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MANAGING CROPPING SYSTEMS TO ENHANCE THE ACTIVE SOIL NITROGEN POOL AND CONTROL ITS MINERALIZATION

By

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ABSTRACT

MANAGING CROPPING SYSTEMS TO ENHANCE THE ACTIVE SOIL NITROGEN POOL AND CONTROL ITS MINERALIZATION

By

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Sustainable nitrogen (N) management requires practices that enhance soil fertility and minimize N loss. This study explores the effects of substrate diversity and living roots on N mineralization, and the influence of management on the seasonal pattern of soil nitrate (NO_3) levels. In part 1 a "diverse system", consisting of a corn (*Zea mays* L.)-corn-soybean (*Glycine max* L.)-wheat (*Triticum aestivum* L.) rotation with cover crops and fertilized with composted dairy manure was compared to a corn monoculture with conventional fertilizers. The specific objectives were to determine if a diverse system would induce higher mineralization rates than would a continuous corn monoculture, and to determine whether these two systems differ in their ability to mineralize added substrate. Net mineralized N in the diverse system was 90 and 40% higher than that of the monoculture at 70 and 150 days of laboratory incubations respectively. A comparable response was found *in situ* where a 70% higher net mineralization was observed at 70 days. The active N pool increased over time, but the ability of soil organisms to break down additional substrate did not change as a result of diversity. In part 2, soil from the diverse system was used to determine if living corn roots influence N mineralization. Corn roots increased the mineral-N supplying capacity of soil from the diverse system by more than 50%. This increase appears to be caused by a stimulation of

N mineralization, but a previous increase in the active N pool seems to be necessary before significant stimulation can be realized. In part 3, soil NO_3 levels were measured throughout six years from April through December in the previously described rotation and continuous corn both receiving compost or fertilizer. The objectives were to determine if NO_3 fluctuation patterns could be used as a suitable indicator of soil fertility changes within or between seasons, and to determine the leaching potential resulting from the management strategies. Seasonal soil NO_3 levels were sensitive to management. Soil NO_3 levels were generally higher in plots receiving fertilizer than those receiving compost. The exceptions were the cover cropped 1st year corn and all soybean plots. Soil NO_3 levels responded not only to fertilizer applications but also to an increased active N pool. The leaching potential in fertilized corn was always significantly higher than in corn receiving compost. In soybean and wheat, no difference between fertility sources was observed. A comparison between leaching potential and NO_3 levels in the following spring revealed that fertilized corn may be susceptible to higher leaching losses. The seasonal NO_3 patterns were sensitive indicators of fertility status. Fertility can be increased by application of conventional fertilizer and also by soil conditioning practices that increases the mineralizable N pool size. Farming practices enhancing substrate diversity can dramatically increase the mineralizable N pool size. The use of rotations, cover crops, and organic amendments from animal sources enhanced the mineralizable N pool. This in combination with a crop specially selected for its ability to stimulate N mineralization has the potential to increase yields, decrease fertilizer requirements, and thus have a beneficial impact on soil and water quality.

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PREFACE

Chapter 1 in this dissertation was written in the style required for publication in the *Soil Science Society of America Journal*. Chapter 2 was written in the style required for publication in the *Agronomy Journal*. Chapter 3 was written in the style required by the *Journal of Environmental Quality*.

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CHAPTER 1

Substrate Diversity Affecting N Mineralization in Long-Term Cropping Systems

ABSTRACT

This study explores the effect of substrate diversity on nitrogen (N) mineralization. A "diverse system", consisting of a corn (*Zea mays* L.)-corn-soybean (*Glycine max* L.)-wheat (*Triticum aestivum* L.) rotation with cover crops and fertilized with composted manure was compared to a corn monoculture with conventional fertilizers. N mineralization was measured *in situ* and in laboratory incubations as was the ability of each soil to mineralize added compost and red clover (*Trifolium pratense*) residue in the 6th and 7th years of rotation. Net mineralized N in the "diverse system" was 90 and 40% higher than that in the monoculture at 70 and 150 days of laboratory incubations respectively. Comparable response was found *in situ* where a 70% higher net mineralization was observed at 70 days. The 70- and 150- day mineralizable N pools increased over time, but the ability of soil organisms to break down additional substrate did not change as result of diversity. The high level of agreement between the laboratory and *in situ* mineralization data is also discussed. A more diverse cropping system may increase the soil's capacity to supply N to a growing crop while maintaining desirable levels of soil organic matter. This is essential for the overall long-term productivity and sustainability of agricultural systems.

