 2001. Tilling was then discontinued due to stabilization in the rate of decrease of TPH in the spill sites. The J6 south prairie was burned prior to sampling in fall 2002, and the J6 north prairie was burned prior to sampling in spring 2003. The tilled sites did not burn during either event due to the discontinuity of plant cover. In addition bison were frequently observed wallowing in the bare areas of J6 north. Under natural conditions bison create and maintain 3-5m wallows (Knapp *et al.*, 1999). Two areas of bare ground in J6 north matching this description persisted throughout the duration of the study.

The second site (G5) consisted of three parallel areas on a 6.5% west-facing slope contaminated with crude oil and brine from separate leaks during the fall of 1999. From North to South the three spill areas were designated G5N, G5M, and G5S respectively. The approximate dimensions were: 15 m by 60 m for G5S; 7 m by 33 m for G5M; and 8 m by 80 m for G5N. The longest dimensions extended downslope to the West. The initial TPH concentrations were 27,000, 16,000, and 14,000 mg kg^{-1} in the G5N, G5M, and G5S areas respectively. The chloride concentrations in these areas ranged from 8,300 – 23,000 mg kg^{-1}, and sodium concentrations ranged from 1,550 – 10,900 mg kg^{-1}. Prairie hay was added to improve the soil structure and hydraulic conductivity in order to allow the leaching of excessive salts, and G5N and G5S were also fertilized. Prairie hay,

fertilizer, and tilling were applied as with J6. The G5 prairie was burned prior to sampling in spring 2003, but the tilled sites did not burn due to the discontinuity of plant cover.

Replicated controls

No nutrient analyses were performed on soils from the contaminated sites prior to this study. In order to demonstrate differences between the soils of the weakly replicated spill treatments and native prairie, I collected samples from 20 untilled grassland sites in the summer of 2002 and 40 untilled grassland sites in the summer of 2003 to serve as replicated controls (Oksanen, 2001). In 2002 I collected soils from 20 grassland sites (Figure 1) selected randomly from a total of 151 that were located at the intersections of the 1 km x 1 km UTM grid in the TGPP. In 2002 8 of these sites were still grazed by cattle instead of bison. In 2003 I collected soils from 40 grassland sites (Figure 2) in the TGPP's oldest bison unit. I limited the locations of these sites to soil complexes similar to those found at the J6 and G5 spill sites (Coweta-Bates and Steedman-Coweta complexes) as shown by the Soil Survey of Osage County, Oklahoma (Bourlier *et al.*, 1979).

Field collections

I gathered soil samples from J6 and G5 during spring (late March-early April), summer (June), and fall (late September-early October) starting in the fall of 2001. I sampled the 2002 and 2003 multiple control sites during the summer only. To overcome some of the error associated with spatial heterogeneity of soil nutrients (Robertson *et al.*, 1988, Jackson and Caldwell, 1993, Davidson *et al.*, 1993), five subsamples were composited for laboratory analysis in each whirl-pack sample bag. The soil cores have a

2.5 cm diameter and a depth of 15 cm. I collected seven groups of five samples from the J6 spill site, each composed of five evenly spaced subsamples, during each sampling period. The groups were: native prairie adjacent to J6 north (J6NN), J6 north unfertilized (J6NNF), J6 north fertilized (J6NF), J6 north tilled control (J6NC), native prairie adjacent to J6 south (J6SN), J6 south unfertilized (J6SNF), and J6 south fertilized (J6SF). From the G5 spill site, I collected 9 samples from each of the three spill areas. The samples were collected in groups of three spaced 2 m apart from east to west at upper-slope, mid-slope, and lower-slope positions within each spill area. The sample groups in G5N were located at distances of 8-12 m, 33-37 m, and 60-64 m from the eastern border representing the upper, middle and lower groups respectively. The groups from G5M were spaced 3-7 m, 14-18 m, and 26-30 m from the eastern border, and the G5S groups were spaced 12-16 m, 32-36 m, and 58-62 m from the border. I collected a set of control samples (G5C) parallel to G5S from the prairie adjacent to its southern edge using the same spacing. Each sample from G5 is composed of a mixture of 5 subsamples spaced evenly across the width of each spill area from north to south. The control sites making up the 2002 and 2003 multiple controls were each represented by one sample composed of 4 and 5 subsamples respectively. The airtight whirl-packs were placed in coolers immediately after combining the subsamples and were transported to a laboratory freezer at Oklahoma State University within 7 hours of sampling. I performed all fresh soil analyses within 2 days after collection (Vinton and Burke, 1995). Fresh soils were thoroughly mixed, sieved (2 mm) and weighed into subsamples for 3 analyses: water content, initial inorganic nitrogen, and incubations to determine the potential net N-mineralization and nitrification rates. The remaining soils were allowed to air dry before

performing all other analyses. I quantified vascular plant species abundance by estimating percent cover during the summer sampling in 2003 (Palmer *et al.*, 2002). Nomenclature for plant species follows Diggs *et al.* (1999).

In addition soils were collected for total petroleum hydrocarbon (TPH) and soil salinity analyses and transported to the University of Tulsa. One TPH sample was collected from each site. The soils were placed in glass jars with Teflon-lined lids. Sample jars were placed immediately on ice in the field and later shipped overnight to Continental Laboratories in Salina, Kansas for analysis. Soil salinity samples composed of four composited subsamples were collected from each slope position at each site at G5. Salinity analyses were performed at the University of Tulsa.

Total C and total N, inorganic N, potential net N -mineralization and nitrification rates

All samples were analyzed for total C, total N, and inorganic N. Total C and total N were measured on a percent weight basis with a LECO CN 2000 combustion analyzer (Leco, St. Joseph, MI) by the Soil, Water & Forage Analytical Laboratory at Oklahoma State University. I extracted inorganic N from a 5 g subsample with 50 mL of 2 mol/L KCl for 1 hour on a reciprocating shaker (Maynard and Kalra, 1993). The extracts were centrifuged at 4000 rpm for 10 min and filtered through a Whatman no. 40 paper filter. I refrigerated extracts until they were analyzed for nitrate and ammonium with a Lachat (Milwaukee, Wisconsin) autoanalyzer by the Soil, Water & Forage Analytical Laboratory (EPA, 1979).

I determined potential net N-mineralization and net nitrification for all soil samples from the summer and fall of 2003. For the measurement of potential net N-mineralization and net nitrification, fresh soil samples were incubated at 25° for 30 days.

For the soil incubation, a 25 g subsample was placed in a vial, brought to the estimated field capacity with deionized water, and placed in a closed mason jar with 10 mL of deionized water in the bottom to maintain a saturated atmosphere (Schimel, 1986). At the end of the incubation, soils were extracted with KCl, filtered, and analyzed for nitrate and ammonium as described above. Potential net N-mineralization was calculated as the difference between concentrations of initial and final inorganic N of the soil, and potential net nitrification was calculated as the difference between the concentrations of initial and final nitrate-N of the soil (Vinton and Burke, 1995).

Phosphorus fractionation and Mehlich III phosphorus

Phosphorus fractionation yields a more complete picture of changes in soil P in the short- and long-term than simply measuring total P. I performed phosphorus fractionation on all summer soil samples from 2002 and 2003. I analyzed air-dried, 0.5 g samples for total P and soil inorganic and organic fractions using a modified sequential extraction procedure developed by Hedley *et al.* (1982) and modified by Tiessen and Moir (1993). This method uses a series of increasingly stronger reagents to separate pools of labile and recalcitrant inorganic and organic P, resulting in 9 extracts per sample. The sequence of extracts was resin-extractable P (resin-P_i), inorganic and organic 0.5 *M* $NaHCO_3$-extractable P ($NaHCO_3$-P_i, $NaHCO_3$-P_o), inorganic and organic 0.1 *M* NaOH-extractable P (NaOH-P_i, NaOH-P_o), 1.0 *M* HCl-extractable P (1M HCl-P_i), inorganic and organic concentrated HCl-extractable P (cHCl-P_i, cHCl-P_o), and H_2SO_4-H_2O_2-extractable P (residual-P). I analyzed the extracts for phosphate concentrations using the Murphy and Riley method (1962). The sequential procedure, P-fractions, and proposed properties of each fraction in soil are described in figure 3. Total P values calculated from the sum

of the individual fractions following Tiessen and Moir (1993) averaged 4.3% less than total P values for a subset of samples digested for only total P. In addition one composite sample was analyzed for soil test P using the Mehlich III extractant for each spill treatment for all sampling dates by the Soil, Water & Forage Analytical Laboratory (Mehlich, 1984).

Soil texture, pH, moisture and field capacity

Due to potential correlations between P fractions and soil texture (Tiessen *et al.*, 1983), I performed particle size analysis using the hydrometer method on all samples collected during the summer in 2002 and 2003 (Bouyoucos, 1951, Gavlak *et al.*, 2003). In contrast, texture may not have a strong relationship with N-mineralization observations (Burke *et al.*, 1997). In addition I measured soil pH in water for the 2002 and 2003 summer samples (Thomas, 1996) and soil moisture gravimetrically (Topp, 1993) for all samples. I estimated field capacity for soils collected in the summer of 2003 using the centrifuge method (Cassel and Nielsen, 1986). Soil field capacity is the percentage of water remaining in a soil two or three days following saturation and after free drainage has practically ceased. Field capacity is controlled by soil texture, structure, and organic matter content in uncontaminated soils (Brady and Weil, 1999).

Total Petroleum Hydrocarbons and Soil Salinity

Total petroleum hydrocarbons were analyzed from one composite sample for each treatment at J6 and G5 from the beginning of bioremediation through spring of 2002 using the EPA 418.1 method by Continental Laboratories in Salina, Kansas (EPA, 1983). The G5 soil salinity samples were analyzed for exchangeable sodium (Helmke and

Sparks, 1996) and exchangeable chloride (Frankenberger *et al.*, 1996) concentrations through summer of 2003 at the University of Tulsa.

Statistical analyses

I performed 4 separate redundancy analyses (RDA) on the correlation matrix (Markarenkov and Legendre, 2002) for J6 summer 2002, J6 summer 2003, G5 summer 2002, and G5 summer 2003 using dummy variables for petroleum contamination, brine contamination, tilling, and fertilizer application as explanatory variables and %C, %N, NH_4-N, NO_3-N, N-mineralization and nitrification rates, and P fractions as response variables with soil textural classes of sand, silt, clay (Table 1) and slope entered as covariables. Separate analyses of J6 and G5 allowed the effects of tilling independent of contamination to be more easily distinguished because tilling, petroleum contamination, and brine contamination occur simultaneously at G5. Because G5 was analyzed separately I also was able to include slope position relative to the pipeline as a covariable for G5 RDAs. I analyzed 2002 and 2003 data separately because I was able to include % plant cover as an explanatory variable in 2003 RDAs, and N-mineralization and nitrification were not available as response variables for 2002 RDAs. I log-transformed all soil variables. I performed a principal components analysis (PCA) (Everitt, 1993, Basilevsky, 1994) on the correlation matrix comparing all samples taken during the summers of 2002 and 2003 including replicated controls. The comparison was based on log-transformed measurements of %C, %N, NH_4-N, NO_3-N, and P fractions. I used textural classes as covariables (Table 1; Table 2). All ordinations were performed with CANOCO for Windows software (Ter Braak and Šmilauer, 1998). I also conducted Pearson's correlations comparing field capacity with soil characteristics and comparing

the effects of tilling, fertilizing, petroleum contamination, and plant cover on soil N and P with SPSS FOR WINDOWS (2001).

Results

Total Petroleum Hydrocarbons and Soil Salinity

Total Petroleum Hydrocarbons decreased rapidly at the contaminated sites during the early stages of bioremediation (Figure 4). The rates of decrease stabilized by the beginning of 2002. Decreases in sodium and chloride at G5 were also greatest during the first year (Figure 5). Salinity levels remained relatively high throughout the course of this study. By fall of 2003 G5N had the lowest levels of sodium and chloride among the contaminated sites at G5.

Carbon, nitrogen, and phosphorus

Changes in soil nutrients were related to tilling, fertilizing, hydrocarbon and brine contaminations, and the subsequent revegetation processes. The PCA showing nutrient data from the summers of 2002 and 2003 illustrates the differences in soil C, N, and P due to tilling, fertilizer, and contamination and the return towards prairie conditions during revegetation (Figures 12a, 12b, 12c, and 12d). The first axis accounted for 41% of the variation in soil C, N, and P, and the second axis accounted for 13% of the variation. Inorganic nutrient pools predominate in samples in the upper and left portions of the diagram and organic nutrient pools and nutrient characteristics of undisturbed prairie predominate in the lower and right portions (Figure 12e). Sites experiencing revegetation shifted toward the lower right from 2002 to 2003 (Figures 12a, 12b, 12c and 12d). The relative sample spread of groups demonstrates soil nutrient heterogeneity within sites, which increased from 2002 to 2003 at J6NF, J6NNF, and G5N and decreased at J6SF and

J6SNF (Figures 12a, 12b, 12c, and 12d). By summer 2003 J6NC was most similar to the prairie controls (Figure 12b). The J6SF and J6SNF sample groups were most similar to J6NC and prairie controls (Figure 12b). The J6NF and J6NNF groups shifted toward the upper right from 2002 to 2003 due to increases in P pools (Figures 12a and12b). Unfertilized samples were generally grouped more closely to the controls than fertilized samples due to lower nutrient availability (Figures 12a, 12b, 12c, and 12d). However, by summer 2003 increased plant cover caused the fertilized G5N samples to group more closely with the control samples than the unfertilized G5M samples (Figure 12d).

I performed separate RDAs for J6 summer 2002, J6 summer 2003, G5 summer 2002, and G5 summer 2003 (Figures 13a, 13b, 13c, and 13d) and Pearson's correlations for J6 summer 2003 (Table 7) to explore the different effects of tilling, fertilizing, petroleum contamination, and revegetation on soil C, N, and P pools. The first RDA axes explained a large amount of the variation (30%, 32%, 47%, and 47% respectively) in the measured characteristics of soil C, N, and P in all RDAs. Fertilizer application was the key environmental variable separating nutrient levels on all second RDA axes, which explained 8%, 6%, 7%, and 7% of the variation respectively (Figures 13a, 13b, 13c, and 13d). RDAs showed that tilling was positively associated with NO_3-N and inorganic P and was negatively associated with %N, N-mineralization, organic P, and residual P (Figures 13a, 13b, 13c, and 13d). Tilling was significantly positively correlated with NO_3-N, $NaHCO_3$-P_i, NaOH-P_i, and 1M HCl-P_i and negatively correlated with $NaHCO_3$-P_o and NaOH-P_o (Table 7). RDAs showed that fertilizer was positively associated with inorganic P and NO_3-N (Figures 13a, 13b, 13c, and 13d). Fertilizer was significantly positively correlated with resin-P_i, $NaHCO_3$-P_i, NaOH-P_i, 1M HCl-P_i, cHCl-P_i, and

cHCl-P_o (Table 7). RDAs showed that petroleum contamination was positively associated with high C:N ratios and variables associated with tilling (Figures 13a, 13b, 13c, and 13d). Petroleum contamination was significantly positively correlated with C:N, $NaHCO_3$-P_i, NaOH-P_i, and 1M HCl-P_i (Table 7). RDAs showed that plant cover was positively associated with N-mineralization and organic P and was negatively associated with NO_3-N, NH_4-N, inorganic P, and high C:N ratios (Figures 13a, 13b, 13c, and 13d). Plant cover was significantly positively correlated with cHCl-P_o and negatively correlated with %C, C:N, $NaHCO_3$-P_i, NaOH-P_i, and 1M HCl-P_i (Table 7)

Soil C decreased gradually through time on all tilled sites (Figures 6a, 6b, and 6c). By fall of 2003 the bioremediation treatments lowered soil C at all contaminated sites to levels common in this ecosystem. (Table 2; Figures 6a, 6b, and 6c). By fall of 2003 J6NNF and J6NF still had higher C than J6NN, but all other contaminated sites had C levels less than or not substantially different from their respective prairie controls and the mean %C of the replicated controls (Table 2; Figures 6a, 6b, and 6c).

Total N also decreased gradually through time on tilled sites (Figures 7a, 7b, and 7c). Unfertilized, contaminated sites (J6NNF, J6SNF, G5M) had substantially lower N than other sites by fall 2003 (Figures 7a, 7b, and 7c). Mean %N was highest at prairie controls. However, fertilized spill sites had %N levels that were similar to those of their respective prairie controls, suggesting that fertilizer offset N losses (Figures 7a, 7b, and 7c). All levels of total N fell within the range represented by the replicated controls throughout the study, but J6NNF, J6SNF, and G5M had means of %N that were more than 1 standard deviation lower than the mean of the replicated controls by 2003 (Table 2; Figures 7a, 7b, and 7c).

The contaminated sites, J6NNF, J6NF, and G5M, had substantially higher C:N ratios than their respective prairie controls throughout the sampling dates (Figures 8a, 8b, and 8c). The mean C:N ratio at J6NNF continued to be higher than any sample from the replicated control groups through the fall of 2003 (Table 2; Figure 8a).

Inorganic N decreased significantly during revegetation. Levels of inorganic N at tilled and prairie sites converged by summer 2003 at J6 south (Figure 9b) and fall 2003 at J6 north (Figure 9a). All tilled sites at G5 had substantially higher inorganic N than the maximum of the replicated controls through fall 2003 (Table 2; Figure 9c). G5S had the highest inorganic N and the fertilized site G5N had the lowest inorganic N of the tilled sites at G5 by fall 2003 (Figure 9c). G5N showed the greatest decrease in inorganic N at G5 during the study period, while G5S and G5M showed little change (Figure 9c).

All potential net N-mineralization rates fell within the range of rates measured from the replicated controls (Table 2; Table 3). The lowest rates were generally found in unfertilized, hydrocarbon contaminated soils. In contrast, potential net nitrification rates in the tilled sites of J6 north from summer 2003 exceeded the highest rates of the replicated controls (Table 2; Table 3). Nitrification was extremely low compared to the replicated controls in soils from the brine contaminated G5 sites, especially G5M (Table 2; Table 3). Sites experiencing recent revegetation had higher N-mineralization in fall 2003 than poorly vegetated sites and sites with longer establishment of vegetation (Table 3).

In contrast to N, inorganic P remained unusually high in fertilized sites through the 2003 sampling seasons regardless of revegetation status. Slope position strongly affected P availability and total P (Table 4; Table 5). J6NF, J6SF, and all tilled sites at

G5 had substantially higher Mehlich III extractable P than their respective controls through fall 2003 (Figures 10a, 10b, and 10c). Fertilized sites generally had higher total P than their respective controls, and unfertilized tilled sites had lower total P than controls in both 2002 and 2003 (Table 4; Table 5). Total P was substantially higher at J6NF and J6NNF in 2003 than 2002; the greatest portions of these increases occurred in the $NaHCO_3$-P_i, NaOH-P_i, and NaOH-P_o fractions (Table 4; Table 5). J6NF had the highest levels of resin-P_i, $NaHCO_3$-P_i, NaOH-P_i, and 1M HCl-P_i in 2002 and 2003 (Table 4; Table 5). The mean sum (50.6 ± 3.7 mg P kg^{-1} soil) of labile P forms (resin-P_i, $NaHCO_3$-P_i, $NaHCO_3$-P_o) at J6NF in 2003 still exceeded the maximum observed in the replicated controls (42.5 mg P kg^{-1} soil). All fertilized sites had unusually high levels (outside of the range of replicated controls) (Table 2; Table 4; Table 5) of $NaHCO_3$-P_i and/or NaOH-P_i in both 2002 and 2003 (Table 6; Table 7). Tilled sites had substantially lower $NaHCO_3$-P_o and NaOH-P_o and higher inorganic P in comparison to their respective prairie controls (Table 4; Table 5).

Soil hydrology

Soil moisture increased as sites became revegetated (Figures 11a, 11b, and 11c). By summer 2003 soil moisture at J6NC was not significantly different from native prairie (Figure 11a). Soil field capacity was significantly correlated with previous TPH concentrations (Table 8). However, field capacity showed no significant correlation with soil texture or %C (Table 8). Petroleum contaminated sites had lower soil field capacities than uncontaminated sites (Table 1). J6NC did not have a lower field capacity than native prairie (Table 1). Field capacity was lowest with the greatest standard deviations at J6NF, J6NNF, and G5M (Table 1). In addition I observed surface beading of water

and KCl solutions on soils from contaminated sites during extraction procedures throughout the course of this study.

Revegetation and community structure

At the end of the 2002 growing season, J6NF, J6NNF, and all tilled sites at G5 had between 70% and 90% bare ground. At the time of vegetation analysis in the summer of 2003 the same sites still had substantial areas of bare ground (Table 6). J6SF, J6SNF, and J6NC had plant cover greater than 50% by the end of 2002 and were nearly completely revegetated by summer 2003 (Table 6). The respective prairie controls were dominated by prairie grasses including *Andropogon gerardii*, *Panicum virgatum*, *Sorghastrum nutans*, and *Festuca arundinacea*, and in some areas by the forbs *Solidago canadensis*, *Helianthus mollis*, and *Gutierrezia dracunculoides* (Table 6). Colonization of J6SF, J6SNF, and J6NC by plants resulted in a fairly random spatial distribution of individuals. The dominant recolonizing species in these plots by summer 2003 continued to be ruderal forbs including *Ambrosia artemisiifolia*, *Ambrosia psilostachya*, *G. dracunculoides*, and *Xanthium strumarium* with the exotic annual grass *Bromus japonicus* (Table 6). In the more slowly colonized sites (J6NF, J6NNF, G5) colonization progressed from the edges through vegetative growth, followed by seedling establishment. J6NF and J6NNF contained the only remaining bison wallows among the tilled treatments by summer 2003. The edges of J6NF and J6NNF contained many of the same ruderals found in J6SF, J6SNF, and J6NC (Table 6). However the exotic perennial grass *Cynodon dactylon* and the creeping forb *Polygonum aviculare* were most common species towards the center of the plots (Table 6). *C. dactylon* and *P. aviculare* were the

dominant species in the G5 tilled sites (Table 6). *C. dactylon* was especially important at G5N, which had the greatest plant cover of the tilled sites at G5 (Table 6).

Discussion

Soil carbon, nitrogen, and phosphorus

Petroleum contamination and bioremediation cause significant changes in nutrient dynamics. The changes in soil nutrients observed during this study are similar to those observed elsewhere due to petroleum contamination (Chaîneau *et al.*, 2003), high salinity (Pathak and Rao, 1998, Curtin *et al.*, 1992), tilling (Jug *et al.*, 1999), fertilizing (Richards *et al.*, 1995, Motavalli and Miles, 2002), and grazing by large ungulates (Pastor *et al.*, 1993, Frank and Evans, 1997, Augustine, 2003) in a variety of laboratory and field contexts. It has been acknowledged that field data are necessary for comparison to laboratory and modeling results to evaluate the success of bioremediation processes at a practical scale (Sturman *et al.*, 1995). In contrast to past research, this study demonstrates the occurrence of processes *in situ* during remediation of a native plant community. The trends observed here support some hypotheses based on previous *ex situ* observations.

Tilling causes mineralization of organic N and P forms over a relatively short period of time and reduces plant available nutrient pools through N losses and soil adsorption of inorganic P. The decreased N observed in this study following tilling is consistent with studies comparing tillage systems (Kandler and Böhm, 1996). Net N-mineralization following removal of plants through tilling should account for N losses because NO_3-N is susceptible to leaching and denitrification (Kandler and Böhm, 1996, Schlesinger, 1997). The organic and inorganic P levels in native tallgrass prairie soils at

the TGPP are similar to those found in other grassland soils (Tiessen and Moir, 1993, Cross and Schlesinger, 2001, Motavalli and Miles, 2002). The relationships between soil P, texture, and slope position are consistent with previous studies (Agbenin and Tiessen, 1995, Leinweber *et al.*, 1997). Also in agreement with other studies, tilling had greater effect than fertilization on P_o (Rubæk *et al.*, 1999), and organic P was a larger proportion of the total P in native prairie sites compared with the cultivated plots, especially in the form of NaOH-P_o (O'Halloran *et al.*, 1987b, Motavalli and Miles, 2002). Increases in $NaHCO_3$-P_i due to tilling were consistent with previous findings (Dormaar and Willms, 2000). This suggests that large portions of the organic P pools have been converted to P_i during the four years since tilling began. Increases in NaOH-P_i suggest that $NaHCO_3$-P_i tends to be adsorbed by hydroxide-soluble minerals (Bolin *et al.*, 1983, Ramirez and Rose, 1992). Because organic pools are the primary source of plant available P in non-fertilized systems, these changes may represent a long-term decrease in P availability (Beck and Sanchez, 1994, Buresh *et al.*, 1997). In addition the negative association between residual-P and tilling observed in this study has been demonstrated in other studies by significant differences in soils with prolonged periods of cultivation (Hedley *et al.*, 1982, Schoenau *et al.*, 1989). The results of this and other studies suggest that the tilling causes slow changes in residual fractions through long-term weathering whereas changes in the other P fractions may occur relatively quickly through mineralization of organic material in previously untilled grasslands. In addition to increased mineralization and weathering, decreases in total P in unfertilized, tilled soils on sloped sites were similar to previous studies that suggested that losses were due to erosion and/or leaching (Hedley *et al.*, 1982, Frossard *et al.*, 1989)

Fertilizer application increases the initial rate of hydrocarbon degradation and offsets nutrient losses due to tilling during bioremediation. Total N and Net N-mineralization rates and the sum of labile P fractions were similar in fertilized and prairie sites. In agreement to previous studies resin-P_i, $NaHCO_3$-P_i, and NaOH-P_i were more strongly correlated with fertilizer application than with tilling alone (Beck and Sanchez, 1994, Richards *et al.*, 1995, Motavalli and Miles, 2002). However, significant correlations between fertilizing and cHCl-P_i and cHCl-P_o suggest that some correlations are influenced by slope position. The results of this study do not support the hypotheses that fertilization will cause locally decreased diversity through site eutrophication or persistence of invasive species through positive feedbacks between species composition and nutrient availability.

In addition to P increases through fertilizing, unexpected increases in total P at J6NF and J6NNF may have been caused by bison. Studies have reported that sites frequented by livestock and other ungulates experienced enrichments of N and P, in which high P persisted while N declined through time (Frank and Evans, 1997, Augustine, 2003). The presence of bison wallows only at these sites suggests a similar relationship in this study. These substantial increases in P resulting in unusually high P availability at J6NF could change community dynamics either directly or through effects on N cycling (Janssens *et al.*, 1998).

High salinity may also have significant effects on soil nutrients. Reduced N-mineralization and strongly inhibited nitrification were observed in saline soils during this and previous studies (Laura, 1977, Pathak and Rao, 1998). Azam and Muller (2003) suggested that sites with high inorganic N like G5 may continue to experience significant

N losses because denitrifying enzyme activity is not strongly inhibited by salinity. Although P losses were related to tilling and topography (Hedley *et al.*, 1982), salinity may also have contributed by increasing P solubility and the susceptibility of the soil to erosion (Curtin *et al.*, 1992).