INTRODUCTION

Nitrogen mineralization is an important indicator of soil quality (Duxbury and Nkambule, 1994; Seybold et al., 1998), and its management could represent an excellent tool in achieving a sustainable N supply. Managing N mineralization efficiently is likely to result in a more synchronized N release, and have the potential to reduce N fertilizer dependence while promoting N recycling within agroecosystem boundaries.

N mineralization is regulated by abiotic factors such as soil moisture, temperature, and texture; and by the supply of above- and below-ground substrates (Jenny, 1980). In a practical sense, managing N mineralization implies managing organic inputs. The way in which organic materials influence mineralization is closely related to their quality (Swift et al., 1979). The quality of a particular material is defined by its chemical composition, including C/N ratio, lignin, and polyphenol contents (Tian et al., 1997). Substrates with low N and high concentration of lignin and polyphenols decompose and release N slowly (Cornforth and Davis, 1968). In contrast, those rich in N with low lignin and polyphenol concentrations decompose rapidly (Handayanto et al., 1997). Therefore, a well-balanced diversity of materials entering the soil is expected to favor N availability for a growing crop while maintaining desirable levels of soil organic matter.

It is accepted from an ecological point of view that enhanced species richness is beneficial for ecosystem performance (Kareiva, 1996, Tilman et al., 1996), but the question remains whether that is the case for below-ground subsystems (Wardle et al., 1996). Diversity can be primarily achieved with the use of crop rotations, cover crops, and application of organic amendments (Gliessman, 1998). In this work we investigated how substrate diversity influences N mineralization. Two cropping systems were

selected according to their level of diversity. The "diverse system" received residues of corn, soybean, wheat, red clover, crimson clover (*Trifolium incarnatum*), and annual ryegrass (*Lolium multiflorum*), in combination with composted manure during each rotation cycle. This system was compared to a continuous corn monoculture where commercial fertilizer was the fertility source. Specific objectives of this study were to determine if the "diverse system" would induce higher mineralization rates than the continuous corn monoculture, and to determine whether these two systems differ in their ability to mineralize added substrate. The first objective deals with the potential of the cropping system to supply N to a growing crop. The second asks whether there have been important changes in microbial performance relating to the decomposing ability of each microbial community.

In 1998 and 1999, laboratory and *in situ* incubation experiments were initiated to determine N mineralization potentials. While *in situ* incubations were expected to generate data that more closely reflect actual field conditions, laboratory incubations require less effort and cost. A regression analysis of N mineralization over time was used to compare them. Learning more about the relationship between the two methods has the potential to facilitate the calibration of predictive models based on commonly used laboratory incubations (Smith et al., 1977; Bhogal et al., 1999).

MATERIALS AND METHODS

Site Description

This study was conducted in the Living Field Laboratory (LFL), a long-term experiment established in 1993 at the W. K. Kellogg Biological Station located in Southwest Michigan. The site experiences cold moist winters and warm humid summers. Average precipitation has been 860 mm y^{-1} (1988-1999), and potential evapotranspiration typically exceeds precipitation from May through September. The soil is a mixture of Kalamazoo and Oshtemo sandy loams (Typic Hapludalfs). The depth of the Ap horizon is 20 to 25 cm, and pH ranges from 6.3-6.8. The LFL was designed to test various combinations of rotation and cover crops under several agronomic management regimes (Jones, 1996). The main goal is to test alternative strategies for achieving nutrient cycling efficiency and reducing chemical input requirements. The experimental design is a four-replication split-split-plot, randomized complete block, with main plots for each management type. The plots used for this study were in the Integrated Compost and Integrated Fertilizer management types. Both management types include minimal application of pesticides, and banded herbicide plus cultivation for weed control. The main difference is in their fertility source: Integrated Fertilizer uses commercial fertilizer and Integrated Compost uses composted dairy manure. Subplots represent each crop in a corn-corn-soybean-wheat rotation, plus continuous corn. All crops except soybean are grown with and without a cover crop. Cover crops include red clover frost-seeded into wheat, crimson clover interseeded into 1st year and continuous corn, and annual ryegrass interseeded into 2nd year corn. Before planting, approximately 4 Mg ha^{-1} of composted

manure is added annually to all crops except soybean. Reduced tillage (chisel plow) is used throughout the LFL.