Many of the biogeochemical characteristics of prairie soils were partially restored through revegetation. Plant species can affect soil chemistry, and native plant growth has been observed in previous studies to drive ecosystem processes in the trajectory of the original system in restored grasslands (Vinton and Burke, 1997, Baer *et al.*, 2002, Knops *et al.*, 2002). In this study RDAs and PCA show that vegetation cover was strongly associated with nutrient conditions characteristic of prairie soils. These observations support the importance of the plant community in the restoration of ecosystem processes.

Revegetation

Factors affecting revegetation at these sites include disturbance by bison, high salinity, and residual petroleum hydrocarbons.

Although bison can play an important role in maintaining the prairie plant community (Knapp *et al.*, 1999), they appear to have a negative affect on community restoration following bioremediation. The tilled surfaces served as temporary wallowing sites, and remaining bare spaces at J6NNF and J6NF may be at least partially maintained by ongoing disturbance and compaction by the animals. The effects of bison on community restoration are also suggested by the revegetation patterns and species composition of sites containing wallows. Previous studies have found that trampling is an important factor in seedling establishment (Sun, 1991) and that plants with tall growth forms are more sensitive to trampling (Sun and Liddle, 1993). In this study the

revegetated sites without bison wallows had a relatively random pattern of plant propagule establishment. The species in these sites were characterized both by reproduction primarily through seed dispersal and erect growth habits. In contrast, J6NNF and J6NF were colonized through vegetative growth from the edges followed by seedling germination. The primary species growing into open spaces at J6NNF and J6NF (*C. dactylon*, *P. aviculare*) were characterized by prostrate growth habits and have been described as trampling tolerant (Dan and Liddle, 1991, Parker, 2004). Although the communities near bison wallows appear to be adapted to this form of disturbance, the complete revegetation of J6NC by annual species with erect growth forms directly adjacent to J6NNF and J6NF suggests that the presence of bison cannot be the only factor contributing to the difficulty of seedling establishment and revegetation.

Conventional bioremediation methods did not promote substantial community restoration in brine-contaminated soils during the course of this study. G5N had the greatest plant cover and a substantially lower salinity than G5S and G5M by fall 2003. This implies that there is a relationship between revegetation and salinity at these sites. In addition the primary colonizing species at G5 (*C. dactylon*, *P. aviculare*) are salt tolerant (Marcum, 1999, Foderaro and Ungar, 1997, Lee *et al.*, 2000). Significant disturbance from bison wallowing was not observed at G5, possibly due to the rockiness and steep slopes at this site. However, it is possible that residual hydrocarbons might influence the distribution of saline patches and contribute to the inhibition of revegetation through mechanisms other than salt stress. Given the success of *C. dactylon* through the fall of 2003 and slow decreases in salinity, *C. dactylon* will likely remain the dominant species at G5 throughout the near future.

Soils with high initial TPH concentrations had high nutrient availability and continued to exhibit poor plant growth 4 years after the beginning of bioremediation. Residual hydrocarbons have been hypothesized to limit plant growth through nutrient limitation (Amadi *et al.*, 1993, Xu and Johnson, 1997, Rentz *et al.*, 2003), inherent toxicity (deOng *et al.*, 1927, Crafts and Reiber, 1948, Baker, 1970, Chaîneau *et al.*, 1997, Chaîneau *et al.*, 2003), and water stress (Baker, 1970, Udo and Fayemi, 1975, Brown *et al.*, 1982, Amakiri and Onofeghara, 1983, Klokk, 1984, Bossert and Bartha, 1985, Li *et al.*, 1997). Previous studies have demonstrated that hydrocarbons can limit plant growth by stimulating competition for nutrients by microorganisms (Xu and Johnson, 1997). The high C:N ratios at contaminated sites might be indicative of some N limitation (Xu and Johnson, 1997). However, nutrient limitation seems unlikely at J6 north because plant available N and P and net N-mineralization rates remained high throughout the study. High N availability could be expected to persist even with high C:N ratios if the remaining C is in the form of recalcitrant petroleum hydrocarbons (Brown *et al.*, 1998). Thus, the alternative hypotheses that residual hydrocarbons limited revegetation through inherent toxicity and/or water stress appear to be better supported in this study.

My analyses do not test the independent effects of the inherent toxicity of residual hydrocarbons on plant growth, but more rigorous tests have shown significant effects in other studies. The toxicity of oils is usually attributed to volatile compounds capable of rapid or acute injury to plant tissues. Because weathered crude oil has much lower concentrations of low-boiling and hydrophilic compounds, it is generally less toxic to vegetation than fresh oil (Baker, 1970). However, slow or chronic injury to plants by heavy fractions has been previously documented (deOng *et al.*, 1927, Crafts and Reiber,

1948). In addition, metabolic by-products of hydrocarbon degradation may cause perturbations to cellular metabolisms as a result of structural damage to membranes and chloroplasts (Chaîneau *et al.*, 2003). Elimination of acute soil toxicity through bioremediation has been reported based on earthworm survival and seed germination (Salanitro *et al.*, 1997). However, germination and survival rates are less sensitive indicators than measures of plant growth and photosynthesis (Nwachukwu, *et al.*, 2001, Chaîneau *et al.*, 2003, Suleiman and Bhat, 2003). Chaîneau *et al.* (2003) reported that 4 out of 5 tested plant species exhibited 100% germination, and mortality of earthworms was not increased in crude oil contaminated soils with TPH reduced to residual concentrations (76% reduction). However, plant growth was significantly reduced, and a 25% inhibition of photosynthesis was measured using the Hill reaction (Dicks, 1974, Chaîneau *et al.*, 2003). Because plant growth is both sensitive to residual toxicity and crucial for community restoration, further consideration should be given to inherent toxicity and the choice of toxicity indicators used to determine remediation endpoints.

Evidence collected in this study, while not ruling out growth inhibition through inherent toxicity, indicates that hydrocarbon related water stress may be the most significant factor limiting revegetation. Because the availability of petroleum hydrocarbons to microbial degradation is positively correlated with water solubility, residual hydrocarbons following bioremediation are extremely hydrophobic (Nocentini *et al.*, 2000). Hydrophobic properties resulting from residual hydrocarbons limit the ability of soils to absorb and hold water (Morgan and Watkinson, 1989, Fine *et al.*, 1997, Deka *et al.*, 1997, Sawatsky and Li, 1997). As a result, plants growing in these soils may experience water stress even when rainfall is high (Ritsema and Dekker, 1994, Sawatsky

and Li, 1997). Reduced plant growth has been attributed to water stress in bioremediated soils in several studies (Brown *et al.*, 1982, Li *et al.*, 1997, Chaîneau *et al.*, 2003).

In contrast, some researchers have reported minimal effects on plant growth even with residual hydrocarbon levels above 8000 mg kg^{-1} (Salanitro *et al.*, 1997). However, Li *et al.* (1997) observed that these results were produced with optimal water conditions and small homogeneous soil samples. Under natural conditions soil wetting only occurs with a prolonged contact period (Li *et al.*, 1997), and water will tend to follow the path of least resistance through the soil profile resulting in isolated patches of high moisture which may intensify with time (Morgan and Watkinson, 1989, Ritsema and Dekker, 1994, Sawatsky and Li, 1997). In addition, a soil's water repellency is dependent on its water content. As contaminated soils dry, water repellency shifts from being almost undetectable to very severe (King, 1981, McNabb *et al.*, 1992, Sawatsky and Li, 1997). The moisture level at which this shift occurs has been called the "critical soil water content" (Dekker and Ritsema, 1994). Infiltration of bioremediated soils has been observed to decrease by more than two orders of magnitude as moisture levels dropped below this point (Sawatsky and Li, 1997). In contrast, moisture levels above the critical content resulted in sorbtivity that approached the control soil (King, 1981, Sawatsky and Li, 1997). Due to this phenomenon, nutrient analyses and ecotoxicity assays may not be accurate indicators of the ability of bioremediated soils to support plant growth when results are not observed at a variety of water conditions (Li *et al.*, 1997).

I made several observations suggesting that revegetation is limited by water stress. Water infiltration patterns were poor in contaminated soils in agreement with findings from other studies (Sawatsky and Li, 1997). In addition the significant negative

correlation between field capacity and TPH concentrations and the nonsignificant correlations between field capacity, soil texture and soil C suggest that residual hydrocarbon concentration is the most important soil characteristic controlling water availability in contaminated sites. Greatly reduced revegetation in contaminated sites in comparison to the tilled control suggests that residual hydrocarbons were a more important factor in revegetation than bison wallowing. In addition, *C. dactylon* and *P. aviculare* may be favored in contaminated sites more by their tolerance to water stress than trampling (Dan and Liddle, 1991, Parker, 2004). The interaction of bison wallowing and hydrocarbon-mediated water stress may represent a form of positive feedback in which soil compaction by bison inhibits hydrocarbon degradation and plant growth. In turn, residual hydrocarbons could inhibit the reclamation of bison wallows by native ruderals, thus maintaining a site for repeated visits by bison herds. In addition, I observed that soil moisture content increased dramatically in sites experiencing revegetation. Such increases in soil moisture beneath vegetation at the edges of contaminated sites may explain the pattern of seedling establishment behind the advancing front of vegetative growth but not ahead of it. These observations support the hypotheses that phytotoxicity is due to petroleum hydrocarbon concentrations (Duncan *et al.*, 2003) and that phytotoxicity may persist over a long time following removal of degradable compounds through bioremediation (Chaîneau *et al.*, 2003).

Community structure

This study suggests that restoration of community structure through low-cost *in situ* bioremediation remains a difficult challenge when high levels of contamination occur. The initial TPH concentration can be viewed as two parts: a first part that can be

depleted quickly and a second one that is not affected by bioremediation at all (Nocentini *et al.*, 2000, Allen *et al.*, 1997, Williamson *et al.*, 1997). According to this model of hydrocarbon degradation, spills with large initial concentrations of recalcitrant compounds may results in long term vegetation shifts due to persisting xeric conditions following bioremediation. In addition to the general inhibition of plant growth, there is evidence that hydrocarbon contamination may have a strong effect on species composition (Chaîneau *et al.*, 1997, Xu and Johnson, 1997, Suleiman and Bhat, 2003). In one study, all of the dominant species (including *C. dactylon*) growing in crude oil contaminated soils exhibited vegetative growth as their primary mode of propagation (Baruah and Sarma, 1996). Vegetative growth may be favored in part because the spatial variability of water and solute movement in hydrophobic soils is lower below the repellent layer (Ritsema *et al.*, 1993). This would give a competitive advantage to propagules growing from rhizomes or supplemented by metabolites through clonal connections over isolated seedlings.

In addition to the threat of changes in community structure, bioremediation may favor exotic species. Modifications to existing disturbance regimes might have the largest influence on invasibility (Hobbs and Huenneke, 1992). Disturbance or changes in resource availability may be a prerequisite of establishment of exotic species (Burke and Grime, 1996, Smith and Knapp, 1999, Davis *et al.*, 2000). Unfortunately, vegetative propagation, which appears to favor dominance following disturbance caused by hydrocarbon contamination (Baruah and Sarma, 1996), is also one of the primary characteristics associated with invasive of plant species (Kolar and Lodge, 2001).

It has been suggested that managing for maximum diversity in tallgrass prairie may lead to greater establishment and persistence of exotic species over time (Smith and Knapp, 2001). For example, bison and other ungulates are thought to play a role in maintaining diversity by increasing spatial heterogeneity through redistribution of soil nutrients and behaviors such as preferential grazing and wallowing (Collins and Steinauer, 1998, Knapp *et al.*, 1999, Augustine, 2003). However, bison wallowing may also favor exotic species such as *C. dactylon*. In the future, managers of restored grasslands may need to choose an appropriate balance between practices promoting biological diversity and those reducing ecosystem invasibility.

Conclusions

Petroleum contamination and bioremediation cause substantial changes in biogeochemical processes and community structure. Tilling causes mineralization of organic N and P forms over relatively short period of time and reduces plant available nutrient pools by N losses and soil adsorbtion of inorganic P. Conventional bioremediation methods did not promote substantial community restoration in brine-contaminated soils during the course of this study. Soils with high initial TPH concentrations had high nutrient availability but continued to exhibit poor plant growth 4 years after the beginning of bioremediation. These results support the hypothesis that phytotoxicity is due changes in the physical properties of soils due to high petroleum hydrocarbon concentrations (Duncan *et al.*, 2003), and that phytotoxicity may negatively affect community restoration for a long time following removal of degradable compounds through bioremediation (Chaîneau *et al.*, 2003).

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Table 1. Sand, silt, clay, pH, and field capacity (mean ± standard deviation) for J6 and G5 sites in 2002 and 2003. G5 slope positions: (1) = upslope; (2) = mid slope; (3) = downslope.

Plot	Code	n	2002				2003				
			% Sand	% Silt	% Clay	Soil pH	% Sand	% Silt	% Clay	Soil pH	Field Capacity (%)
J6											
North fertilized	J6NF	5	27.0 ± 4.1	50.0 ± 6.4	23.0 ± 8.9	6.22 ± 0.17	29.5 ± 4.5	44.0 ± 1.4	26.5 ± 3.8	5.97 ± 0.38	19.3 ± 1.6
North no fertilizer	J6NNF	5	31.5 ± 2.9	59.0 ± 1.4	9.5 ± 2.1	6.42 ± 0.13	31.5 ± 2.2	58.0 ± 3.7	10.5 ± 2.7	6.14 ± 0.16	18.0 ± 1.7
North tilled control	J6NC	5	31.0 ± 1.4	44.5 ± 3.3	24.5 ± 2.1	6.39 ± 0.16	31.0 ± 2.2	44.5 ± 2.7	24.5 ± 1.1	6.23 ± 0.13	21.3 ± 1.1
North prairie	J6NN	5	27.5 ± 1.8	46.0 ± 3.4	26.5 ± 2.2	6.11 ± 0.29	28.0 ± 4.1	45.5 ± 2.7	26.5 ± 3.8	5.77 ± 0.08	19.9 ± 0.8
South fertilized	J6SF	5	26.0 ± 2.2	42.0 ± 4.5	32.0 ± 2.7	6.54 ± 0.30	27.0 ± 3.3	39.5 ± 2.1	33.5 ± 2.2	6.29 ± 0.12	22.1 ± 0.4
South no fertilizer	J6SNF	5	29.0 ± 1.4	36.5 ± 1.4	34.5 ± 2.1	7.46 ± 0.20	31.0 ± 1.4	36.0 ± 1.4	33.0 ± 1.1	7.16 ± 0.27	21.9 ± 1.0
South prairie	J6SN	5	28.5 ± 2.2	43.0 ± 2.1	28.5 ± 2.2	6.28 ± 0.08	29.5 ± 2.1	43.0 ± 2.1	27.5 ± 3.5	5.81 ± 0.07	21.9 ± 1.0
G5											
North (1)	G5N	3	34.2 ± 1.4	42.5± 4.3	23.3 ± 2.9	5.27 ± 0.18	30.0 ± 0.0	44.2 ± 6.3	25.8 ± 6.3	5.49 ± 0.12	16.6 ± 0.9
North (2)	G5N	3	33.3 ± 2.9	40.8 ± 1.4	25.8 ± 1.4	5.64 ± 0.02	28.3 ± 1.4	46.7 ± 3.8	25.0± 4.3	5.77 ± 0.06	20.5 ± 0.7
North (3)	G5N	3	35.8 ± 1.4	40.8 ± 1.4	23.3 ± 2.9	5.52 ± 0.09	34.2 ± 5.2	40.0 ± 2.5	25.8 ± 7.2	5.46 ± 0.09	20.1 ± 0.7
Middle (1)	G5M	3	34.2 ± 1.4	38.3 ± 1.4	27.5 ± 2.5	5.43 ± 0.05	35.0 ± 5.0	40.8 ± 1.4	24.2 ± 3.8	5.73 ± 0.07	15.3 ± 1.6
Middle (2)	G5M	3	33.3 ± 1.4	40.0 ± 0.0	26.7 ± 1.4	5.18 ± 0.04	31.7 ± 1.4	40.0 ± 2.5	28.3 ± 1.4	5.21 ± 0.06	16.3 ± 1.1
Middle (3)	G5M	3	38.3± 1.4	38.3 ± 3.8	23.3 ± 3.8	5.18 ± 0.02	32.5 ± 3.5	37.5 ± 0.0	30.0 ± 3.5	5.29 ± 0.10	18.8 ± 0.4
South (1)	G5S	3	35.0 ± 0.0	38.3 ± 2.9	26.7 ± 2.9	5.51 ± 0.17	34.2 ± 1.4	39.2 ± 3.8	26.7 ± 5.2	5.72 ± 0.22	16.9 ± 0.4
South (2)	G5S	3	35.8 ± 1.4	37.5 ± 2.5	26.7± 1.4	5.42 ± 0.03	37.5 ± 2.5	40.8 ± 2.9	21.7 ± 5.2	5.46 ± 0.15	20.5 ± 0.5
South (3)	G5S	3	35.8 ± 1.4	41.7 ± 1.4	22.5 ± 2.5	5.33 ± 0.04	35.8 ± 5.8	38.3 ± 1.4	25.8 ± 5.2	5.32 ± 0.04	21.5 ± 1.4
Prairie (1)	G5C	3	37.5 ± 2.5	35.8 ± 1.4	26.7 ± 1.4	5.70 ± 0.13	38.3 ± 2.9	36.7 ± 2.9	25.0 ± 5.0	5.81 ± 0.18	20.3 ± 0.7
Prairie (2)	G5C	3	30.8 ± 1.4	45.8 ± 1.4	23.3 ± 1.4	5.59 ± 0.14	38.3 ± 1.4	36.7 ± 3.8	25.0 ± 4.3	7.26 ± 0.10	20.5 ± 1.9
Prairie (3)	G5C	3	38.3 ± 1.4	41.7 ± 1.4	20.0 ± 0.0	5.59 ± 0.06	34.2 ± 1.4	38.3 ± 1.4	27.5 ± 0.0	6.70 ± 0.13	20.6 ± 0.8

Table 2. Total carbon and nitrogen, KCl extractable inorganic N, potential N-cycling rates, inorganic (Pi) and organic (Po) sequential P fractions, sand, silt, clay, pH, and field capacity (mean ± standard deviation) for prairie controls.

	2002 (n = 20)			2003 (n = 40)		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Total C (%)	2.97 ± 0.64	1.35	4.22	3.13 ± 0.54	1.32	4.11
C:N ratio	14.2 ± 0.7	12.5	15.5	12.3 ± 1.5	8.6	16.2
Total N (%)	0.21 ± 0.04	0.09	0.31	0.30 ± 0.05	0.13	0.38
NO_3-N (mg Kg^{-1})	0.6 ± 0.3	0.1	1.2	0.9 ± 0.8	0.4	5.5
NH_4-N (mg Kg^{-1})	6.7 ± 2.6	3.2	10.9	4.0 ± 2.5	2.0	17.7
N-mineralization (mg Kg^{-1} d^{-1})	--	--	--	0.24 ± 0.17	-0.10	0.69
Nitrification (mg Kg^{-1} d^{-1})			--	0.31 ± 0.13	0.02	0.55
Total P (mg Kg^{-1})	259.1 ± 77.6	144.5	489.0	277.2 ± 79.1	164.2	648.1
Resin P_i (mg Kg^{-1})	9.3 ± 2.4	5.6	15.9	7.9 ± 1.9	3.6	14.1
$NaHCO_3$ P_i (mg Kg^{-1})	5.9 ± 1.3	4.2	10.1	5.5 ± 0.7	3.8	7.6
NaOH P_i (mg Kg^{-1})	15.7 ± 3.8	10.7	26.9	16.6 ± 3.5	10.3	28.8
1M HCl P_i (mg Kg^{-1})	15.2 ± 12.1	7.2	57.3	14.0 ± 20.4	2.4	132.6
cHCl P_i (mg Kg^{-1})	37.6 ± 15.0	11.1	76.7	35.3 ± 14.6	19.0	106.6
$NaHCO_3$ P_o (mg Kg^{-1})	10.2 ± 5.7	1.2	21.4	9.4 ± 6.2	0.6	29.3
NaOH P_o (mg Kg^{-1})	61.0 ± 22.0	33.5	114.2	69.0 ± 22.7	20.7	117.0
cHCl P_o (mg Kg^{-1})	51.5 ± 25.4	12.9	125.0	35.3 ± 14.6	19.0	106.6
Residual P (mg Kg^{-1})	52.6 ± 14.6	32.8	83.7	52.7 ± 10.3	32.7	79.2
Sand (%)	25.7 ± 4.8	17.8	36.9	25.9± 11.6	10.0	65.0
Silt (%)	37.4 ± 4.5	28.3	43.4	42.3 ± 8.0	22.5	57.5
Clay (%)	36.9 ± 5.0	20.2	43.3	31.8 ± 7.9	12.5	50.0
Soil pH	6.4 ± 0.5	5.8	7.4	6.6 ± 0.5	6.0	7.9
Field Capacity (%)				21.5 ± 2.5	13.4	26.0

Table 3. Potential N-cycling rates for J6 and G5 sites (mean ± standard deviation).

Plot	Code	n	Summer 2003		Fall 2003	
			N-mineralization rate (mg Kg^{-1} d^{-1})	Nitrification rate (mg Kg^{-1} d^{-1})	N-mineralization rate (mg Kg^{-1} d^{-1})	Nitrification rate (mg Kg^{-1} d^{-1})
J6						
North fertilized	J6NF	5	0.61 ± 0.43	0.60 ± 0.42	0.18 ± 0.09	0.21 ± 0.10
North no fertilizer	J6NNF	5	0.45 ± 0.10	0.54 ± 0.15	0.17 ± 0.12	0.15 ± 0.11
North tilled control	J6NC	5	0.57 ± 0.07	0.57 ± 0.08	0.08 ± 0.17	0.14 ± 0.12
North prairie	J6NN	5	0.63 ± 0.22	0.40 ± 0.12	0.04 ± 0.09	0.08 ± 0.08
South fertilized	J6SF	5	0.52 ± 0.06	0.47 ± 0.06	0.20 ± 0.12	0.13 ± 0.07
South no fertilizer	J6SNF	5	0.46 ± 0.04	0.44 ± 0.04	0.03 ± 0.03	0.01 ± 0.01
South prairie	J6SN	5	0.57 ± 0.07	0.51 ± 0.06	0.07 ± 0.03	0.08 ± 0.04
G5						
North	G5N	9	0.30 ± 0.13	0.03 ± 0.11	0.23 ± 0.10	0.19 ± 0.07
Middle	G5M	9	0.16 ± 0.25	-0.02 ± 0.14	-0.02 ± 0.21	0.00 ± 0.18
South	G5S	9	0.31 ± 0.15	-0.01 ± 0.11	0.10 ± 0.09	0.13 ± 0.10
Prairie	G5C	9	0.46 ± 0.11	0.50 ± 0.09	0.14 ± 0.13	0.18 ± 0.14

Table 4. Inorganic (Pi) and organic (Po) sequential fractions (mg Kg^{-1}) (mean ± standard deviation) from J6 and G5 sites (summer 2002). cHCl = concentrated hydrochloric acid. G5 slope positions: (1) = upslope; (2) = mid slope; (3) = downslope.

Plot	Code	n	Inorganic P (Pi)					Organic P (Po)			Residual P	Total P
			Resin	$NaHCO_3$	NaOH	1M HCl	cHCl	$NaHCO_3$	NaOH	cHCl		
J6												
North fertilized	J6NF	5	20.3 ± 2.8	17.8 ± 2.7	45.6 ± 8.3	47.0 ± 8.5	27.3 ± 3.8	14.9 ± 6.7	52.6 ± 23.4	42.7 ± 5.6	46.3 ± 7.0	314.5 ± 42.6
North no fertilizer	J6NNF	5	6.5 ± 1.0	9.5 ± 1.0	23.2 ± 2.9	20.4 ± 0.7	20.8 ± 1.9	4.5 ± 0.1	45.4 ± 16.3	37.8 ± 4.8	44.5 ± 3.5	212.6 ± 12.6
North tilled control	J6NC	5	7.9 ± 1.0	8.8 ± 0.4	24.5 ± 4.4	17.7 ± 3.3	28.8 ± 3.6	1.7 ± 0.3	48.5 ± 10.4	46.3 ± 8.8	43.0 ± 8.0	227.2 ± 35.4
North prairie	J6NN	5	9.5 ± 1.9	7.4 ± 0.8	18.9 ± 1.9	10.7 ± 2.4	25.7 ± 2.8	6.6 ± 2.5	103.1 ± 11.3	43.9 ± 5.4	52.8 ± 1.6	278.6 ± 11.5
South fertilized	J6SF	5	9.9 ± 3.0	9.7 ± 2.0	27.8 ± 4.5	15.7 ± 5.9	33.8 ± 5.3	6.0 ± 2.5	59.7 ± 14.8	52.0 ± 9.5	54.6 ± 8.3	269.1 ± 41.4
South no fertilizer	J6SNF	5	6.0 ± 0.9	7.1 ± 1.0	20.0 ± 2.2	14.0 ± 4.4	30.4 ± 2.3	1.9 ± 0.1	37.0 ± 6.5	49.0 ± 4.2	61.7 ± 3.8	227.0 ± 15.6
South prairie	J6SN	5	7.9 ± 1.4	6.8 ± 0.5	19.4 ± 0.9	10.9 ± 3.3	28.2 ± 4.3	2.7 ± 1.6	65.8 ± 11.5	52.0 ± 8.4	58.9 ± 3.8	252.4 ± 25.5
G5												
North (1)	G5N	3	8.0 ± 3.1	9.0 ± 0.3	25.8 ± 2.5	9.9 ± 3.0	16.5 ± 1.5	9.2 ± 5.0	61.7 ± 10.1	29.0 ± 3.0	33.7 ± 4.7	202.9 ± 24.0
North (2)	G5N	3	9.9 ± 3.4	9.1 ± 1.6	29.8 ±3.5	10.0 ± 3.0	24.3 ± 3.2	8.1 ± 3.0	63.2 ± 5.3	41.6 ± 6.6	44.9 ± 3.0	240.8 ± 17.7
North (3)	G5N	3	9.8 ± 2.0	9.9 ± 1.8	30.2 ± 5.1	11.5 ± 3.8	21.8 ± 0.9	8.7 ± 5.8	66.2 ± 9.5	43.0 ± 2.7	41.7 ± 3.2	242.8 ± 20.5
Middle (1)	G5M	3	3.2 ± 0.9	6.7 ± 0.8	17.6 ± 2.0	6.0 ± 1.2	21.2 ± 4.5	6.0 ± 2.5	39.7 ± 0.1	30.5 ± 6.6	37.8 ± 6.8	168.7 ± 16.6
Middle (2)	G5M	3	3.5 ± 0.5	6.6 ± 0.3	16.7 ± 1.3	5.1 ± 0.7	22.7 ± 3.5	5.6 ± 3.6	36.6 ± 6.7	38.5 ± 6.3	38.5 ± 2.8	174.1 ± 8.4
Middle (3)	G5M	3	5.1 ± 1.0	7.0 ± 0.5	17.7 ± 0.6	5.5 ± 1.4	22.8 ± 1.1	1.7 ± 0.3	40.5 ± 3.7	47.2 ± 6.2	42.6 ± 1.6	190.1 ± 5.1
South (1)	G5S	3	7.6 ± 2.2	9.4 ± 1.1	28.7 ± 5.2	10.3 ± 3.8	31.0 ± 0.5	6.1 ± 2.7	43.0 ± 5.1	50.8 ± 5.7	39.6 ± 2.0	226.6 ± 24.2
South (2)	G5S	3	8.6 ± 0.7	8.3 ± 0.3	24.5 ± 2.9	8.7 ± 1.4	35.9 ± 9.9	2.8 ± 1.5	46.3 ± 7.3	51.7 ± 6.2	38.5 ± 2.9	225.2 ± 22.9
South (3)	G5S	3	7.0 ± 2.3	7.8 ± 0.3	23.5 ± 0.4	5.5 ± 0.7	26.6 ± 0.2	4.7 ± 3.0	62.0 ± 7.4	49.6 ± 5.2	40.4 ± 1.8	227.2 ± 15.6
Prairie (1)	G5C	3	7.6 ± 1.4	7.0 ± 1.0	17.1 ± 0.8	12.2 ± 1.3	27.7 ± 7.0	4.1 ± 2.2	53.3 ± 10.5	58.0 ± 9.7	36.6 ± 3.7	223.6 ± 33.7
Prairie (2)	G5C	3	6.2 ± 1.5	6.5 ± 0.9	14.5 ± 1.9	4.4 ± 0.7	29.7 ± 0.8	1.8 ± 0.6	47.2 ± 5.3	53.5 ± 8.5	42.6 ± 4.2	206.4 ± 17.1
Prairie (3)	G5C	3	5.3 ± 0.8	6.6 ± 0.3	15.8 ± 2.5	5.5 ± 1.8	26.0 ± 2.6	1.7 ± 0.3	54.1 ± 10.0	51.0 ± 5.0	43.4 ± 3.2	209.4 ± 22.1

Table 5. Inorganic (Pi) and organic (Po) sequential fractions (mg Kg^{-1}) (mean ± standard deviation) from J6 and G5 sites (summer 2003). cHCl = concentrated hydrochloric acid. G5 slope positions: (1) = upslope; (2) = mid slope; (3) = downslope.