This study used 1st year corn plots with cover crops of the Integrated Compost, and the continuous corn plots without cover crop of the Integrated Fertilizer management. The crop rotation in the Integrated Compost was selected as the "diverse system" because residues of six plant species in combination with composted manure are incorporated into the soil throughout the rotation cycle. These substrates have a wide range of C/N ratio. The lowest C/N ratio provided by the red clover cover crop (14:1) and the highest by wheat stubble (80:1). We used the 1st year corn (immediately following wheat + clover) plots because, historically, its grain yields has been comparable to those when fertilizer was used (Jones, 1996). In contrast, the continuous corn plots in the Integrated Fertilizer management only receives corn residues (C/N ratio of 60:1) as substrate. For clarity we will use the terms "diverse system" to describe the 1st year corn, and "monoculture" when referring to continuous corn. The C, N, and C/N ratios of soil from these cropping systems are described in Table 1.

In early May 1998 and 1999, soil samples were taken from the diverse and monoculture cropping systems following tillage and immediately before planting and used in long-term laboratory incubations. Immediately after planting, microplots were established in the plots of the two cropping systems and were used for *in situ* incubations.

Laboratory Incubation

Soil samples were taken at 0-10 cm depth using a 1.9 cm diameter soil probe. Thirty cores from each plot were composited, placed in plastic bag, and stored at approximately 4 °C until processed. Soils were sieved through a 6 mm screen and sub-sampled for moisture determination. Sixty-three 20 g dry weight equivalent aliquots of each sample were weighed into 100 ml plastic specimen cups. Three rates of red clover were added: 0 Mg ha^{-1} (Control), 2.5 Mg ha^{-1} (Clo), and 5 Mg ha^{-1} (2Clo). The rates of compost were 0 Mg ha^{-1} (Control), 5 Mg ha^{-1} (Com), and 10 Mg ha^{-1} (2Com). The combinations Com + Clo, Com + 2Clo, 2Com + Clo, and 2Com + 2Clo were also included to the experiment. Both compost and clover were dried, finely ground, and sub-sampled for chemical analysis using the acid and neutral fiber detergent method (Goering and Van Soest, 1970). Selected chemical properties of added clover and compost are presented in Table 1. Calculations for the actual amount of material added to cups were based on a soil bulk density of 1.3 and depth of 10 cm. The quantity of compost was based on dried weight of non-sand material. Following the additions, each cup was manually agitated to uniformly mix the soil and substrates. The soils were brought to 50 % of water holding capacity. The specimen cups were stored in plastic storage containers that had a thin layer of water on the bottom to maintain humidity. These containers were then placed in a controlled temperature room at 25 °C for 10, 20, 30, 50, 70, 100, and 150 days. At the end of each incubation interval, the corresponding samples were removed and frozen temporarily to stop microbial activity. $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ concentrations were determined using the extraction technique described by Kenney and Nelson (1982) and a Lachat automated colorimetric analyzer (Lachat Instruments Inc. Milwaukee, WI).

In Situ Incubation

Four microplots of 2 m^2 were established in each plot and used for *in situ* incubation experiments (Raison et al., 1987). Two levels of red clover: 0 Mg ha^{-1} (Control) and 5 Mg ha^{-1} (2Clo), two rates of compost: 0 Mg ha^{-1} (Control) and 10 Mg ha^{-1} (2Com), and the combination 2Com + 2Clo were used in this experiment. The clover was dried, and applied unground. The treatments were randomly assigned to microplots, the materials uniformly added to the soil surface, and incorporated using a long-tined hand cultivator (Ben Meadows Co. Atlanta, GA). Fifteen PVC tubes (30-cm long and 5-cm i.d.) were inserted 25 cm in the corn row of each microplot to prevent root in-growth. After tube insertion, soil samples were taken at 0-10 cm from each microplot to measure initial mineral N. Soil moisture was controlled in the cylinder, using temporary rain shelters and periodic addition of water, to prevent extreme wetting-drying events, and to minimize nitrate leaching and denitrification. Soil moisture was gravimetrically determined every two weeks. The amount of water to be added was calculated by subtracting the actual soil moisture from the estimated water holding capacity for the 0-10 cm depth. Half of the calculated amount was added at each of two different days during the two-week period.

On days 14, 28, 42, 56, and 70 after insertion, three cylinders from each microplot were randomly selected and removed. Samples were taken from 0-10 cm depth within each cylinder using a 1.9-cm dia soil probe. The three cores were composited, placed in plastic bag, and stored at approximately 4 °C until processed. Mineral N was determined according to the method mentioned in the laboratory incubation section.

Mineralized N Calculations

Net N mineralization using laboratory and *in situ* incubations was calculated using the difference between inorganic N content at the end of the incubation period and at day 0. For the *in situ* incubation we assumed that deep N leaching was prevented and gaseous N loss minimized. Linn and Doran (1984) reported that in a well-drained soil the relative amount of anaerobic denitrification is negligible. Also, a recent long-term study in an adjacent field indicated that N loss due to denitrification ranged from 1.3 to 0.4 kg ha^{-1} per year (Robertson et al., 2000). Our agronomic treatments were nearly identical to that study.

The amount of net N mineralized from added substrate was calculated by subtracting the mineralized N of the control from the treatments with added substrate. The calculated amount is expressed as a percentage of the initial amounts of N added as clover, and/or compost.

Data Analysis

The same statistical model was applied to the laboratory and *in situ* data sets. Factors used in the model were: year, replication, cropping system, treatment, and incubation time. We performed analysis of variance for each data set, using the SAS Mixed procedure (SAS Institute, 1999) in which the data from each incubation interval was treated as a repeated measurement of the corresponding experimental unit. The optimal covariance structure was determined using Schwarz's Bayesian Criterion (Littell et at., 1997). The laboratory incubations data set was appropriately explained by a compound symmetry (CS) covariance structure, while its heterogeneous version (CSH) better corresponded to the *in situ* data set. CS assumes constant variance and covariance,

and CSH uses a different variance parameter for each diagonal element, and assigns the square roots of these parameters in the off-diagonal entries (SAS Institute, 1999). The appropriateness of CSH for *in situ* data set may be related to the inherent variability commonly found in the field.

The presented results focus on the 70- and 150-day N pools, obtained from mineralization at those days. These incubation periods were selected due to their agronomic importance. The 70-day N pool was expected to closely represent the portion of organic-N available for mineralization during a growing season. The 150-day pool contains that of the 70-day plus a more resistant fraction and is likely to be available for mineralization in a more intermediate term.

In situ incubations were expected to generate data that more closely reflect actual field conditions, but laboratory incubations are more widely used. Regressions for the laboratory and *in situ* incubations were constructed using the least square means (LS-means) of common treatments at incubations periods less than 70 days.

RESULTS

Laboratory Incubation

Results of laboratory incubation are shown in Figure 1. Net mineralized N in the diverse system was 90 and 40% higher than the monoculture at 70 and 150 days of incubation respectively. When substrates were added, the diverse system still mineralized more N than the monoculture when comparing the same treatment and incubation time. The net N mineralization was highest when 5 Mg ha^{-1} of clover was applied to this cropping system. The lowest net mineralization was observed in the monoculture without clover. The monoculture was able to produce as much mineral-N as the diverse system when it received an extra 2.5 Mg ha^{-1} of clover. In general, the addition of compost did not significantly alter net mineralization.

Table 1 provides some important chemical properties of the soils and added clover and composted manure. They are critical elements in explaining the observed mineralization rates for the two materials. The laboratory information in Table 2 shows the percentage of N (from added substrates) mineralized at 70 and 150 days of incubation. There were no significant differences between cropping systems in N mineralization from added clover and/or compost. Clover additions released considerable N during the first 70 days, and modest amounts from 70-150 days of incubation. The proportion of N mineralized from each substrate remained constant as the rate of added substrate was doubled. The proportion of each substrate N mineralized increased, as calculated from weighted average calculation, when the substrate were combined (calculation not shown).

In Situ Incubation

Mineralized N for the two cropping systems at 70 days of *in situ* incubation is shown in Figure 2. The net N mineralization in the diverse system was 70% higher than that of the monoculture, agreeing with the pattern observed in the laboratory. When substrates were applied, the diverse system also produced more N than the monoculture. The highest N mineralizations were obtained when 5 Mg ha^{-1} of clover was added to the diverse system. The monoculture without clover produced the lowest net mineralized N. In the absence of clover, the monoculture required considerably more (compared to the 2.5 Mg ha^{-1} in the laboratory) clover to release comparable amount of N than the diverse system. The addition of compost did not significantly increase mineralized N in any treatment.

In situ results of mineralized N from added substrates are included in Table 2. Mineralized N values from the additions were consistent with those measured in the laboratory. Thus, no significant differences between the two cropping systems in mineralization of added materials were observed. The low mineralization rates of compost caused a dilution effect on the percentage of mineralized N from the combined materials.