Plot	Code	n	Inorganic P (Pi)					Organic P (Po)			Residual P	Total P
			Resin	$NaHCO_3$	NaOH	1M HCl	cHCl	$NaHCO_3$	NaOH	cHCl		
J6												
North fertilized	J6NF	5	17.7 ± 1.8	29.1 ± 3.0	68.0 ± 9.1	38.1 ± 9.2	31.0 ± 2.2	3.9 ± 3.2	83.4 ± 42.0	46.6 ± 9.2	43.5 ± 4.1	361.2 ± 50.9
North no fertilizer	J6NNF	5	7.8 ± 1.2	13.6 ± 1.2	33.9 ± 3.4	23.2 ± 3.0	22.5 ± 2.4	3.0 ± 1.7	83.1 ± 25.4	31.2 ± 6.8	43.9 ± 1.3	262.3 ± 22.9
North tilled control	J6NC	5	8.7 ± 1.6	7.5 ± 0.9	21.6 ± 1.3	12.4 ± 2.0	29.3 ± 1.7	1.5 ± 0.7	62.2 ± 10.8	44.8 ± 3.1	43.4 ± 4.2	231.4 ± 12.9
North prairie	J6NN	5	8.5 ± 1.0	5.2 ± 0.6	17.5 ± 1.5	9.5 ± 0.1	23.7 ± 3.8	7.1 ± 2.4	101.7 ± 32.3	38.3 ± 9.9	48.5 ± 3.0	260.0 ± 31.7
South fertilized	J6SF	5	9.6 ± 2.6	9.6 ± 2.9	30.5 ± 7.8	15.1 ± 3.5	37.1 ± 2.2	4.6 ± 2.2	77.0 ± 6.5	57.6 ± 5.0	51.5 ± 4.6	292.6 ± 17.0
South no fertilizer	J6SNF	5	7.0 ± 1.4	4.6 ± 1.1	20.8 ± 4.3	13.7 ± 5.7	32.6 ± 2.9	2.7 ± 1.4	44.4 ± 7.4	48.0 ± 8.8	51.9 ± 4.2	225.7 ± 32.6
South prairie	J6SN	5	7.6 ± 0.9	4.6 ± 1.0	19.0 ± 2.0	7.6 ± 1.0	28.3 ± 3.7	11.5 ± 2.6	92.7 ± 25.3	41.8 ± 10.0	48.7 ± 3.2	261.7 ± 30.7
G5												
North (1)	G5N	3	5.6 ± 1.7	7.3 ± 0.3	28.9 ± 3.0	6.8 ± 3.0	20.8 ± 2.4	6.8 ± 3.2	58.5 ± 14.5	31.2 ± 1.2	31.2 ± 5.9	197.1 ± 21.0
North (2)	G5N	3	6.3 ± 2.5	7.3 ± 1.3	27.2 ± 1.6	6.0 ± 2.6	34.3 ± 4.6	4.8 ± 1.6	64.3 ± 10.5	45.3 ± 5.2	45.0 ± 1.4	240.4 ± 15.9
North (3)	G5N	3	7.4 ± 0.2	8.4 ± 0.6	30.0 ± 1.2	11.6 ± 3.5	31.1 ± 1.3	5.4 ± 0.8	68.2 ± 7.8	44.2 ± 3.3	36.3 ± 1.4	242.4 ± 8.9
Middle (1)	G5M	3	3.3 ± 0.8	6.1 ± 0.8	21.1 ± 3.7	5.6 ± 2.0	21.2 ± 2.3	3.0 ± 0.7	48.8 ± 13.8	30.4 ± 5.2	32.4 ± 2.9	171.8 ± 27.7
Middle (2)	G5M	3	3.0 ± 0.2	4.6 ± 0.3	17.1 ± 2.0	5.5 ± 2.7	32.3 ± 1.6	2.4 ± 1.2	32.6 ± 7.9	40.1 ± 3.4	38.1 ± 13.1	175.7 ± 1.0
Middle (3)	G5M	3	4.2 ± 1.1	6.2 ± 0.8	19.8 ± 4.4	5.5 ± 0.8	32.6 ± 1.7	3.2 ± 1.2	46.8 ± 5.5	45.0 ± 1.5	40.1 ± 3.0	203.4 ± 11.2
South (1)	G5S	3	6.3 ± 1.7	7.7 ± 1.1	28.2 ± 2.3	11.6 ± 1.4	41.1 ± 2.2	3.3 ± 1.4	42.1 ± 9.8	49.7 ± 3.0	32.6 ± 0.3	222.7 ± 14.5
South (2)	G5S	3	6.9 ± 1.0	7.1 ± 0.7	25.2 ± 2.3	6.4 ± 0.1	47.5 ± 10.6	4.6 ± 1.2	41.2 ± 4.0	55.4 ± 7.1	47.1 ± 2.6	241.4 ± 21.7
South (3)	G5S	3	4.6 ± 0.8	7.5 ± 0.9	25.9 ± 1.4	5.2 ± 1.3	34.2 ± 1.8	6.3 ± 2.5	61.2 ± 11.5	49.4 ± 1.4	38.5 ± 5.5	232.7 ± 7.3
Prairie (1)	G5C	3	4.1 ± 1.0	5.0 ± 0.3	16.1 ± 0.5	4.2 ± 0.9	24.0 ± 5.0	3.3 ± 1.4	56.7 ± 7.7	33.8 ± 1.6	32.5 ± 5.3	179.6 ± 4.9
Prairie (2)	G5C	3	5.0 ± 0.6	5.6 ± 0.6	14.2 ± 1.3	17.2 ± 4.0	41.9 ± 4.0	3.1 ± 2.4	34.5 ± 1.0	58.4 ± 0.2	47.5 ± 10.6	227.4 ± 11.3
Prairie (3)	G5C	3	8.3 ± 0.5	6.7 ± 0.3	15.3 ± 4.9	10.7 ± 0.8	40.6 ± 3.2	1.5 ± 0.3	62.3 ± 5.9	65.5 ± 2.4	40.3 ± 3.5	251.8 ± 5.9

Table 6. Plant species characteristics and relative percentage canopy cover of most abundant plant species and bare ground during summer 2003. Phenology and Life-span : w = warm season, c = cool season, a = annual, p = perennial (Diggs et al. 1999). NF = J6 north fertilized, NNF = J6 north no fertilizer, NC = J6 tilled control, NN = J6 north prairie, SF = J6 south fertilized, SNF = J6 south no fertilizer, SN = J6 south prairie, N = G5 north fertilized, M = G5 middle no fertilizer, S = G5 south fertilized, C = G5 prairie.

		Estimated relative canopy cover (%)										
Species, phenology, & life-span	Growth form	NF	NNF	NC	NN	SF	SNF	SN	N	M	S	C
Ambrosia artemisiifolia ^{wa}	erect forb	20	15	20	0.5	0.5	10	–	–	–	1	0.5
Ambrosia psilostachya ^{wp}	erect forb	0.5	1	20	3	40	10	1				
Gutierrezia dracunculoides ^{wa}	erect forb	1	5	15	0.5	10	20	7	–	0.5	–	0.5
Andropogon gerardii ^{wp}	cespitose/rhizomatous grass	–	–	0.5	20	–	0.5	7	–	–	–	30
Bromus japonicus ^{ca}	cespitose grass	20	7	10	2	5	15	1	0.5	0.5	0.5	0.5
Cynodon dactylon ^{wp}	stoloniferous/rhizomatous grass	2	1	0.5	–	1	0.5	–	35	1	5	1
Digitaria sanguinalis ^{wa}	stoloniferous grass	–	2	0.5	–	0.5	3	–	–	–	–	–
Elymus virginicus ^{cp}	cespitose grass	–	–	0.5	0.5	0.5	0.5	3	–	–	–	–
Festuca arundincea ^{cp}	cespitose grass	1	0.5	0.5	3	0.5	0.5	10	1	–	5	30
Helianthus mollis ^{wp}	erect/rhizomatous forb	0.5	0.5	0.5	30	–	–	–	–	–	–	–
Panicum virgatum ^{wp}	rhizomatous grass	–	–	–	7	0.5	–	5	–	–	0.5	5
Polygonum aviculare ^{wa}	prostrate forb	15	1	0.5	–	–	0.5	–	3	2	3	–
Polygonum lapathifolium ^{wa}	erect forb	–	–	–	–	–	–	–	2	–	–	–
Setaria parviflora ^{wp}	rhizomatous grass	0.5	–	–	1	–	–	1	–	–	0.5	3
Solidago canadensis ^{wp}	erect/rhizomatous forb	0.5	–	0.5	5	0.5	–	20	–	–	–	0.5
Sorghastrum nutans ^{wp}	rhizomatous grass	–	–	–	3	–	–	3	–	–	–	2
Xanthium strumarium ^{wa}	erect forb	3	2	5	0.5	30	50	0.5	–	–	1	0.5
Bare ground		35	50	2	0.5	10	10	10	50	90	80	1

Table 7. Pearson's correlation coefficients between tilling, fertilizing, petroleum contamination, % plant cover, and soil C, N, and P from J6 site during summer 2003. n=35

	Tilled	Fertilized	Petroleum	% Cover
Total C	-0.014	0.216	0.101	-0.495**
C:N	0.329	0.121	0.506*	-0.890**
Total N	-0.303	0.195	-0.317	0.163
NO_3-N	0.489**	0.299	0.307	-0.622**
NH_4-N	-0.056	0.125	-0.021	-0.328
N-mineralization	-0.233	0.034	-0.196	0.162
Nitrification	0.164	0.138	0.094	-0.161
Resin P_i	0.245	0.688**	0.262	-0.305
$NaHCO_3$ P_i	0.525**	0.652**	0.532**	-0.712**
NaOH P_i	0.538**	0.715**	0.627**	-0.693**
1M HCl P_i	0.638**	0.555**	0.677**	-0.726**
cHCl P_i	0.378*	0.545**	0.312	0.276
$NaHCO_3$ P_o	-0.688**	-0.014	-0.284	0.166
NaOH P_o	-0.377*	0.007	-0.243	-0.018
cHCl P_o	0.227	0.461**	0.156	0.347*
Residual P	-0.182	0.008	0.081	0.368*

** Significant at the 0.01 level (2-tailed) (not corrected for multiple comparisons)

* Significant at the 0.05 level

Table 8. Pearson's correlation coefficients between field capacity, total petroleum hydrocarbons (TPH), soil C, and soil texture. Significances not corrected for multiple comparisons. n = 19

	TPH	C	Sand	Silt	Clay
Field capacity	-0.529	-0.051	-0.211	-0.010	0.141
Significance (2-tailed)	0.020	0.834	0.385	0.968	0.566

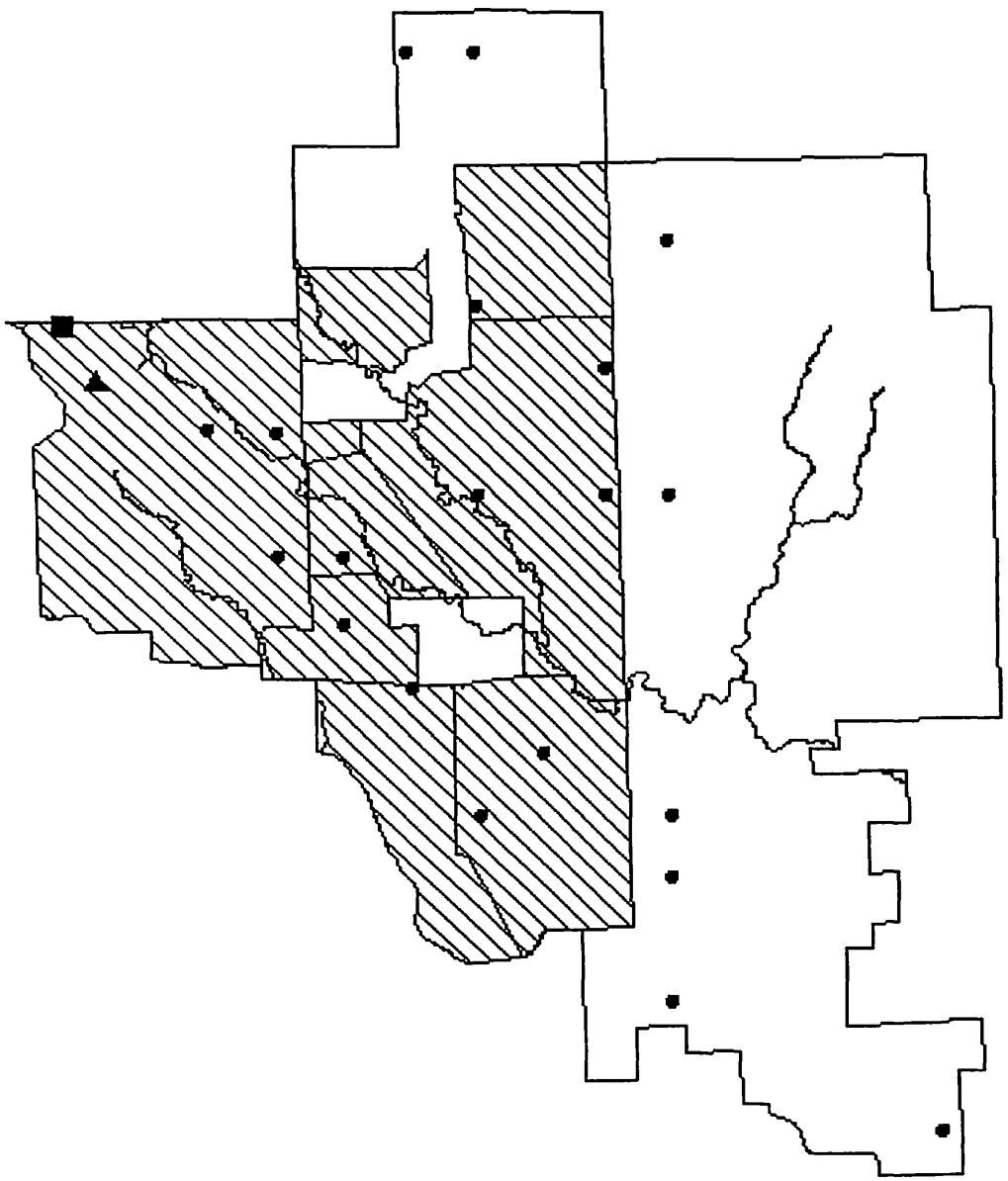

Figure 1. 2002 prairie controls in the Nature Conservancy's Tallgrass Prairie Preserve. Small circles = Control sites. Triangle = J6 site. Large square = G5 site. Shaded = bison pastures.

Figure 2. 2003 prairie controls in the Nature Conservancy's Tallgrass Prairie Preserves's bison unit 1. Small circles = Control sites. Triangles = J6 north and south sites. Large Square = G5 site. Shaded = Coweta-Bates and Steedman-Coweta soil complexes.

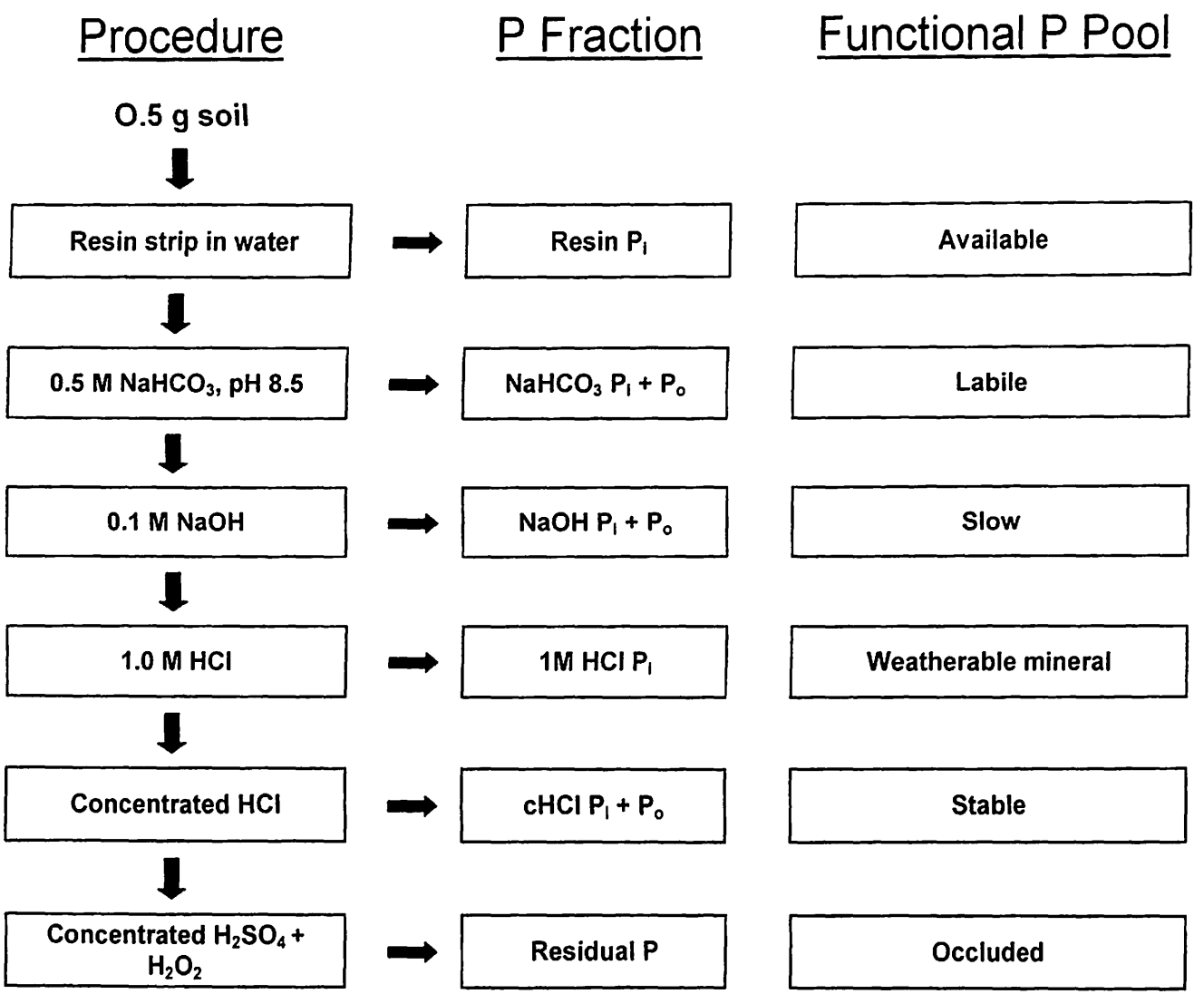

Figure 3. Sequential P extraction procedure and functional significance of extracted soil P fractions (Pi: inorganic P, Po: organic P) (Adapted from Tiessen and Moir, 1993)

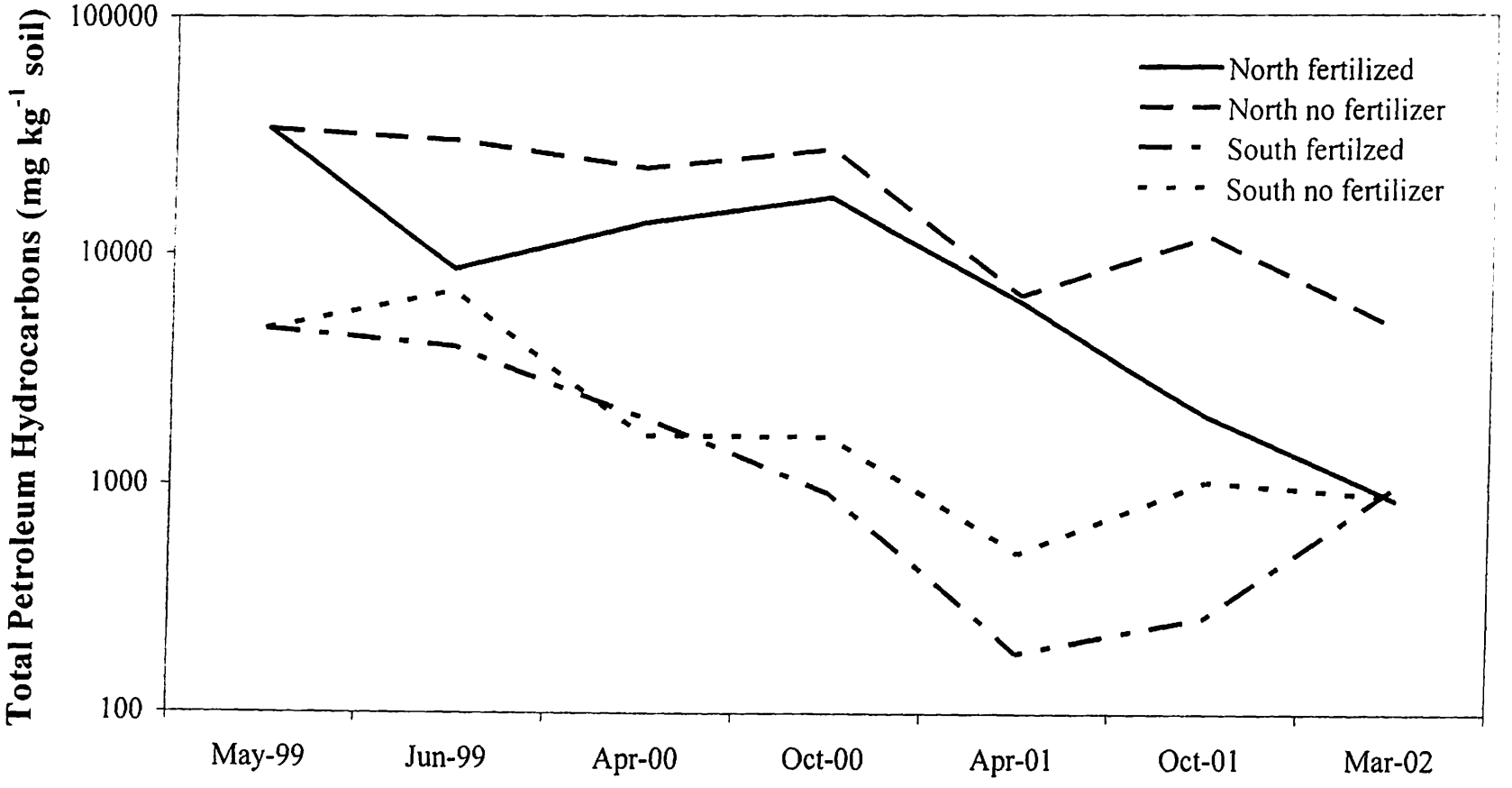

Figure 4a

Figure 4. Soil total petroleum hydrocarbons (1-15 cm depth). (a) J6 site. (b) G5 site.

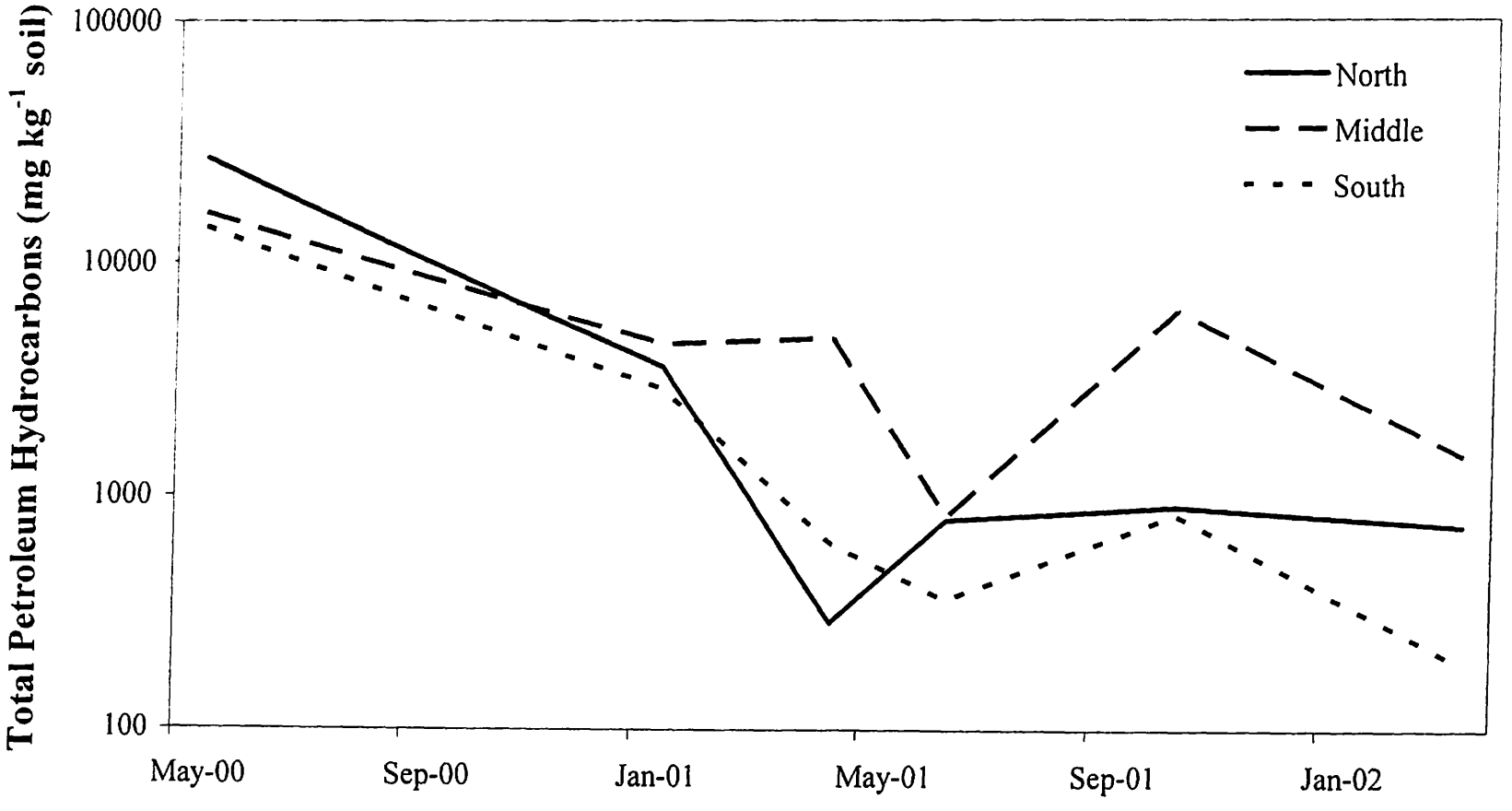
Total Petroleum Hydrocarbons (mg kg-1 soil)
100000
10000
1000
100
May-00
Sep-00
Jan-01
May-01
Sep-01
Jan-02
North
Middle
South

Figure 4b

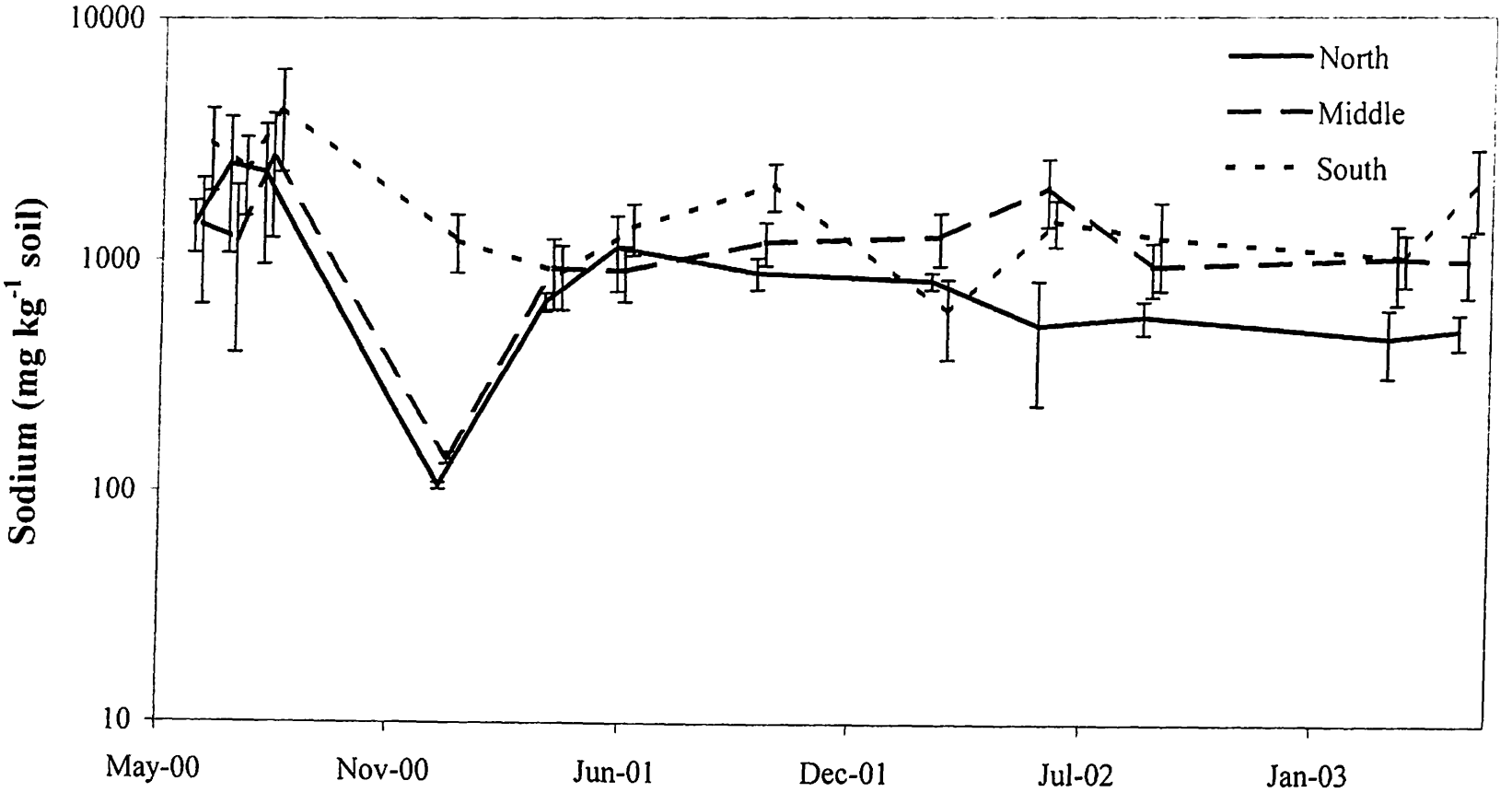

Figure 5a

Figure 5. Soil salinity at G5 site (1-15 cm depth). Values are means of three samples ± 1 S. E. Data points are staggered to enhance visibility of error bars. (a) Exchangeable sodium. (b) Exchangeable chlorine.

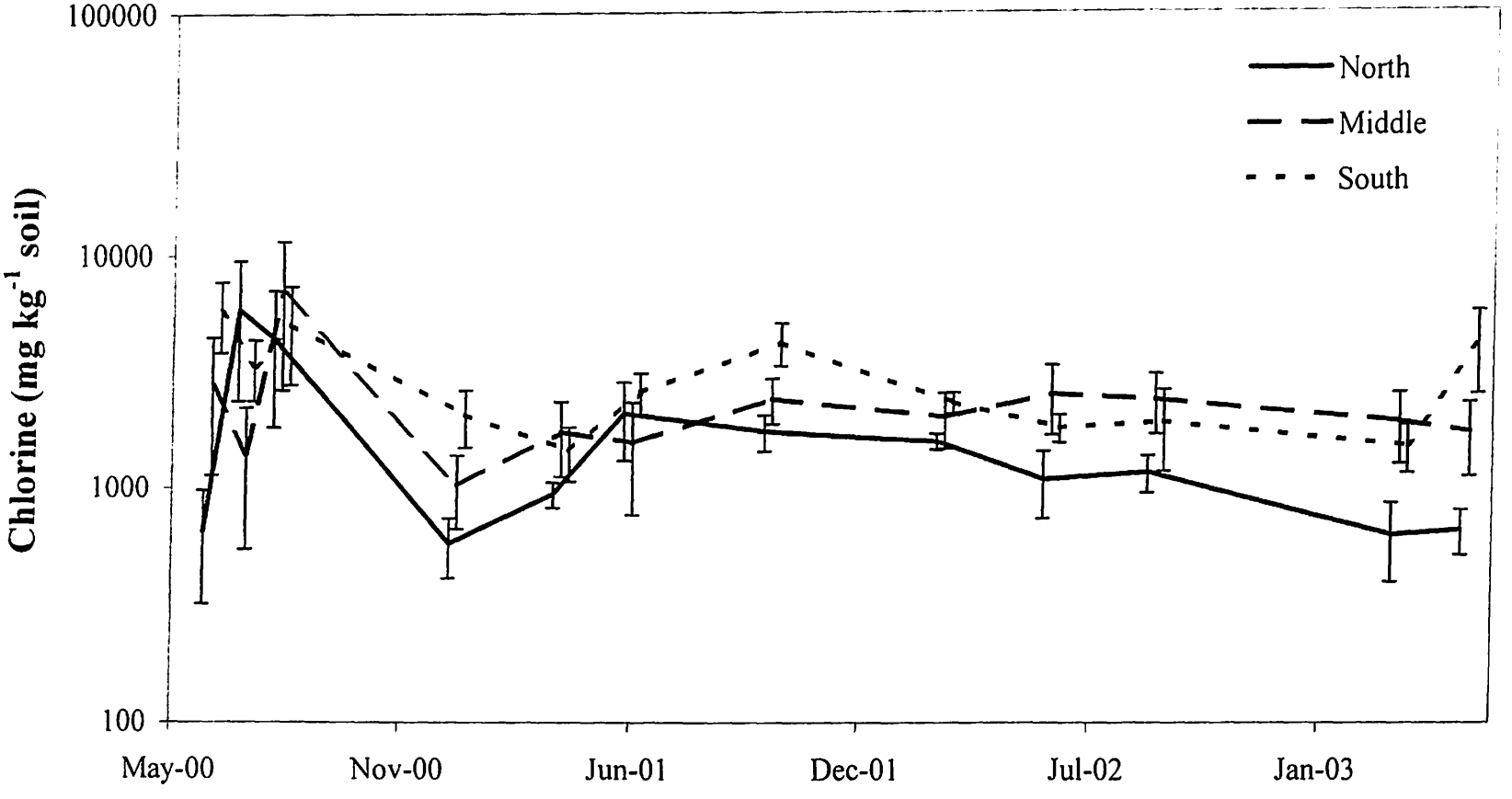
100000
10000
1000
100
Chlorine (mg kg-1 soil)
May-00
Nov-00
Jun-01
Dec-01
Jul-02
Jan-03
North
Middle
South

Figure 5b

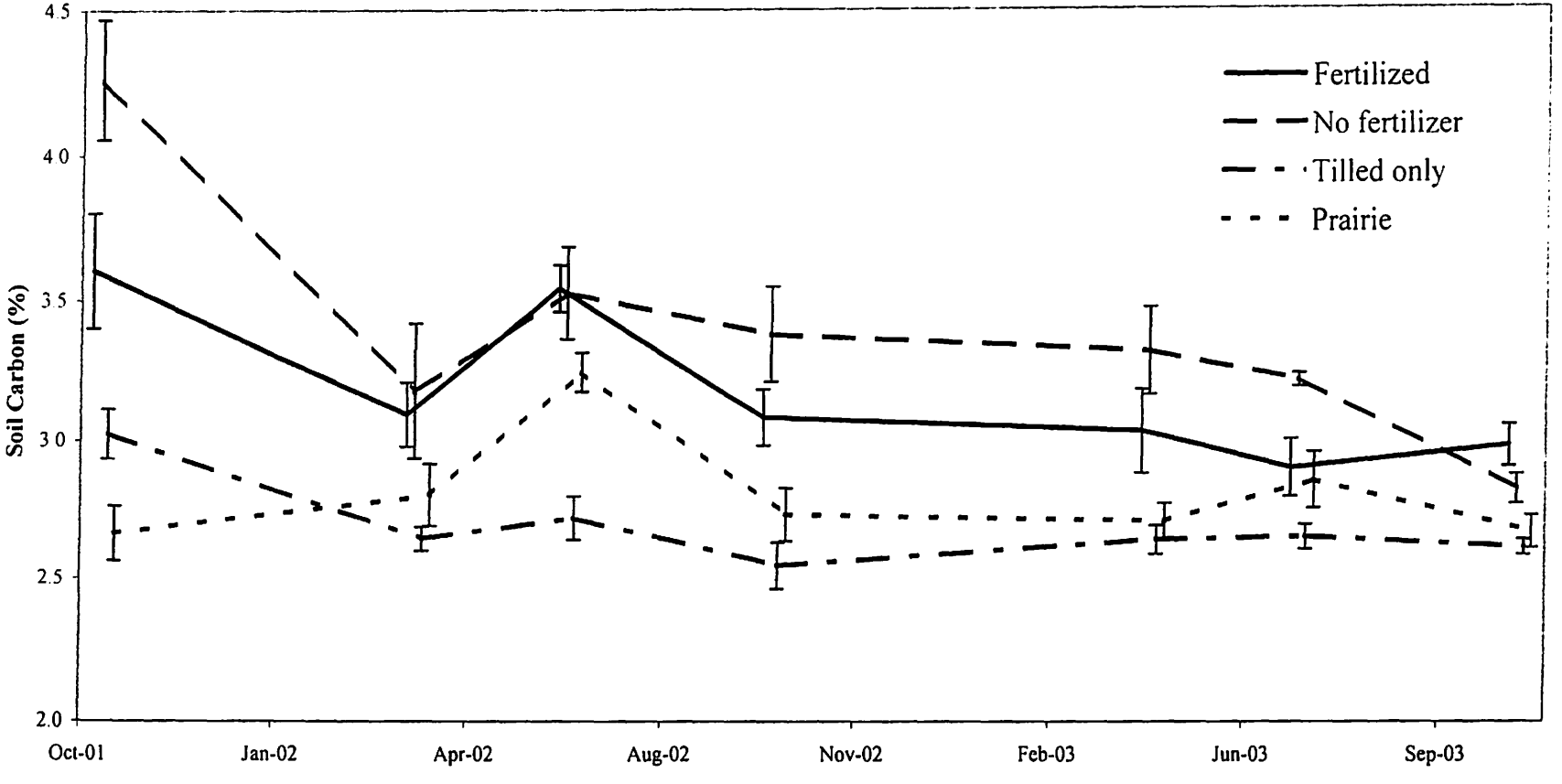

Figure 6a

Figure 6. Total carbon (as percentage of soil by weight) (1-15 cm depth). Values are means of five samples ± 1 S. E. Data points are staggered to enhance visibility of error bars. (a) J6 north site. (b) J6 south site. (c) G5 site.

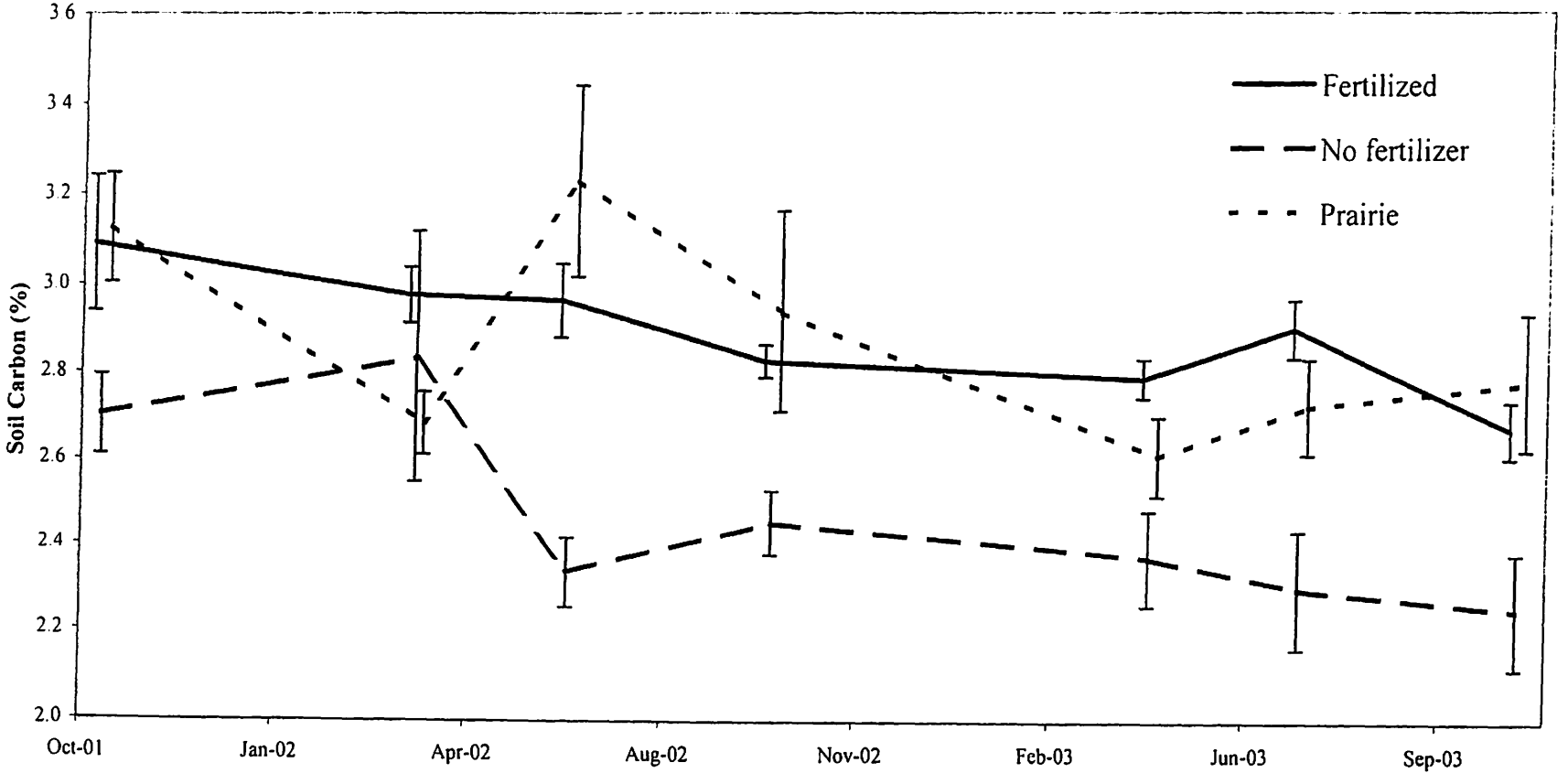
Soil Carbon (%)
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
Oct-01
Jan-02
Apr-02
Aug-02
Nov-02
Feb-03
Jun-03
Sep-03
Fertilized
No fertilizer
Prairie

Figure 6b

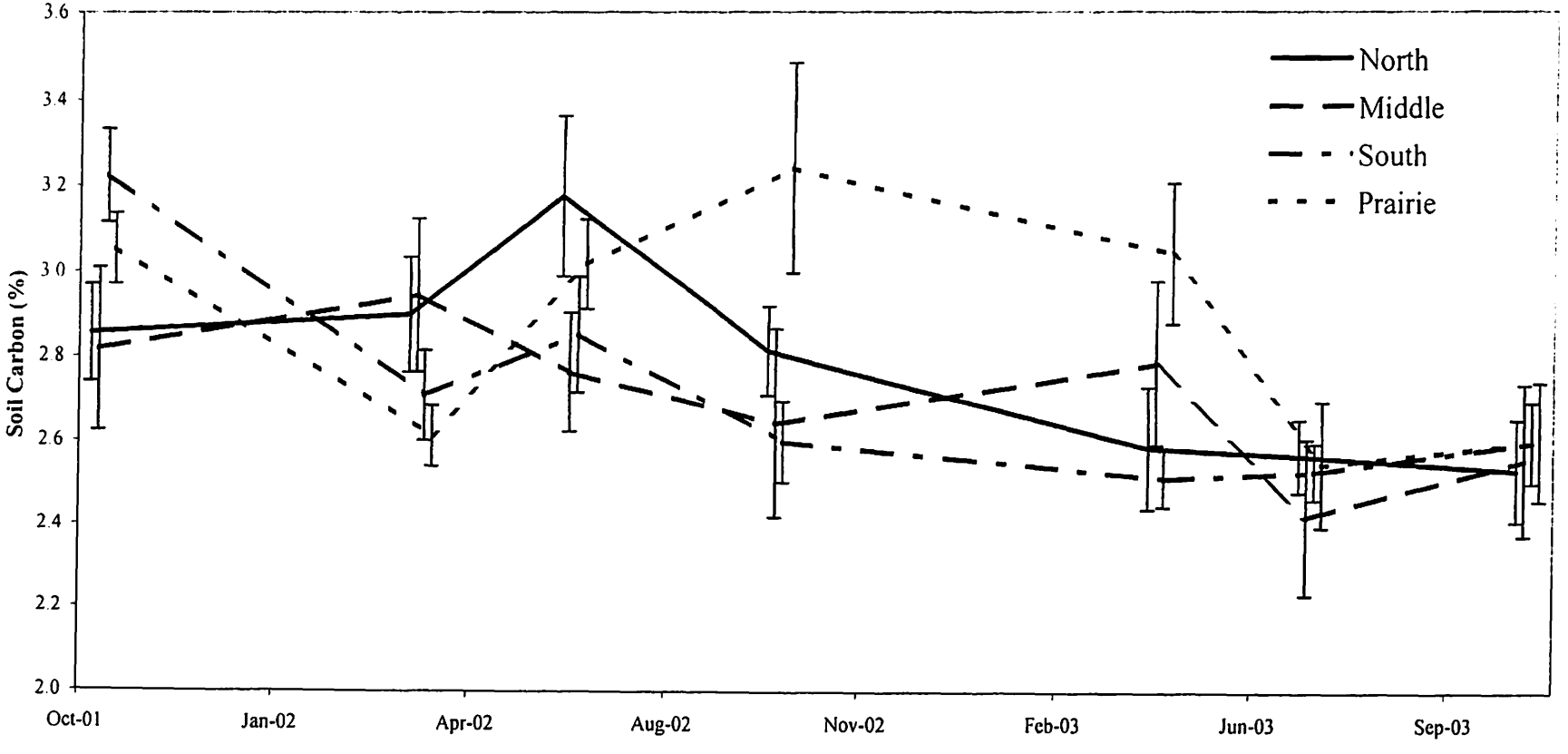
Soil Carbon (%)
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
Oct-01
Jan-02
Apr-02
Aug-02
Nov-02
Feb-03
Jun-03
Sep-03
North
Middle
South
Prairie

Figure 6c

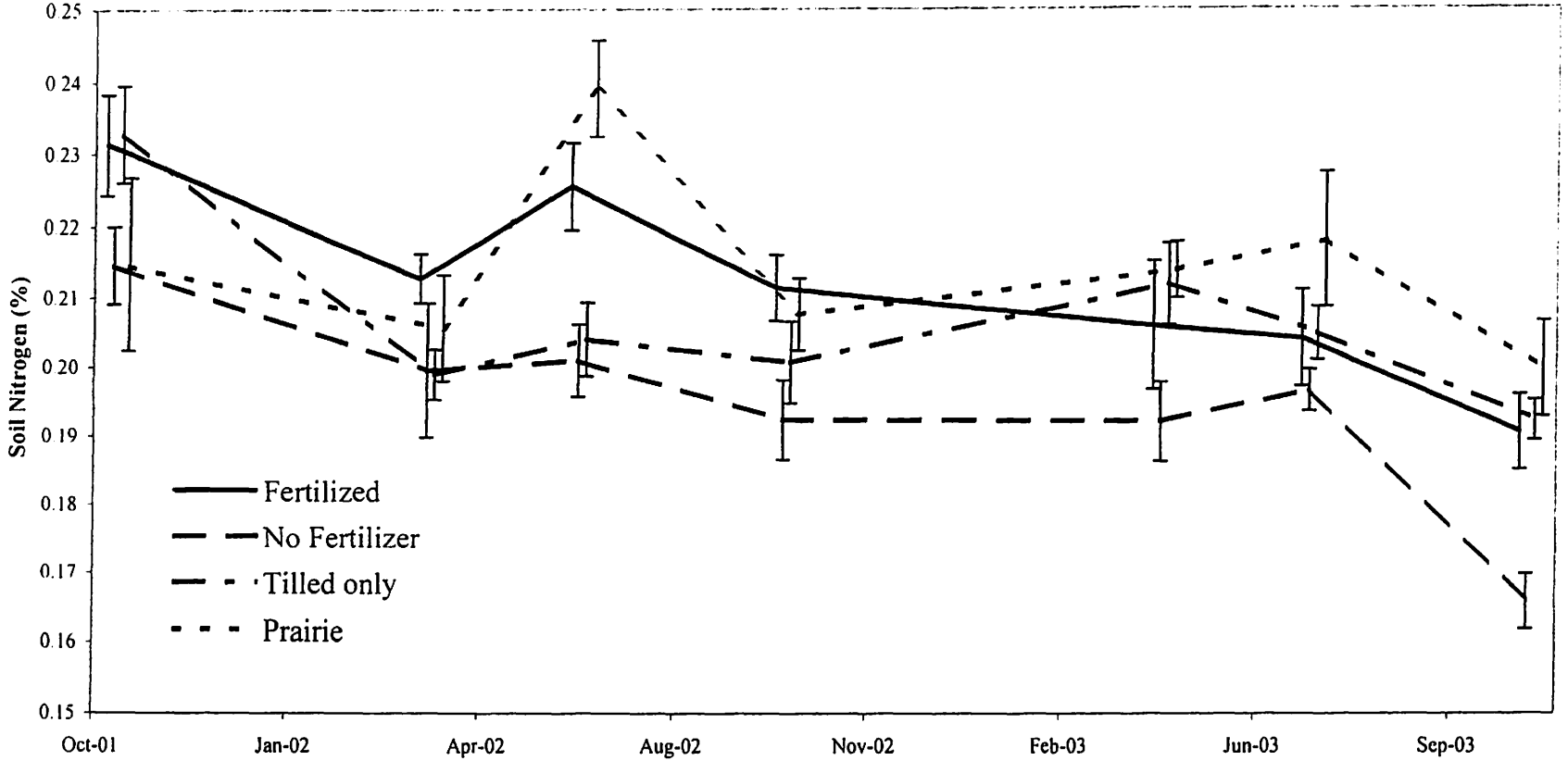

Figure 7a

Figure 7. Total nitrogen (as percentage of soil by weight) (1-15 cm depth). Values are means of five samples ± 1 S. E. Data points are staggered to enhance visibility of error bars. (a) J6 north site. (b) J6 south site. (c) G5 site.

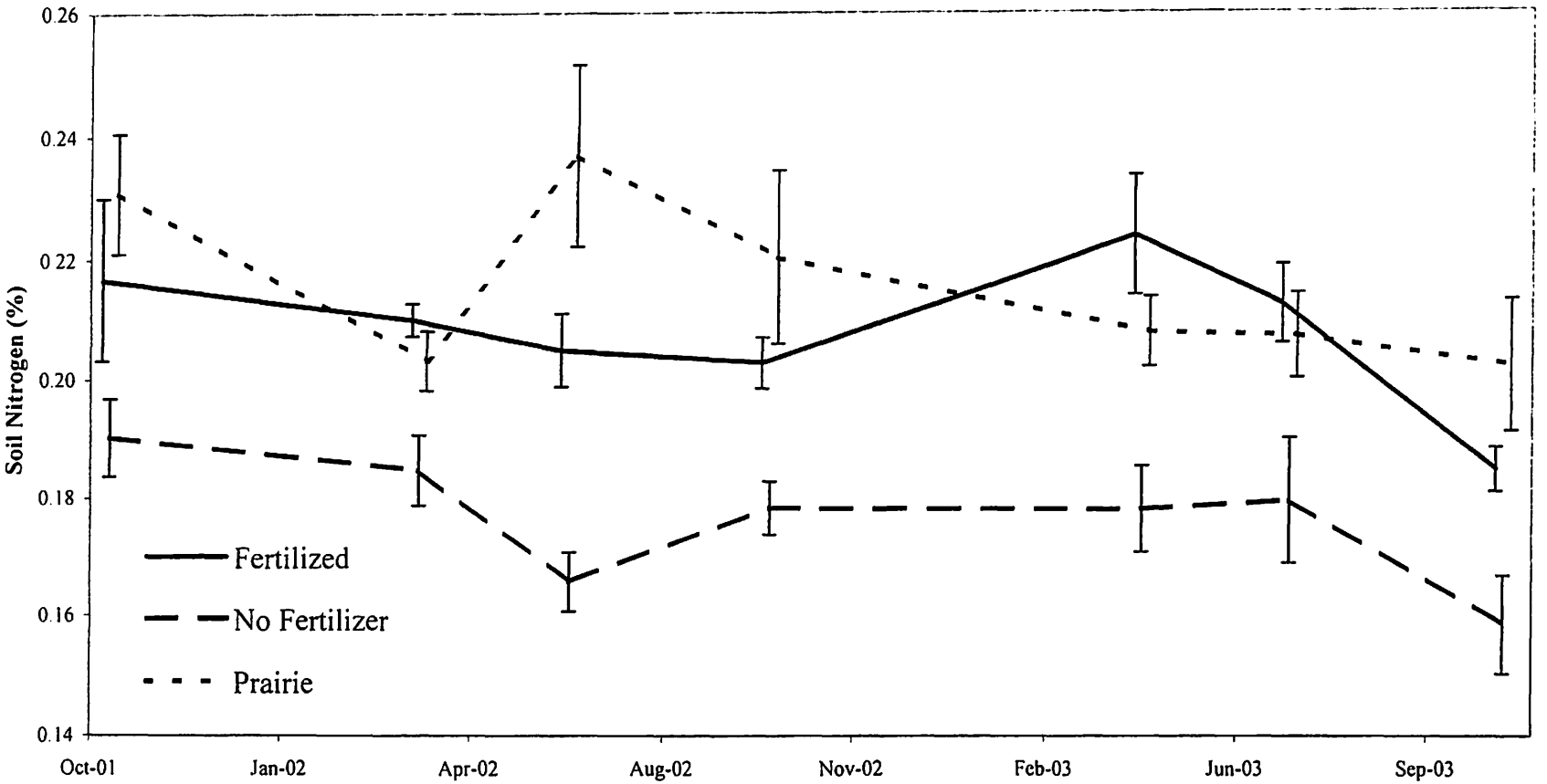
0.26
0.24
0.22
0.20
0.18
0.16
0.14
Soil Nitrogen (%)
Fertilized
No Fertilizer
Prairie
Oct-01
Jan-02
Apr-02
Aug-02
Nov-02
Feb-03
Jun-03
Sep-03

Figure 7b

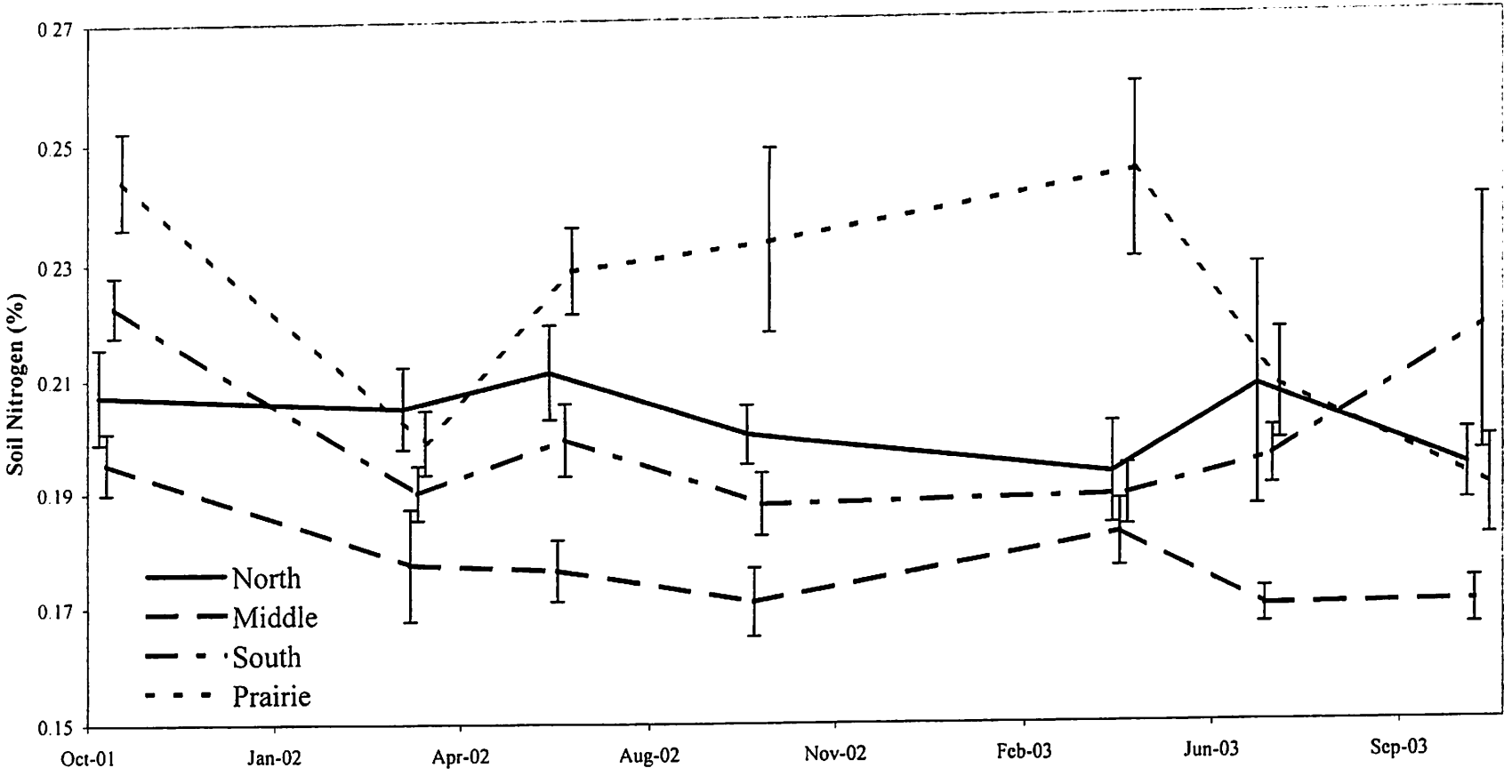
0.27
0.25
0.23
0.21
0.19
0.17
0.15
Soil Nitrogen (%)
North
Middle
South
Prairie
Oct-01
Jan-02
Apr-02
Aug-02
Nov-02
Feb-03
Jun-03
Sep-03

Figure 7c

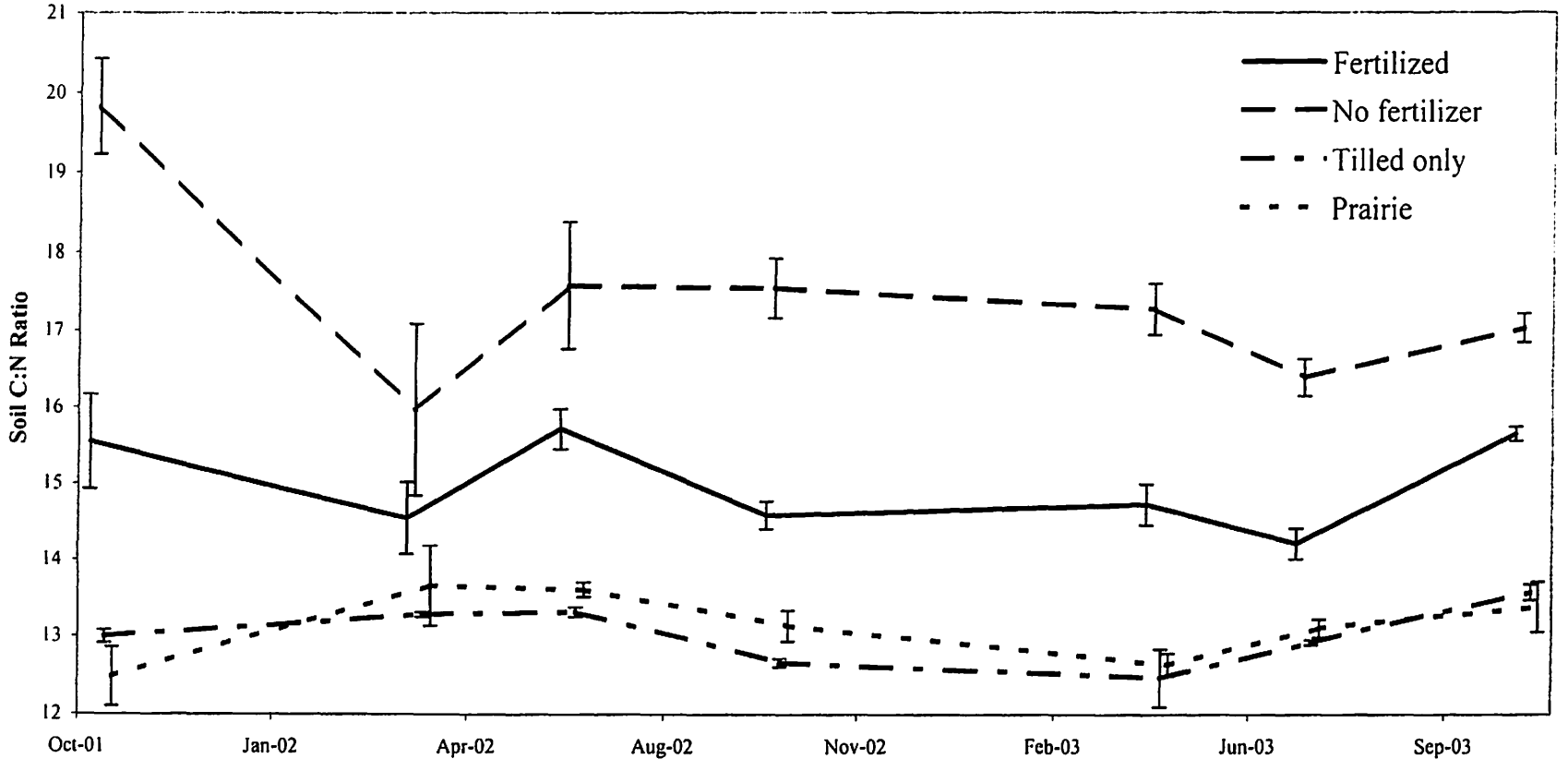

Figure 8a

Figure 8. Soil carbon : nitrogen ratios (1-15 cm depth). Values are means of five samples ± 1 S. E. Data points are staggered to enhance visibility of error bars. (a) J6 north site. (b) J6 south site. (c) G5 site.

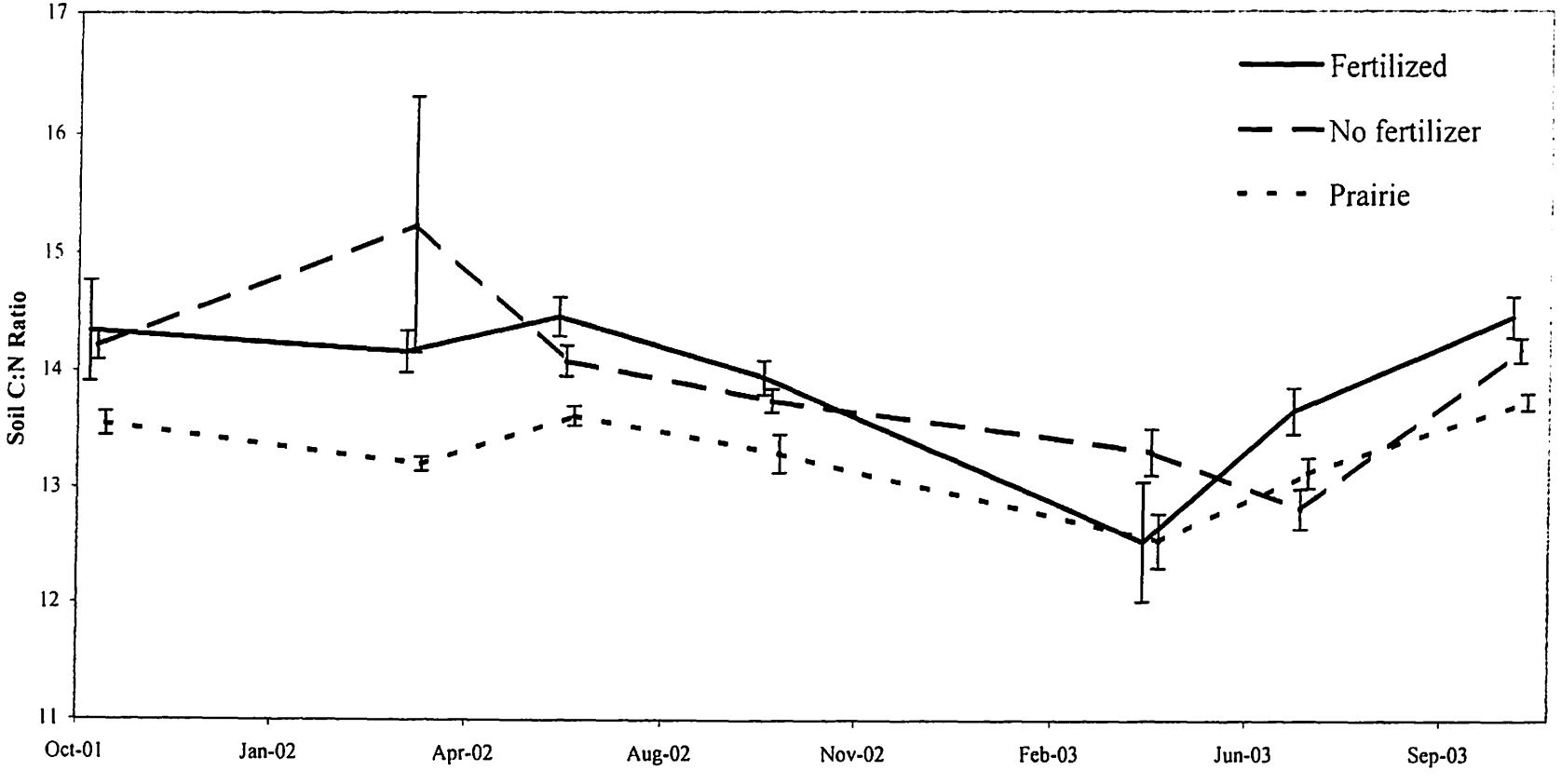
Soil C:N Ratio
17
16
15
14
13
12
11
Oct-01
Jan-02
Apr-02
Aug-02
Nov-02
Feb-03
Jun-03
Sep-03
Fertilized
No fertilizer
Prairie

Figure 8b

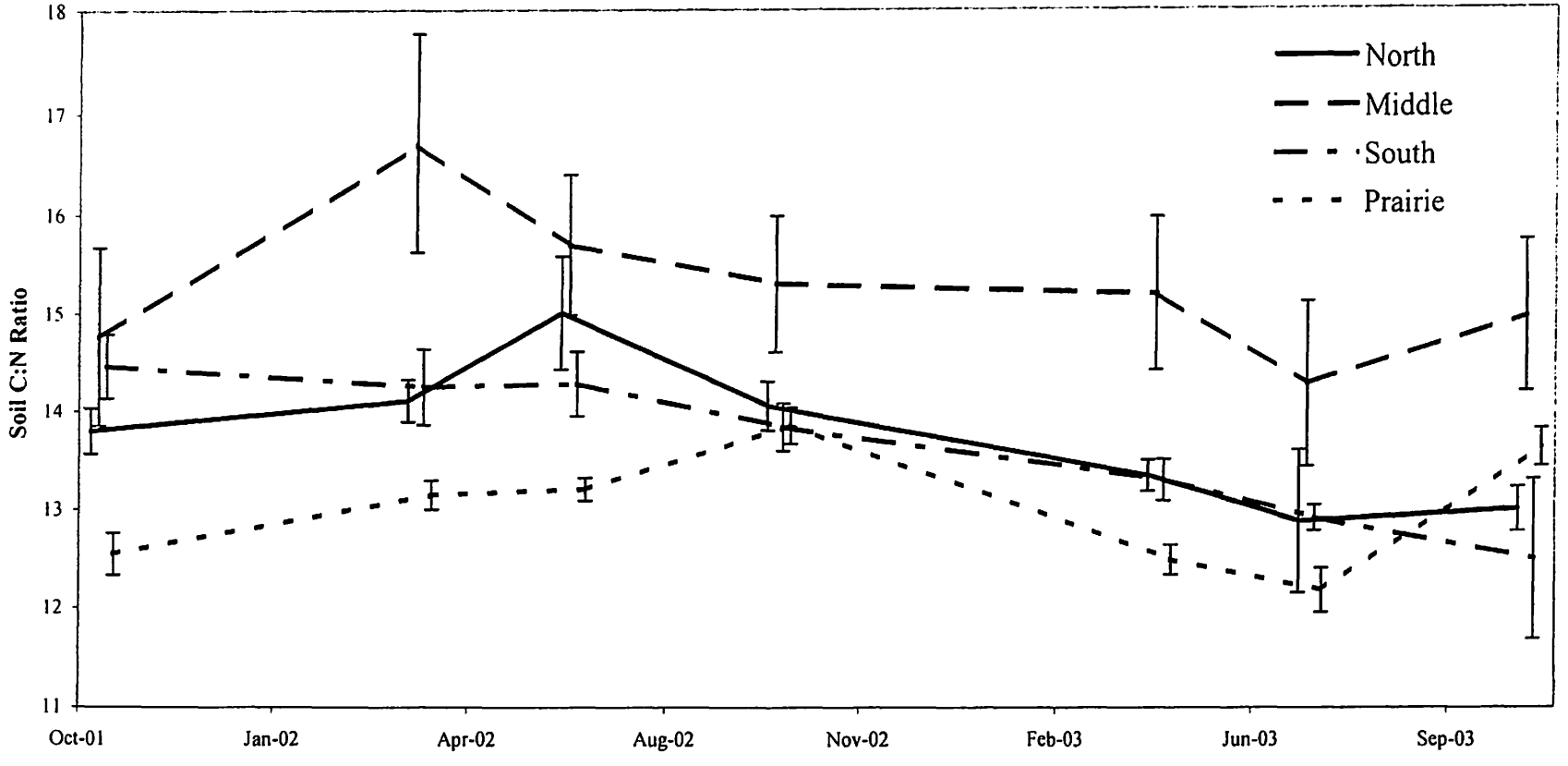
Soil C:N Ratio
18
17
16
15
14
13
12
11
Oct-01
Jan-02
Apr-02
Aug-02
Nov-02
Feb-03
Jun-03
Sep-03
North
Middle
South
Prairie

Figure 8c

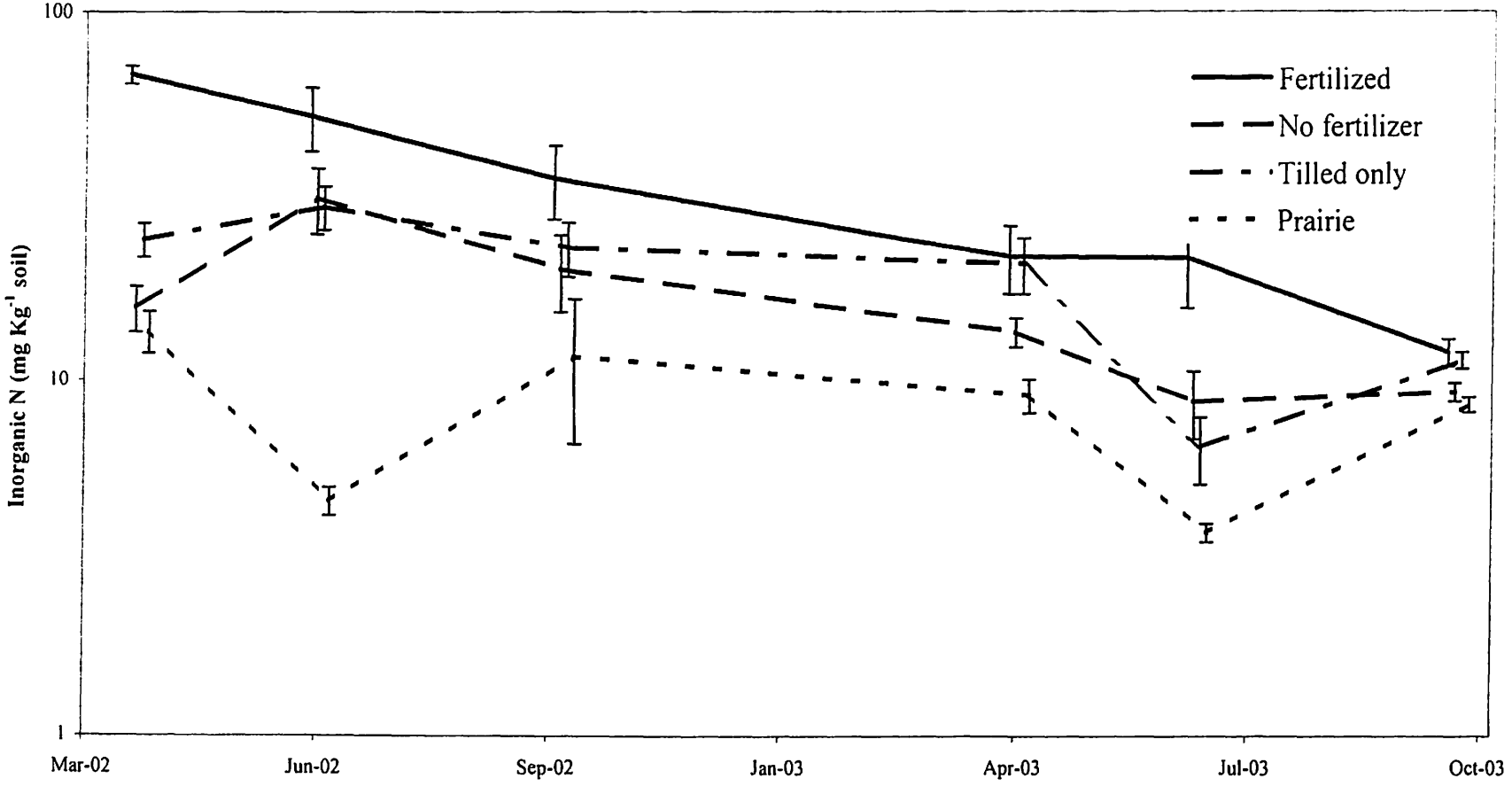

Figure 9a

Figure 9. Soil inorganic N (1-15 cm depth). Values are means of five samples ± 1 S. E. Data points are staggered to enhance visibility of error bars. (a) J6 north site. (b) J6 south site. (c) G5 site.

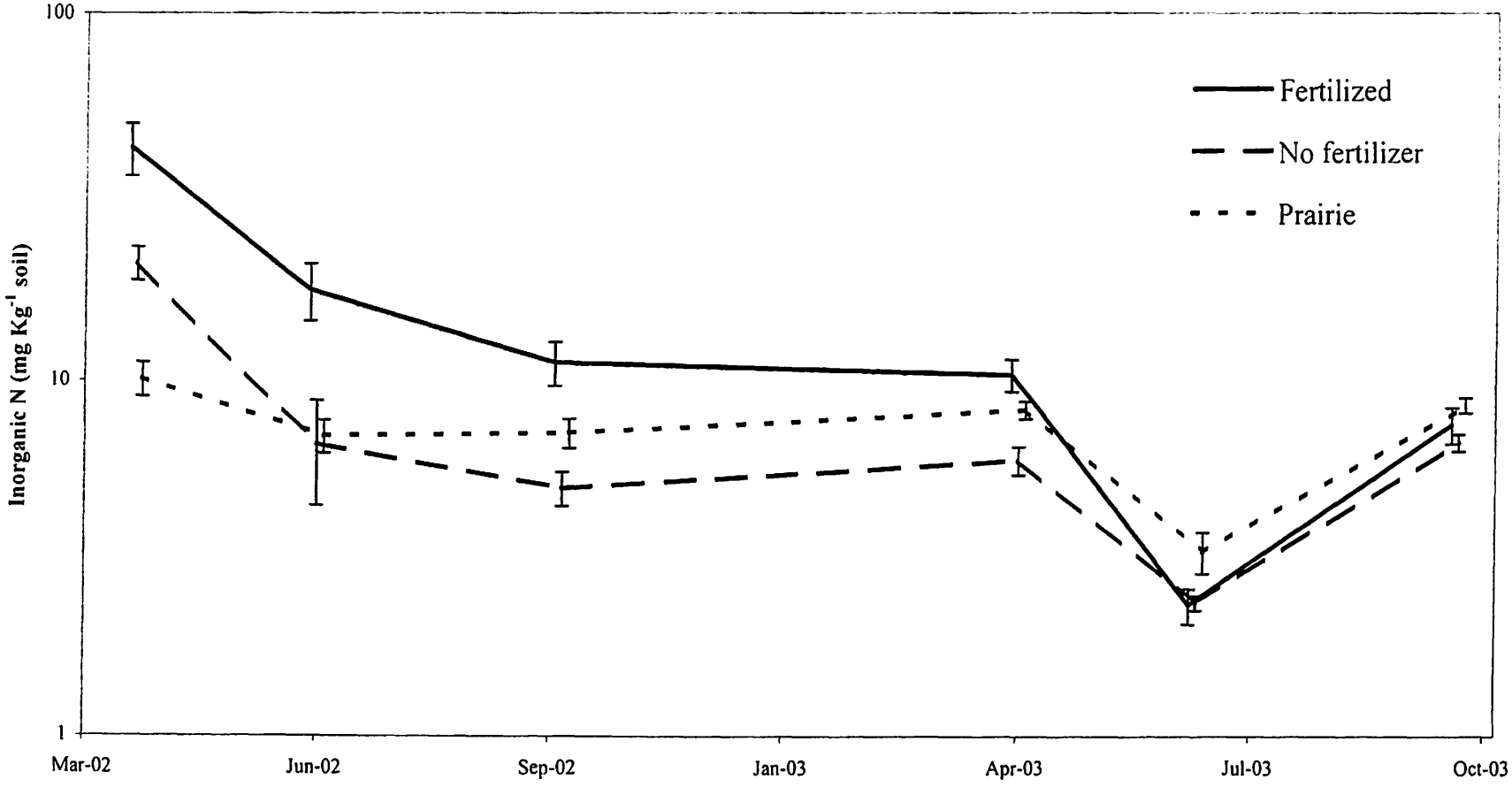
100
10
1
Inorganic N (mg Kg-1 soil)
Fertilized
No fertilizer
Prairie
Mar-02
Jun-02
Sep-02
Jan-03
Apr-03
Jul-03
Oct-03

Figure 9b

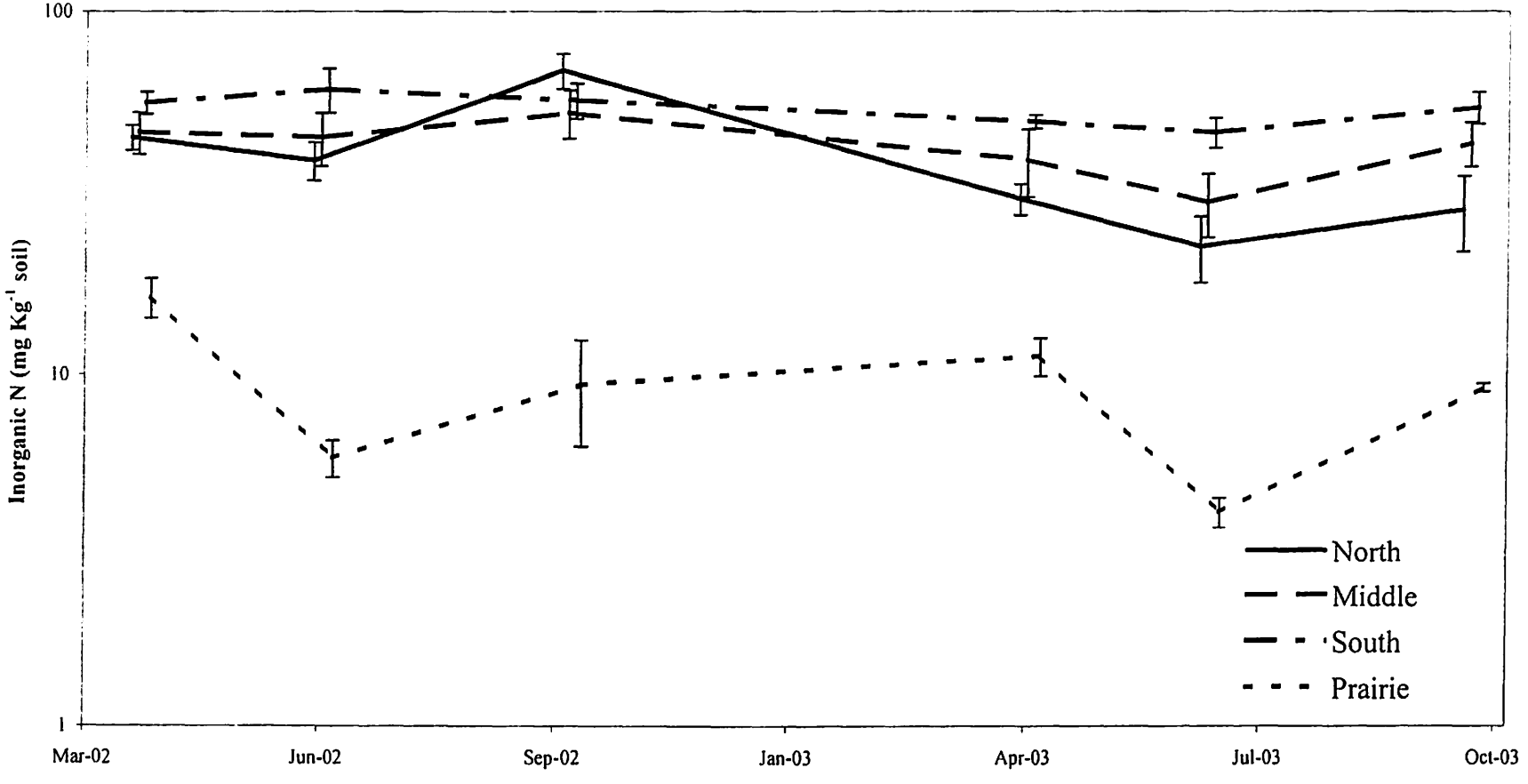
100
10
1
Inorganic N (mg Kg-1 soil)
Mar-02
Jun-02
Sep-02
Jan-03
Apr-03
Jul-03
Oct-03
North
Middle
South
Prairie

Figure 9c

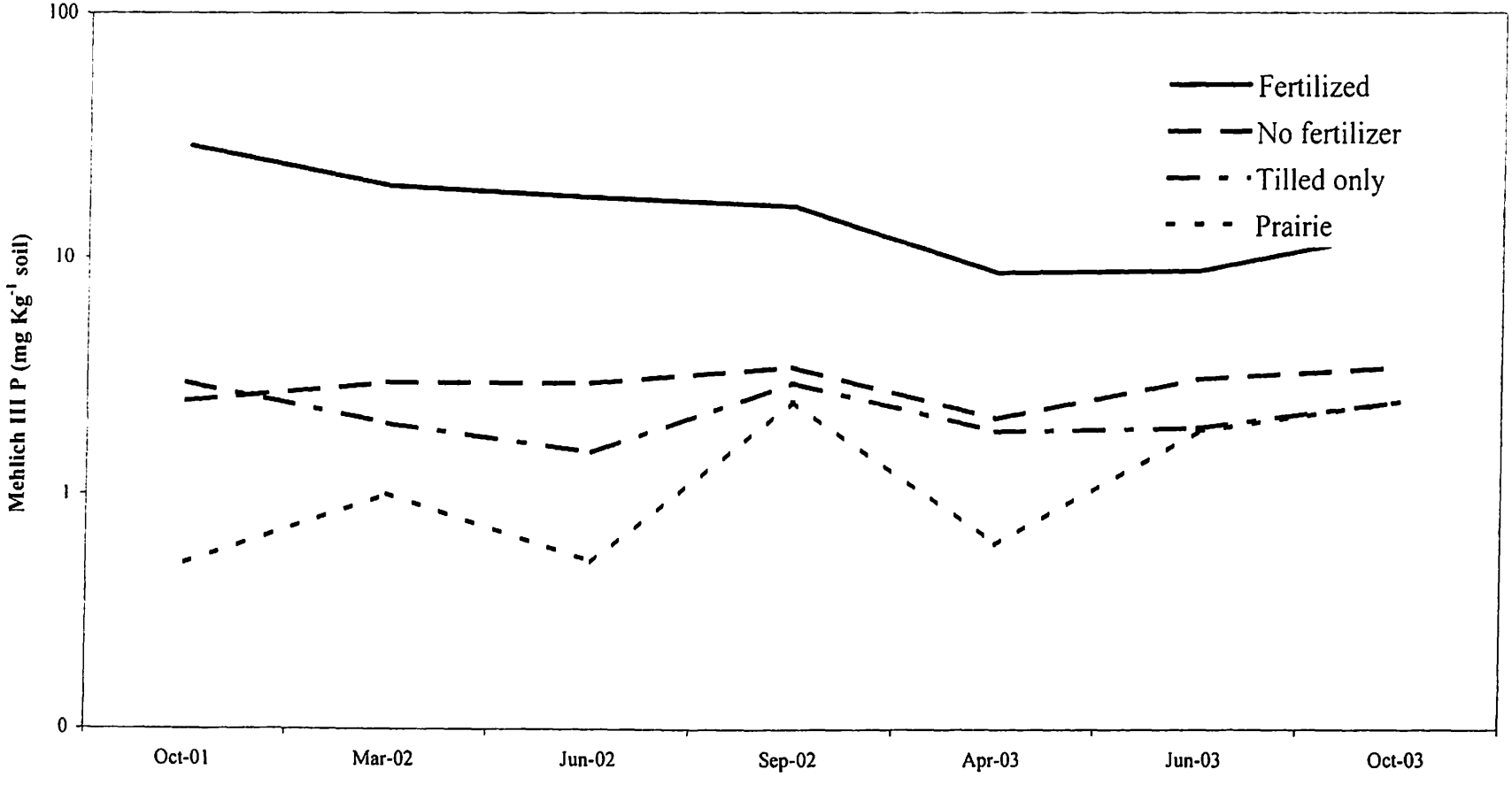

Figure 10a

Figure 10. Soil Mehlich III P (1-15 cm depth). (a) J6 north site. (b) J6 south site. (c) G5 site.

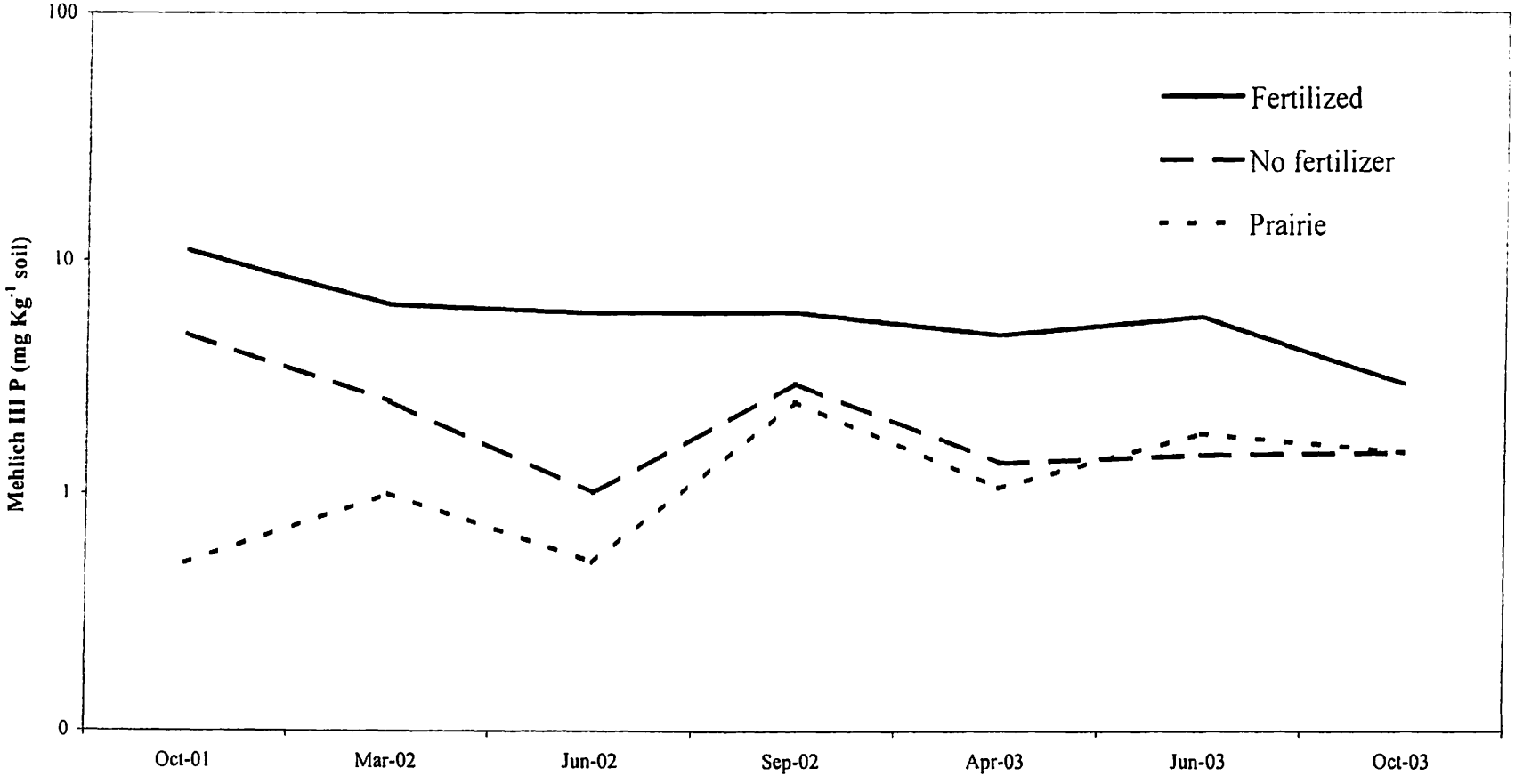
100
10
1
0
Mehlich III P (mg Kg-1 soil)
Fertilized
No fertilizer
Prairie
Oct-01
Mar-02
Jun-02
Sep-02
Apr-03
Jun-03
Oct-03

Figure 10b

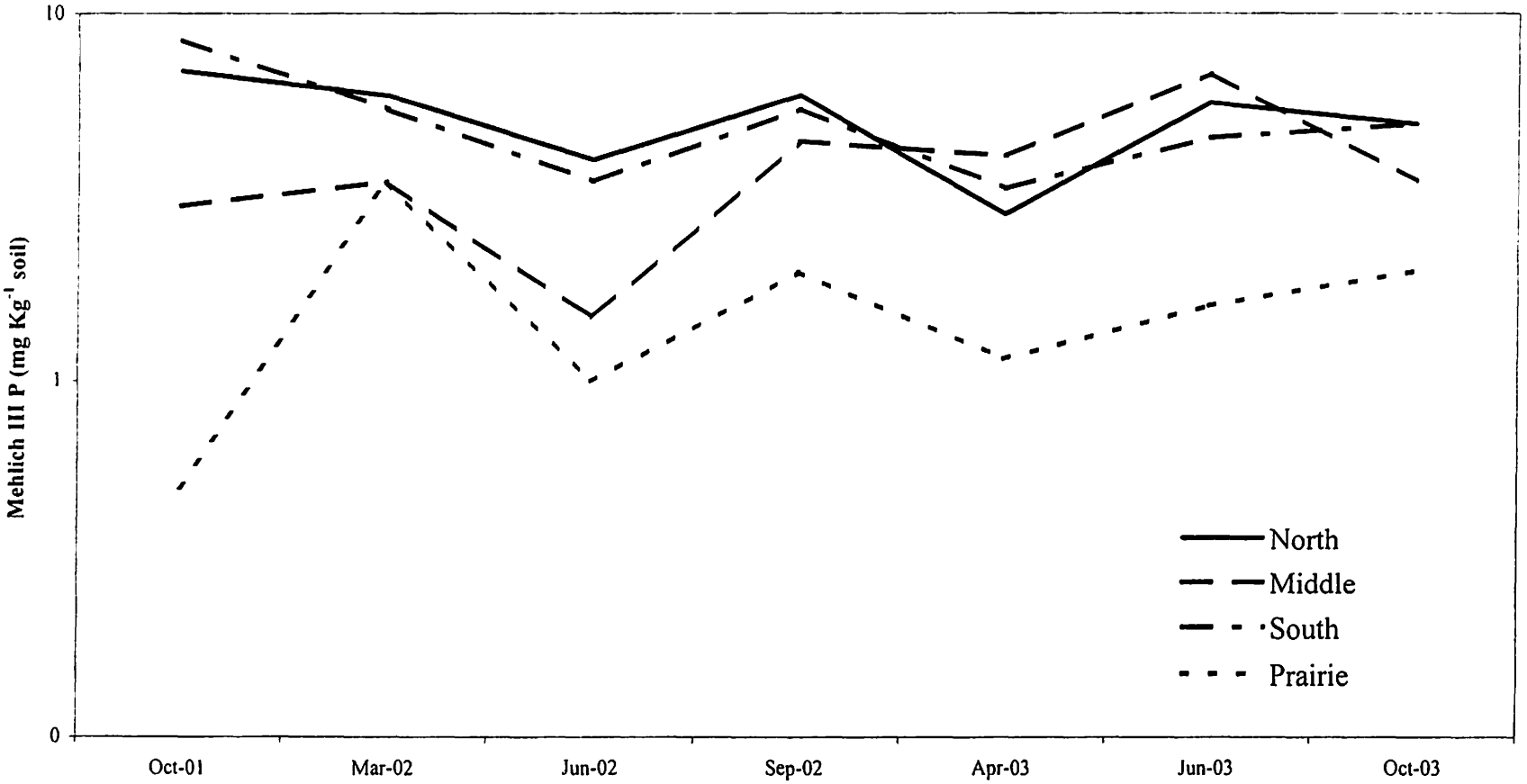
10
1
0
Mehlich III P (mg Kg-1 soil)
Oct-01
Mar-02
Jun-02
Sep-02
Apr-03
Jun-03
Oct-03
North
Middle
South
Prairie

Figure 10c

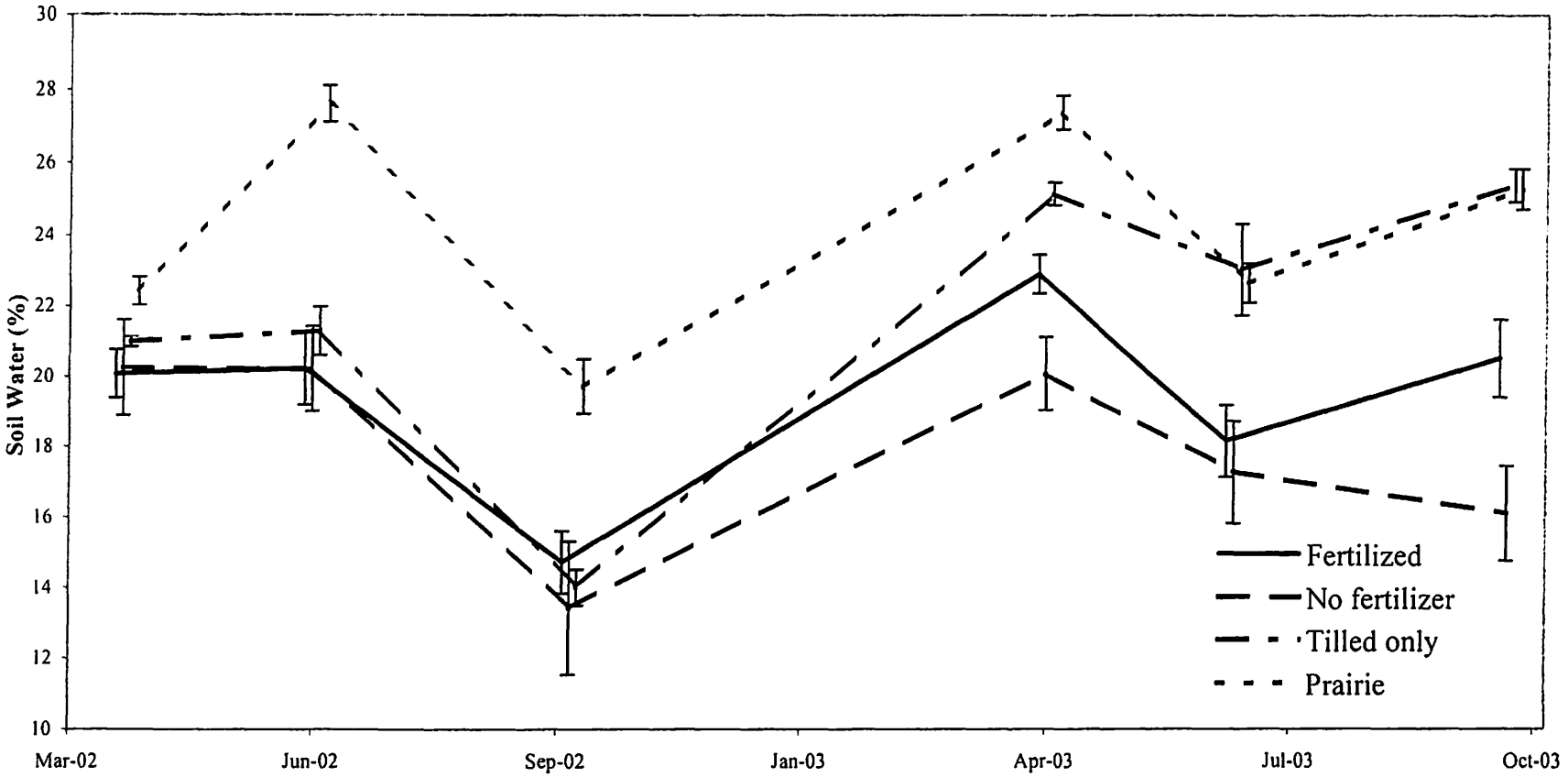

Figure 11a

Figure 11. Soil water content (1-15 cm depth). Values are means of five samples ± 1 S. E. Data points are staggered to enhance visibility of error bars. (a) J6 north site. (b) J6 south site. (c) G5 site.

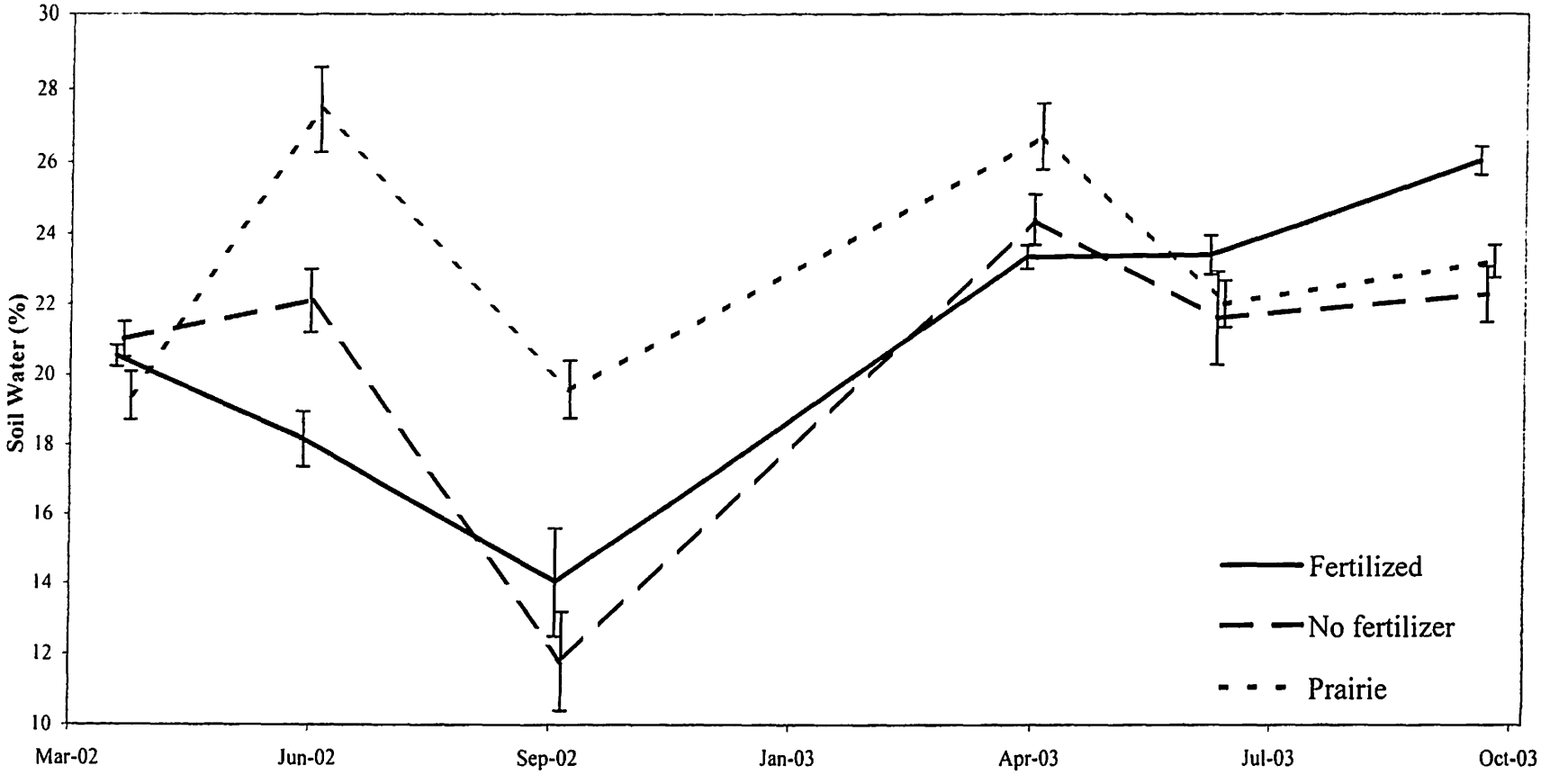
30
28
26
24
22
20
18
16
14
12
10
Soil Water (%)
Mar-02
Jun-02
Sep-02
Jan-03
Apr-03
Jul-03
Oct-03
Fertilized
No fertilizer
Prairie

Figure 11b

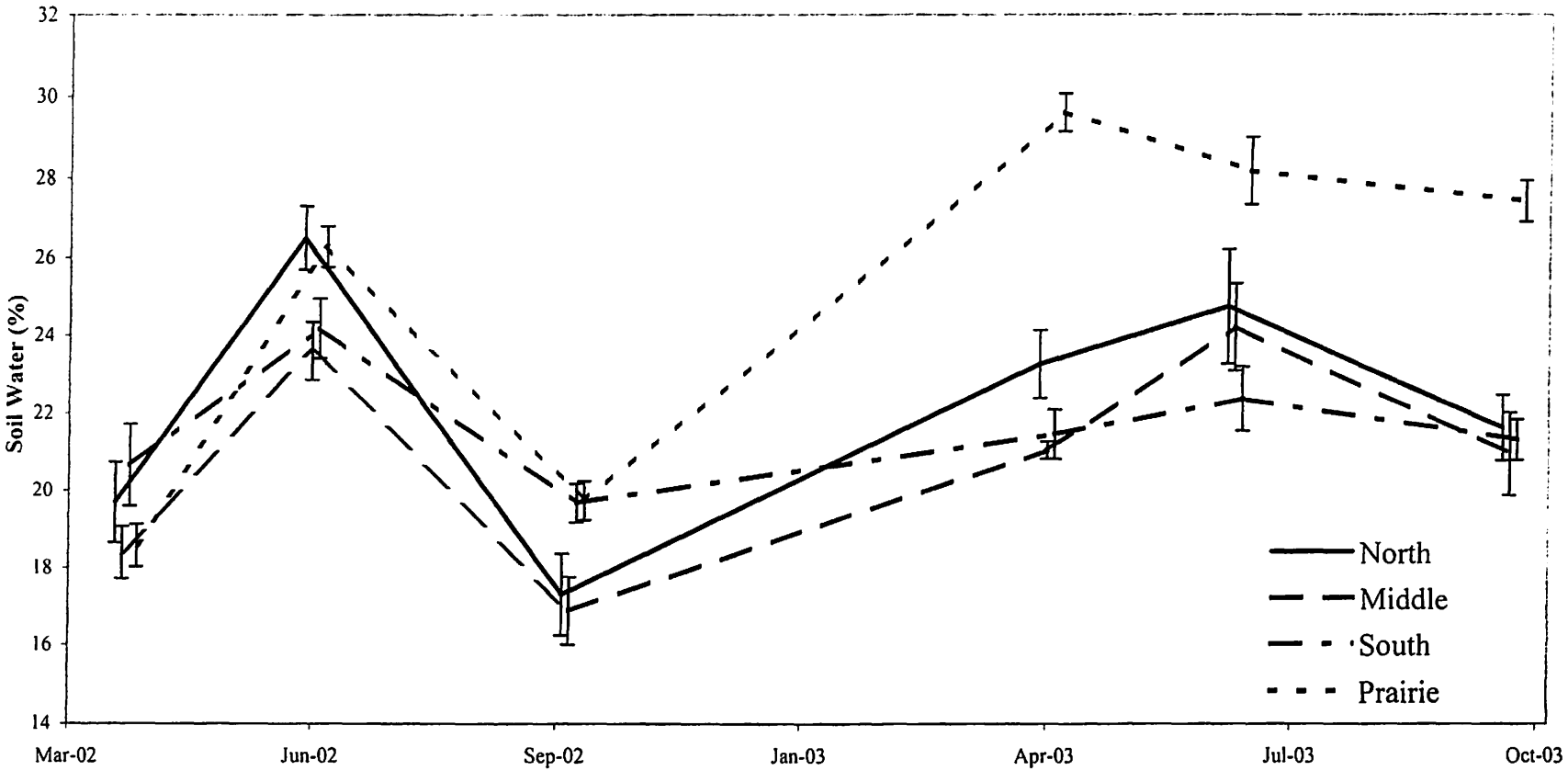
32
30
28
26
24
22
20
18
16
14
Soil Water (%)
Mar-02
Jun-02
Sep-02
Jan-03
Apr-03
Jul-03
Oct-03
North
Middle
South
Prairie

Figure 11c

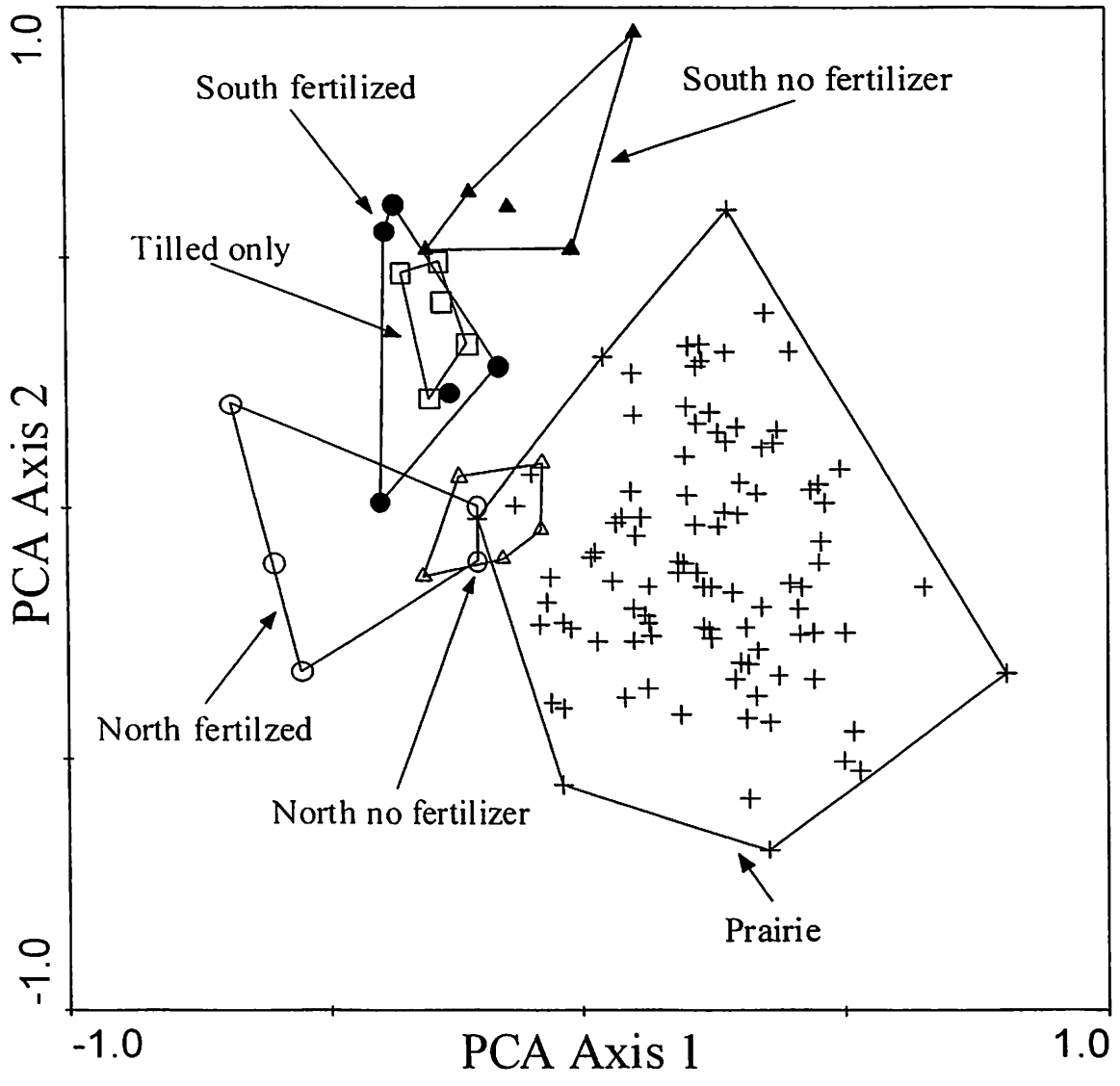

Figure 12a

Figure 12. PCA of J6 (summer 2002 and 2003), G5 (summer 2002 and 2003), and all prairie controls. (a) J6 summer 2002 and controls. (b) J6 summer 2003 and controls. (c) G5 summer 2002 and controls. (d) G5 summer 2003 and controls. (e) Environmental variables.

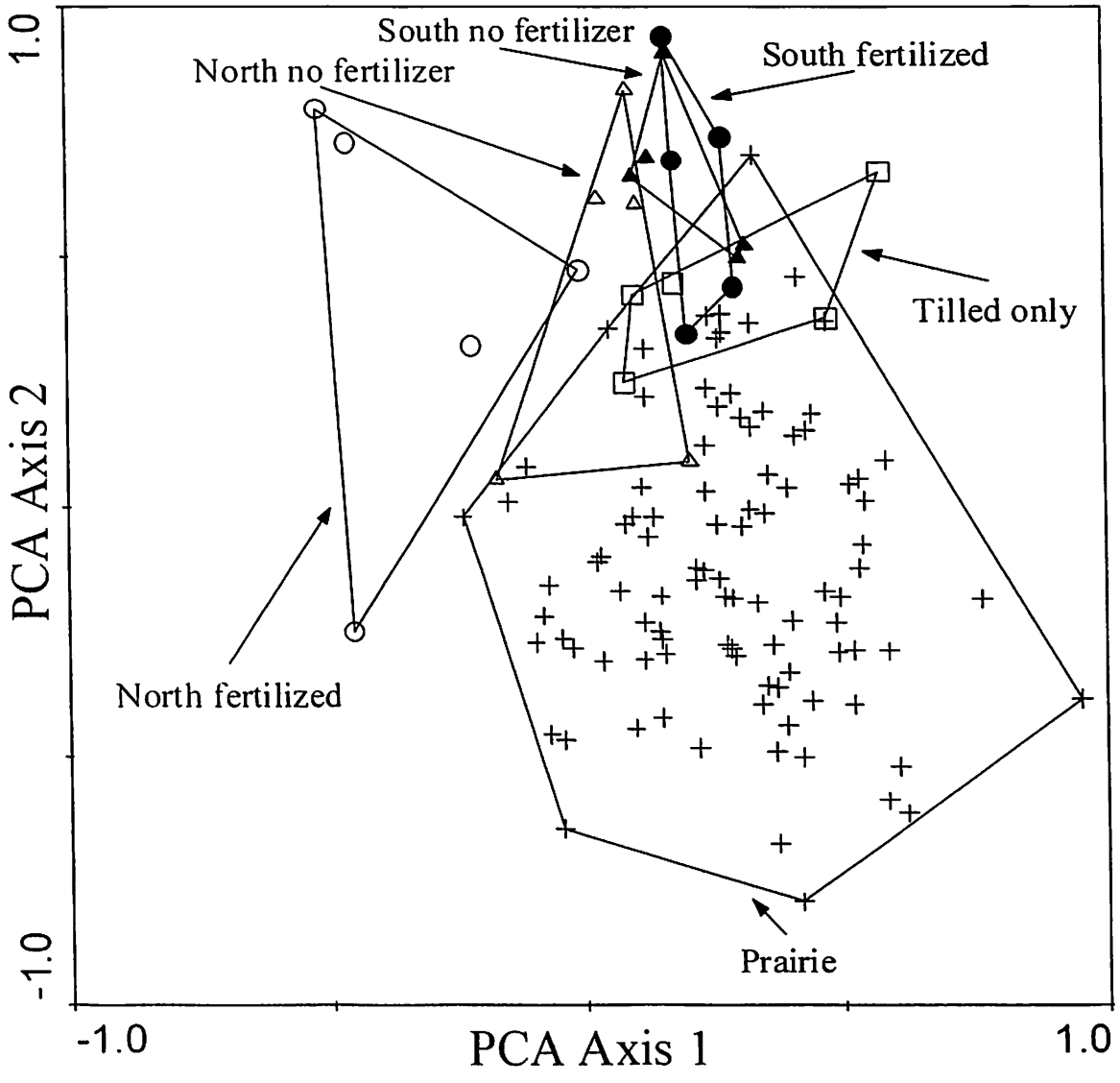
South no fertilizer
North no fertilizer
South fertilized
Tilled only
North fertilized
Prairie
1.0
-1.0
PCA Axis 2
-1.0
PCA Axis 1
1.0

Figure 12b

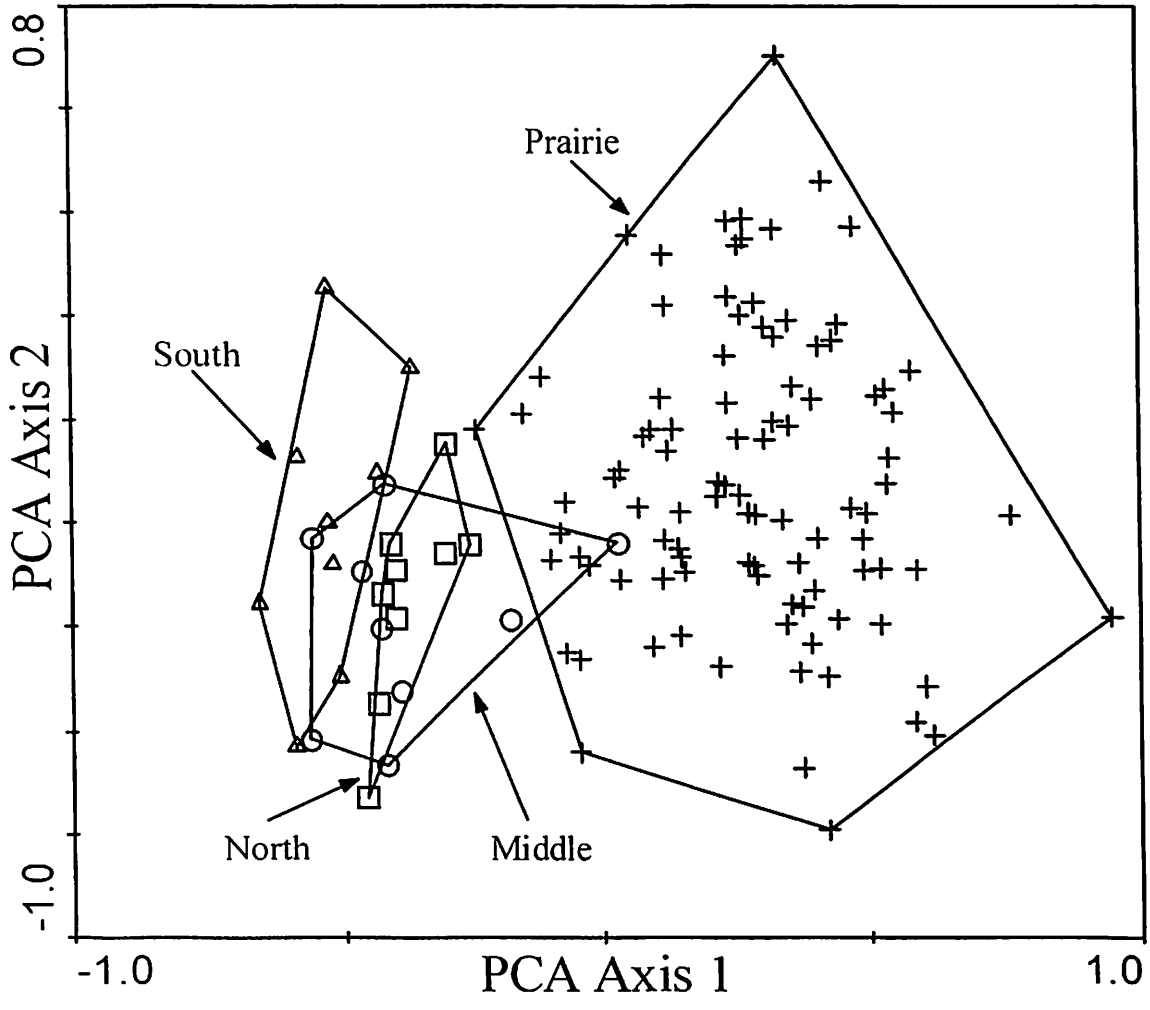

Figure 12c

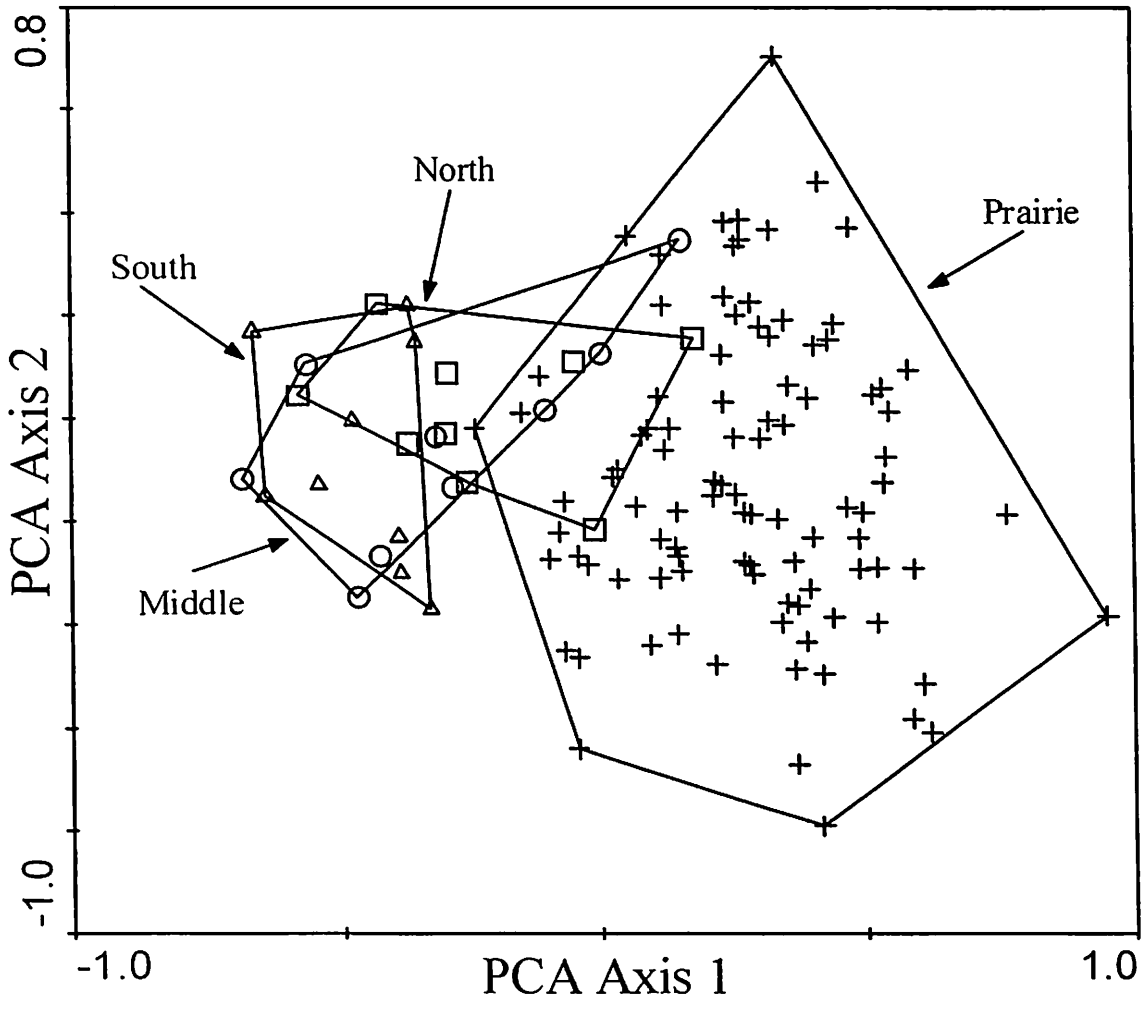

Figure 12d

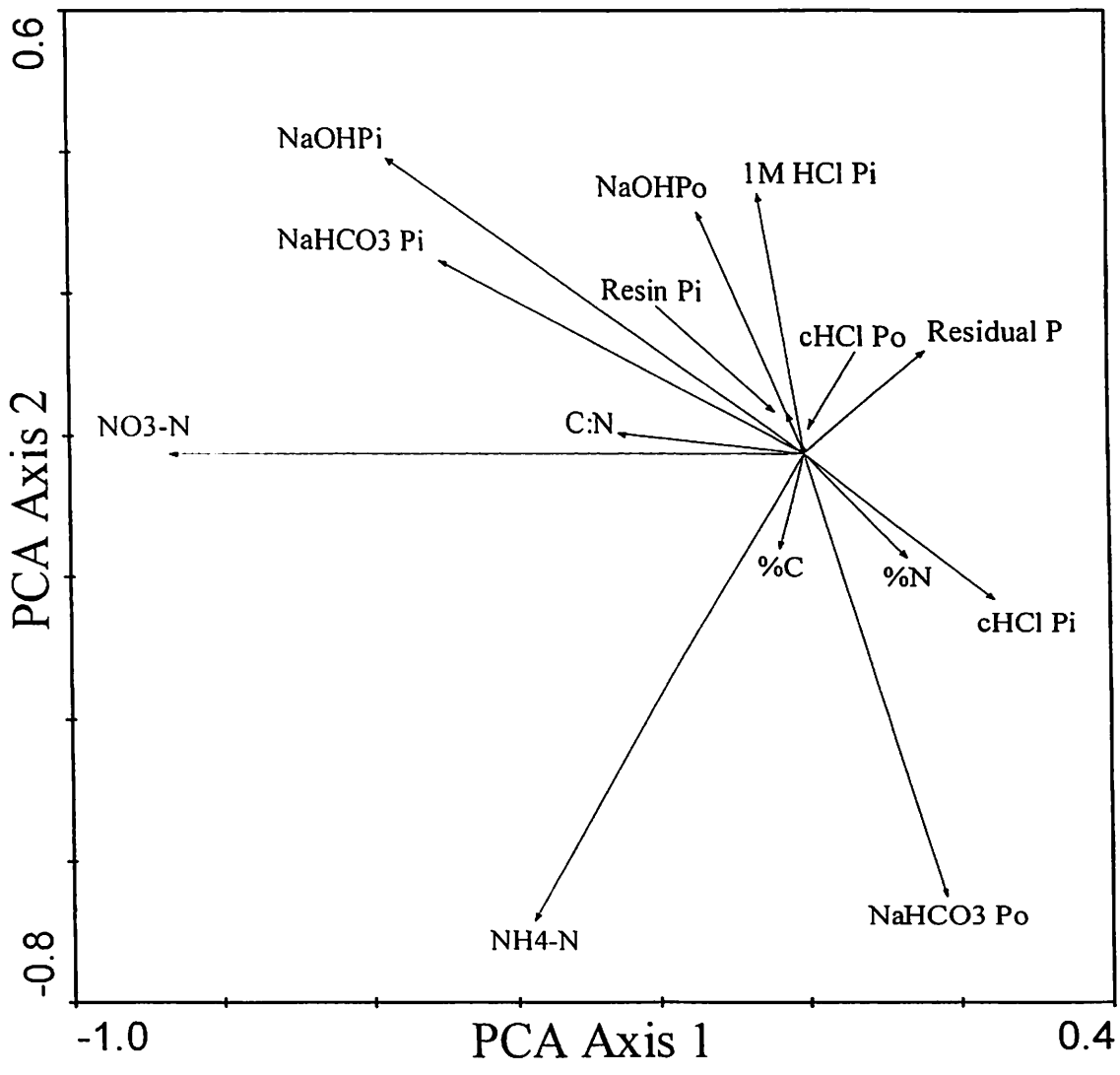
0.6
-0.8
PCA Axis 2
-1.0
PCA Axis 1
0.4
NaOHPi
NaOHPo
1M HCl Pi
NaHCO3 Pi
Resin Pi
cHCl Po
Residual P
NO3-N
C:N
%C
%N
cHCl Pi
NH4-N
NaHCO3 Po

Figure 12e

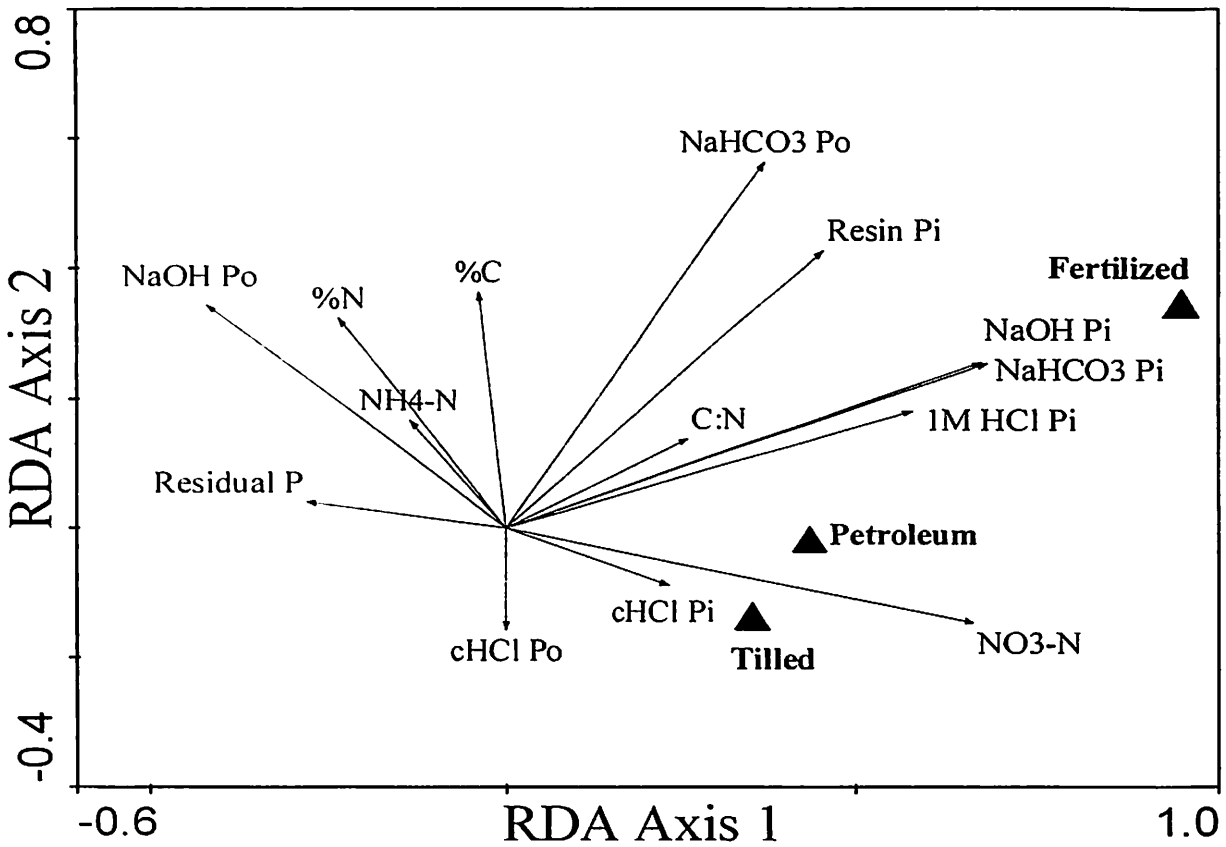

Figure 13a

Figure 13. RDAs soil nutrients at J6 and G5. (a) J6 summer 2002. (b) J6 summer 2003. (c) G5 summer 2002. (d) G5 summer 2003.

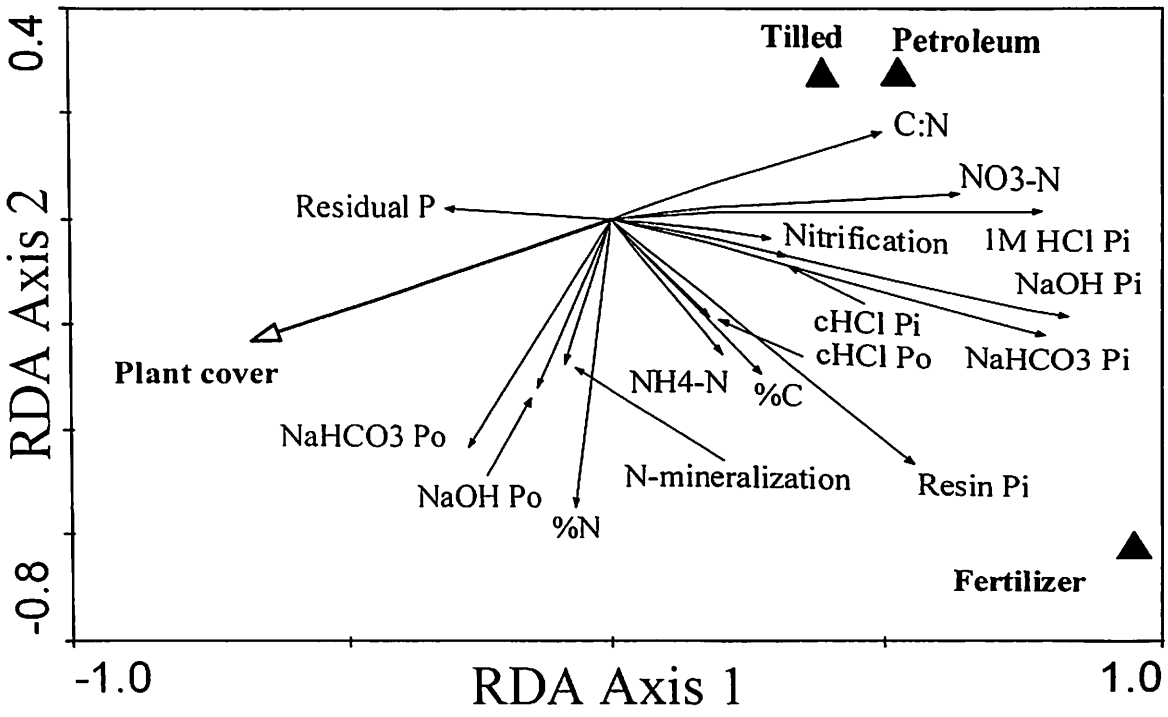
Tilled
Petroleum
C:N
NO3-N
Residual P
Nitrification
1M HCl Pi
NaOH Pi
cHCl Pi
cHCl Po
NaHCO3 Pi
Plant cover
NH4-N
%C
NaHCO3 Po
N-mineralization
Resin Pi
NaOH Po
%N
Fertilizer
0.4
-0.8
RDA Axis 2
-1.0
RDA Axis 1
1.0

Figure 13b

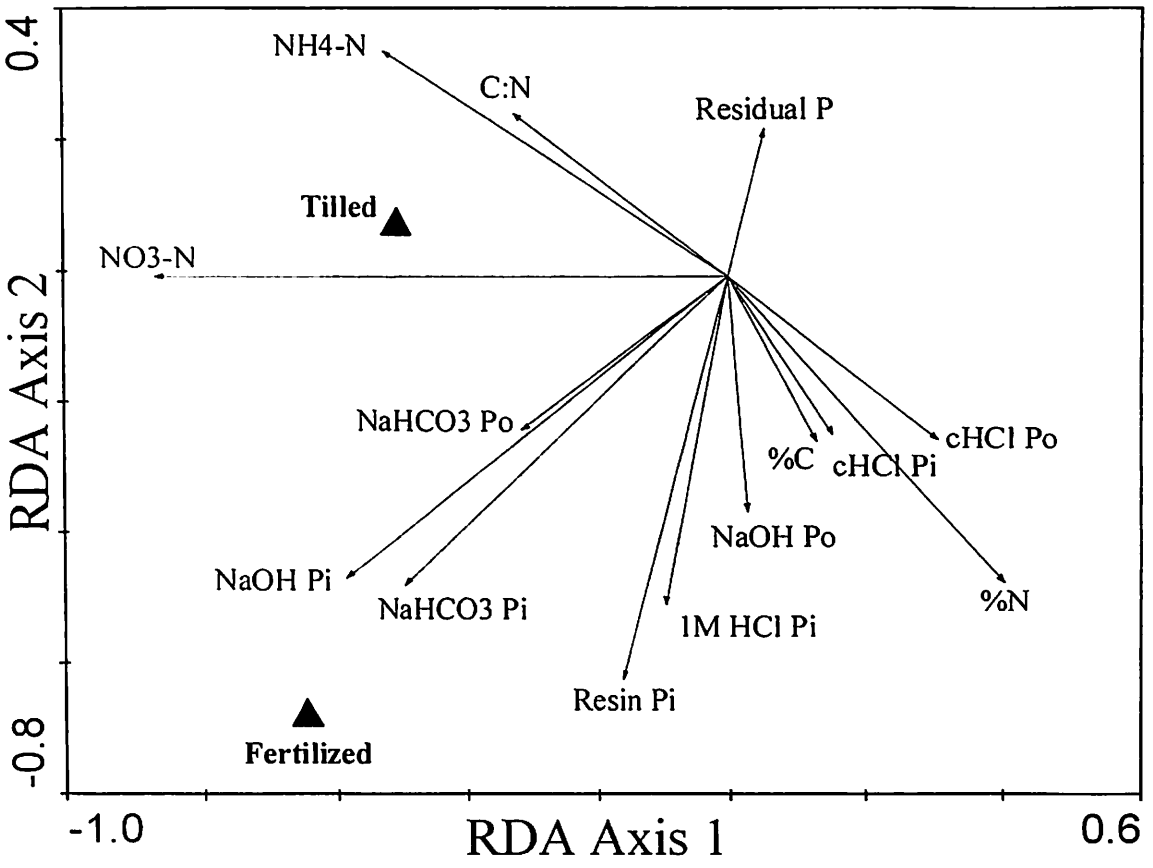
0.4
-0.8
RDA Axis 2
NH4-N
C:N
Residual P
Tilled
NO3-N
NaHCO3 Po
%C
cHCl Po
cHCl Pi
NaOH Po
NaOH Pi
NaHCO3 Pi
%N
1M HCl Pi
Resin Pi
Fertilized
-1.0
RDA Axis 1
0.6

Figure 13c

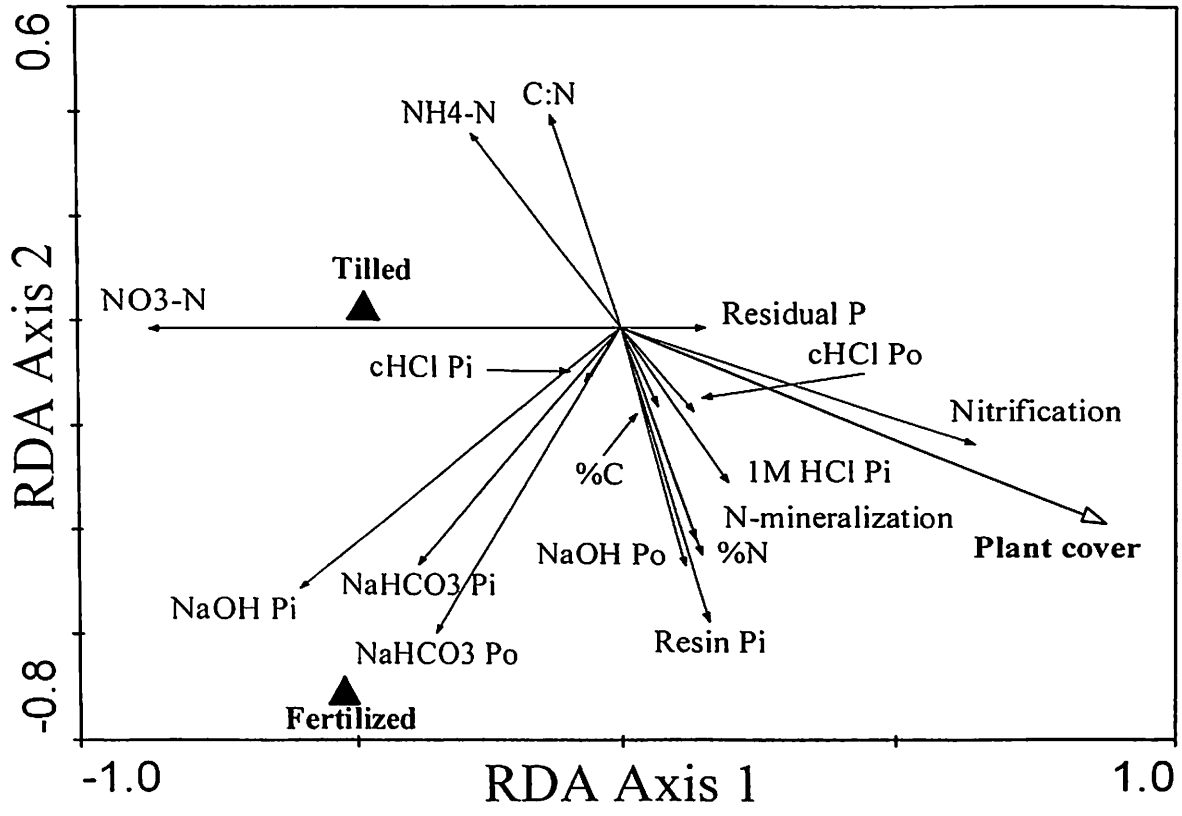
0.6
-0.8
RDA Axis 2
-1.0
RDA Axis 1
1.0
NH4-N
C:N
Tilled
NO3-N
Residual P
cHCl Pi
cHCl Po
Nitrification
%C
1M HCl Pi
N-mineralization
NaOH Po
%N
Plant cover
NaHCO3 Pi
NaOH Pi
Resin Pi
NaHCO3 Po
Fertilized

Figure 13d

CHAPTER II

COMPARING EXPLANATORY VARIABLES IN THE ANALYSIS OF SPECIES COMPOSITION OF A TALLGRASS PRAIRIE

Abstract

Although the relationship between soil characteristics and plant species composition has been well studied, exploratory analyses have been limited by the cost and/or difficulty of soil analyses. In this study I used Canonical Correspondence Analysis to determine whether a set of potentially important but difficult to measure (or "new") soil variables (total C and N, inorganic N, potential net N-mineralization and net nitrification rates, P fractions, and soil textural classes) explain composition of tallgrass prairie species beyond that explained by an easier and more routinely collected (or "old") set. Through forward selection I chose five environmental variables (total C (C_t), pH, Fe, residual P (P_r), and Zn) that explained a significant ($\alpha = .05$) portion of variation in species composition. Using variance partitioning I found that the "new" variables, C_t and P_r, independently accounted for 35.9% of the explained variation in species composition. However, when a second forward selection was performed using only the "old" variables, soil organic matter (SOM) and slope, were chosen in place of C_t and P_r. The explanatory power of the "new" variables (C_t, P_r) was not significantly greater than that of SOM and slope. In addition, the large number of significant correlations between C_t and P_r and essential resources suggest that these variables are only indirectly linked to species composition. This study demonstrates that difficult-to-measure variables are superfluous in some cases.

Introduction

The relationship between soil characteristics and species composition is useful to understand for restoration because the success of maintaining or restoring a specific community depends on how management impacts such characteristics (Critchley, 2002). Canonical Correspondence Analysis, a form of direct gradient analysis, has proven to be a useful tool for comparing plant species composition of communities with many environmental gradients (Ter Braak, 1987, Palmer, 1993, Lepš and Šmilauer, 2003). Thus, it is a potentially useful technique for restoration ecology. However, exploratory analyses of species composition are limited because soil analysis is often expensive or labor intensive.

Nitrogen and phosphorus are of particular interest as potential explanatory variables because they are the two most commonly limiting soil nutrients in grasslands (Seastedt *et al.*, 1991, Boeye *et al.*, 1997, Brenner, 2001, Turner, 2003). Nutrient limitation is one of the most important factors affecting plant communities (Grime *et al.*, 1997). This might be due to competition for the one most limiting nutrient such as nitrogen or differential limitation between species by different nutrients (Koersleman and Meuleman, 1996). For example, higher phosphorus availability may favor legumes, whereas higher nitrogen availability could favor grasses (Janssens *et al.*, 1998). The quantity of available nitrogen can have a major influence on species composition and diversity (Willems *et al.* 1993, Mountford *et al.* 1993), and phosphorus can control vegetation type and soil organic matter (Smeck, 1973). Ratios of these nutrients with organic carbon may also have substantial effects on vegetation (Koerselman and Meuleman, 1996). For instance, a C:N ratio of 14 has been observed to cause a N-

mineralization rate that maximized plant productivity whereas lower ratios caused nitrate leaching and higher ratios caused litter accumulation (Alvarez *et al.* 1998).

Not all variables are equally useful in explaining plant species composition (Palmer *et al.*, 2002). Previous exploratory analyses of tallgrass prairie have included nitrogen and phosphorus gradients that correlate well with productivity in agronomic systems. However, natural communities occur across a wide range of nutrient regimes, and measurements of highly available nutrient pools might not represent the major sources of plant available N and P in unfertilized systems with coevolved niche differentiation (Critchley, 2002, Schmidt *et al.*, 1996). Such systems may rely heavily on the mineralization of organic N and P through microbial activity. Distinctions between mineralization processes such as ammonification and nitrification could provide greater explanatory value because plant competition is affected by the form of available N (Schimel *et al.*, 1989, Jackson *et al.*, 1989, Bloom *et al.*, 2003). There is substantial temporal heterogeneity and microbial redistribution of P (Magid and Nielson, 1992, Hedley *et al.*, 1982). Although P-mineralization is difficult to measure, the total P in soils can be divided into inorganic and organic fractions and quantified based on levels of bioavailability (Hedley *et al.*, 1982). Agriculture-based P measurements quantify the combined total of immediately soluble P plus portions of the more easily extracted, insoluble fractions (Mehlich, 1978a, Mehlich, 1978b, Mehlich, 1984), however, biologically active P has been found in several fractions (Schmidt *et al.*, 1996, Nichols, 1984). Fractionation of total P allows for comparisons of pools of P that may be plant available (Abrams and Jarrell, 1992) and recalcitrant P forms that may explain

community structure through their correlation to soil weathering (Smeck, 1973, Newman, 1995).

It is also important to explore relationships between important environmental gradients. Many variables that display high explanatory power in direct gradient analyses may in fact be serving as proxies for one or many variables that have a more direct, causal relationship with plant species composition. The species composition of the plant community is not only controlled by the initial physical environment but also by the modifications to the physical environment imposed by community succession (Odum, 1969). As a result the causal relationships resulting in simple correlations between species composition and environmental variables may be extremely convoluted. For example, P availability may be controlled by the chemical characteristics of the soil parent material or the chemical characteristics of plant litter (Walker and Adams, 1958, Walker *et al.*, 1959, Nichols, 1984). P-availability may affect species composition directly by favoring legumes (Walker and Adams, 1958, Janssens *et al.*, 1998), or it could exert indirect control by affecting N-fixation, N-mineralization and nitrification (Walker and Syers, 1976, McGill and Cole, 1981, Janssens *et al.*, 1998, Hue and Adams, 1984). There is strong evidence for control of N-mineralization rates by the C:N ratio (Aulakh *et al.*, 2000). Textural classes are strongly correlated with P fractions (O'Halloran *et al.*, 1987, Day *et al.*, 1987) and soil organic matter (Hook and Burke, 2000) but less so with soil N and N-mineralization (Burke *et al.*, 1997, Hook and Burke, 2000). Such correlations by proxy may provide useful generalizations, but it is important to demonstrate causality in order to apply information provided through direct gradient analyses to management.

My objectives for this study are: 1) to compare the abilities of several pools of soil carbon, nitrogen, and phosphorus and soil texture to explain plant species composition in tallgrass prairie; and 2) to examine proxy relationships between variables with high explanatory value and other potentially important environmental variables in this community.

Methods

All vascular plant species were recorded in 20 permanent 10m x 10m plots in the Nature Conservancy's Tallgrass Prairie Preserve during June of 2002 (Palmer *et al.*, 2003). The plots were a random sample of grassland plots from a total of 151 that are located at the intersections of the 1km x 1km UTM grid. These 20 plots have been resurveyed annually beginning in 1998. Species abundance was quantified by estimating percent cover (Palmer *et al.*, 2002). Each plot is one sample in the species data for use in Canonical Correspondence Analysis (CCA) (Ter Braak, 1986).

For direct gradient analysis I used estimates of percent slope, aspect, height of grasses, forbs, and woody plants, and percent cover of rock, bare ground, and woody plants. I also collected soil samples from each plot. The samples consisted of four combined cores from the top 10 cm of the soil profile (Palmer, 1990). I divided each sample into two portions. At Oklahoma State University (OSU), I measured P fractions (Figure 5) (Tiessen and Moir, 1993), inorganic N (Maynard and Kalra, 1993), potential net N-mineralization and net nitrification rates (Vinton and Burke, 1995), total carbon and nitrogen with a LECO CN 2000 combustion analyzer (Leco, St. Joseph, MI), and soil texture (Bouyoucos, 1951, Gavlak *et al.*, 2003). The other portions of these samples were sent to Brookside Labs in New Knoxville, Ohio to be analyzed for cation exchange

capacity (CEC), pH, percent soil organic matter (SOM), estimated N-release, soluble S, exchangeable Ca, Mg, K, and Na , and Mehlich III extractable P, Mn, Zn, B, Cu, Fe, and Al (Mehlich, 1984). Unlike potential net N-mineralization, estimated N-release is calculated as a function of SOM. I log transformed all variables, excluding pH, that were derived from these soil analyses (Palmer, 1990). In addition I included easting, northing, and sampling date for each plot sampled in the analysis. For the purposes of discussion I will refer to total carbon and nitrogen, inorganic nitrogen, mineralization and nitrification rates, phosphorus fractions, and soils textural classes as the "new" environmental variables. All other environmental data constitute the "old" variables.

To perform direct gradient analysis (Palmer, 1993) and variance partitioning (Borcard *et al.*, 1992, Økland, 1994, Økland, 1999) with the environmental variables and species data, I used canonical correspondence analysis (CCA) through CANOCO for Windows software (Ter Braak and Šmilauer, 1998). I chose to square-root transform the species data and down weight rare species prior to analysis. Because the number of environmental variables collected was greater than the number of samples, I used stepwise forward selection to choose the environmental variables from the full set that explained the greatest amount of variation in plant species composition within the samples (Ter Braak, 1988b, Hallgren *et al.*, 1999). In addition, I used stepwise forward selection to choose environmental variables from only the "old" set of variables in order to detect those variables that are potentially interchangeable with new variables. I chose variables with p values less than 0.05 derived through Monte-Carlo permutations tests with 999 permutations.

I used variance partitioning (Økland, 2003) to evaluate the redundancy in explanatory value of a set of "old" (O) and 2 "new" (N_1 and N_2) variables chosen through forward selection. The CCA of all selected variables measures the total inertia (TI) of the variation in plant species composition and the inertia explained by the union of the 3 sets ($N_1 \cup N_2 \cup O$). I divided the TI explained by $N_1 \cup N_2 \cup O$ into $2^n - 1 = 2^3 - 1 = 7$ components. I quantified the inertia uniquely explained by each set ($N_1|N_2 \cup O$, $N_2|N_1 \cup O$, and $O|N_1 \cup N_2$) with 3 partial CCAs in which I entered 1 set as environmental variables and the 2 remaining sets as covariables. I quantified the intersections between all 3 sets ($N_1 \cap N_2 \cap O$) and each pair of sets ($N_1 \cap N_2|O$, $N_1 \cap O|N_2$, and $N_2 \cap O|N_1$) indirectly. For example, $N_1 \cap N_2|O$ is the difference between $N_1 \cup N_2|O$ and the sum of $N_1|N_2 \cup O$ and $N_2|N_1 \cup O$, and $N_1 \cap N_2 \cap O$ is the difference between $N_1 \cup N_2 \cup O$ and the sum of $N_1|N_2 \cup O$, $N_2|N_1 \cup O$, $O|N_1 \cup N_2$, $N_1 \cup N_2|O$, $N_2 \cup N_1|O$, and $O \cup N_1|N_2$. I used variance partitioning to test the null hypothesis: the "new" environmental variables do not explain variation in plant species beyond that which is explained by the "old" environmental variables (Ter Braak, 1986, Ter Braak, 1987, Ter Braak, 1988a, Ter Braak and Prentice, 1988).

In addition to CCA, I compared all environmental variables using Pearson correlations with SPSS FOR WINDOWS (2001). As I display these correlation coefficients to assess the strength, rather than the significance of these relationships, I do not correct for the multiple correlation (Legendre and Legendre, 1998).

Results

Forward selection chose environmental variables in the order: total C (C_t) ($p = 0.001$), Mehlich III extractable Fe (Fe) ($p = 0.001$), pH ($p = 0.002$), residual P (P_r) ($p =$

0.031), and Mehlich III extractable Zn (Zn) ($p = 0.041$). The remaining variation in plant species composition could not be significantly explained ($\alpha = 0.05$) with the available set of environmental variables. The selected variables explained 41% of the total inertia of the species data. C_t and P_r were chosen from the "new" set of variables. Forward selection from only the "old" variables set resulted in the selection: soil organic matter (SOM) ($p = 0.001$), Fe ($p = 0.001$), pH ($p = 0.001$), slope ($p = 0.044$), and Zn ($p = 0.044$). These variables also explained 41% of the total inertia of the species data.

Variance partitioning shows that 37.9% of the variation explained by C_t, Fe, pH, P_r, and Zn is uniquely explained by the "new" variables C_t and P_r (Figure14). C_t uniquely accounts for 20.1% of the explained variation, and P_r accounts for 15.0% of the explained variation. The intersection of variation explained by C_t and P_r accounts for 2.8% of the explained variation. Together the three "old" variables (Fe, pH, and Zn) accounted for 53.3% of the explained variation. The intersection of C_t and the "old" variables accounted for 1.9% of the explained variation, and the intersection of P_r and the "old" variables accounted for 0.5% of the explained variation. The intersection of all five variables accounted for 6.3% of the explained variation. I do not display variance partitioning between the selected "new" variables and the "alternate" "old" variables (SOM and slope) because the partial CCAs necessary to produce N|O and O|N had *p* values of 0.16 and 0.21 respectively ($\alpha = 0.05$).

Using two-tailed tests of significance C_t, Fe, pH, P_r, and Zn were significantly correlated ($\alpha = 0.05$) with 25, 12, 18, 17, and 4 of the unused environmental variables in the data set respectively. Total N was the soil nutrient variable most highly correlated with both C_t and P_r (Table 9). All variables are significantly correlated with C_t except

$NaHCO_3$ P_o, NaOH P_o, and Na. Most soil nutrients are significantly correlated with P_r except the labile forms of N and P, and Na. C_t was significantly correlated with all variables associated with biomass and soil type. P_r was significantly correlated with all texture variables, soil organic matter, and forb height. C_t was significantly correlated with all inorganic fractions of soil P and organic soil P extracted with concentrated HCl. P_r was significantly correlated with the other recalcitrant forms of soil P (HCl extractable P and NaOH extractable organic P. The correlation coefficient between P_r and slope was 0.438 ($p = 0.053$).

Discussion

Based on the results of variance partitioning, the "new" environmental variables were unable explain variance in species composition beyond that explained by the "old" environmental variables. The low level of redundancy found between C_t, Fe, pH, P_r, and Zn suggests that C_t and P_r could provide additional, significant explanation of species composition. However, partial CCAs comparing C_t and P_r to SOM and slope show that differences between the "new" variables and "alternate" "old" variables are non-significant.

The lack of significance is primarily due to the strong correlation between C_t and SOM. C_t represents pools of inorganic C, which may be found in abundance in limestone soils, in addition to the organic C represented by SOM. However, organic C also tends to be higher in limestone soils. By combining two of the common characteristics of soils forming from limestone parent material, high inorganic C and high organic content, C_t could provide a marginal increase in explanatory power over variables representing organic content and Ca content in plant communities that are highly influenced by soil

type such as the boundary between cross-timbers and prairie (Francaviglia, 2000). Unfortunately the small sample size in this study is inadequate for outlining the differences in such highly correlated variables.

P_r is not so highly correlated with any single variable in the available set, and its correlation with slope is only marginally significant. However, interpreting its value in explaining plant species composition in problematic. P_r tends to be correlated, although weakly, with many of the same environmental variables as C_t. This is likely because recalcitrant P is also associated with a limestone parent material (Schlesinger, 1997). The explanatory value of P_r beyond that of the other selected variables, though statistically significant, shows no discernable relationship with the ecological traits of the species variables. In addition P_r almost certainly functions as a proxy variable because the pool of soil P represented by P_r is not bioavailable without extensive weathering. As a proxy variable P_r is likely related to soil type and extent of weathering (Smeck, 1973). A stronger causal connection between P_r and species responses is still needed in order to interpret the role of P_r in controlling species composition. It is also possible that the significant explanatory value of P_r is an artifact of a low sample size. Unfortunately the high cost of phosphorus fractionation suggests that P_r is not likely to play a substantial role in the future of direct gradient analysis.

C_t and P_r are strong examples of the use of proxy variables in direct gradient analysis. Unlike indirect gradient analysis, direct gradient analyses such as CCA constrain the scores of the response variables to be linear combinations of explanatory variables. The variation in species composition of the samples is represented in terms of the chosen explanatory variables, but a causal relationship cannot be guaranteed.

Environmental gradients such as C_t, SOM, Ca, and pH tend to be strongly correlated with patterns in plant species composition, but it is difficult to unravel the causal relationships responsible for this correlation. It is likely that most if not all of these variables serve as proxies for environmental conditions contributing to the spatial arrangement of species within the prairie community. Resources traditionally recognized to limit plant growth include space, light, water, and nutrients. C_t and P_r are correlated with environmental variables associated with the availability of space, light, and water (Table 2), and C_t is correlated with plant available nutrients (Table 1, Table 2). The correlation of C_t and P_r with such a large number of potentially influential environmental variables suggests that the relationships between C_t and P_r and plant species composition are probably not directly causal.

It is not surprising that proxy variables representing multiple factors affecting species composition are chosen through stepwise forward selection because we intentionally choose variables that explain the greatest amount of variation in the species data. Future research intended to identify more direct relationships could focus on more homogeneous systems such as only communities within a single soil type. In contrast, when studying a more heterogeneous system such as the prairie-crosstimbers continuum, proxy variables should become more dominant by explaining differences between forest and grassland vegetation. Due to the interdependence of organisms and multiple biogeochemical cycles, proxy variables will likely continue to play an important role in exploratory analysis.

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Table 9. Pearson correlation coefficients between total C, residual P, and soil nutrients, soil texture, and site characteristics.

	Total C	Residual P
Total N	0.977**	0.679**
NO_3	0.464*	-0.017
NH_4	0.534*	0.179
Resin P_i	0.664**	0.353
$NaHCO_3$ P_i	0.455*	0.240
NaOH P_i	0.512*	0.392
1M HCl P_i	0.542*	0.485*
cHCl P_i	0.866**	0.781**
$NaHCO_3$ P_o	-0.361	-0.004
NaOH P_o	0.398	0.546*
cHCl P_o	.0899**	0.790**
K	0.583**	0.551**
SO_4	0.808**	0.632**
Ca	.0868**	0.633**
Mg	0.827**	0.650*
Na	0.234	0.186
Sand	-0.835**	-0.778**
Silt	0.758**	0.689**
Clay	0.892**	0.693**
Soil organic	0.959**	0.713**
Bare ground	-0.496*	-0.337
Grass height	0.588**	0.259
Forb height	0.523*	0.473*

** Significant at the 0.01 level (2-tailed)

* Significant at the 0.05 level (2-tailed)

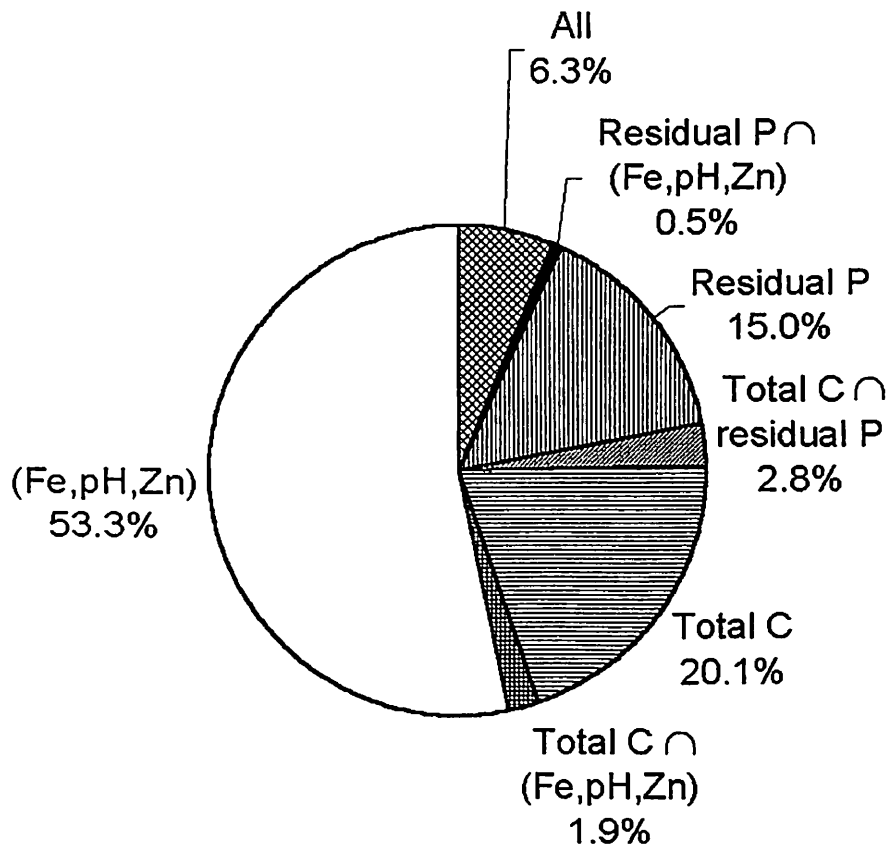

Figure 14. Partitioning of variation in plant species composition explained by the soil variables: total C, Mehlich III extractable Fe, pH, residual P, and Mehlich III extractable Zn. Variation explained by Fe, pH, and Zn is grouped as one unit.
∩ = intersection.

VITA (1)

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