Relating Laboratory and *In Situ* Incubations

Figure 3 indicates the level of agreement between laboratory and *in situ* incubation. Regardless of cropping history and addition of materials, net mineralized N at 70 days *in situ* was 90% of that in the laboratory.

DISCUSSION

The striking differences in net mineralization between the two cropping systems demonstrate the linkage between substrate diversity and the size of the active N pool. Swift (1979) reported that quantitative variation in mineralized N occurs as a result of the variety of organic inputs. Net mineralized N in the diverse system was undoubtedly enhanced by the incorporation of residues from the three legumes, three grasses, and composted manure. Individual contribution varies according to quantity and quality of the substrate. For instance, legumes with narrow C/N ratios and abundant soluble compounds are more likely to mineralize at higher rates than residues from grasses with high C/N ratios. It is reasonable to expect that the legumes are primarily responsible for the increased active N pool. Our data agree with those of Stanford and Smith (1972), Chang and Juma (1996), and Franco-Vizcaino (1997), showing that crop diversity including annual legumes increases the mineralizable N compared to monoculture. The higher mineralization rates in the diverse system with added clover and/or compost were influenced by the initial N pool size of this soil. Despite this accumulative effect across treatments, the corn monoculture showed that the presence of clover can dramatically increase its mineralization potential. This indicates that increasing diversity by incorporating a high quality substrate may cause a considerable improvement in soil fertility.

Net mineralized N from the additions was expressed as percentage of added N to facilitate the comparison between both cropping systems. The similar ability of the corn monoculture and the diverse system to mineralize additional organic N may be an indicator of rapid changes in microbial biomass. N mineralization is a microbiologically

mediated process (Paul and Vorony, 1984), and the microbial biomass is largely controlled by organic C content and by recent substrate additions (Paul and Clark, 1989). Its appears that regardless of their initial status, the microbial biomass positively reacts to the abundance of easily-decomposable compounds (e.g., from clover) by increasing its size and activity (immobilization and mineralization). This response was reported in a previous study performed at the same experimental site (Cavigelli, 1998). The sizeable clover-N mineralized during the first 70 days and the small or undetected amount mineralized from 70-150 days suggest that N release may coincide with crop growth. Results of mineralized clover-N are comparable to those reported by Ladd et al. (1983), Bremer and van Kessel (1992), and Biederbeck et al. (1996). It is possible that the addition of compost did not stimulate the microbial biomass as may happen with clover. In a previous study, Willson et al. (2000) found that microbial biomass did not increase immediately following compost additions. Even if the C/N ratio of clover and compost were somehow similar, decomposition rates were different because different C compounds exhibit different decomposition rates (Somda et al., 1991). We suggest that the low, sometimes-negligible, release of compost-N was related to the presence of a large quantity of more stable compounds. During the composting process manure-N is stabilized through microbial assimilation and humification and thus the end product (compost) mineralizes N at a considerably slower rate (Castellanos and Pratt, 1981).

Under laboratory conditions, the monoculture required about 2.5 Mg ha^{-1} of clover to release as much N as the diverse system (in absence of clover). But *in situ*, much more clover was apparently needed. Under field conditions, N mineralization for the diverse system may have been enhanced by meso-, macro-, and mega-fauna activity.

These components of the soil biota are responsible for increased fragmentation of organic materials. Fragmentation increases substrate surface area for further microbial colonization, thereby speeding decomposition and mineralization (Linden et al., 1994). Faunal biomass appears to be more extensive in diverse soils and is related to the amount and quality of organic materials in the soil (Andren et al., 1990).

The combination of substrates always mineralized more N than have been anticipated from the "weighted" means of their individual mineralization. This was especially striking when 5 Mg ha^{-1} of compost was combined with 5 Mg ha^{-1} of clover, where the percentage mineralized was nearly double that anticipated from their individual amounts. We suggested the possibility of a synergistic relationship but this effect was not seen in the more limited *in situ* experiment.

The results of the laboratory incubations were generally comparable to those of the *in situ* experiment. This can be explained by the similarity of their environmental conditions *in situ*. Selected climatic data obtained from the nearby Long-Term Ecological Research weather station is shown in Table 3. Moisture was controlled in both experiments and the average soil temperatures in the field and in the laboratory were also coincidently alike. The greater differences in N mineralizations were observed early in the incubation period. Early season lower average temperatures probably influenced the *in situ* N mineralization to be lower compared to those from the laboratory where temperature was kept constant. The similarity of results generated by these two methods agrees with those reported by Hadas et al. (1989).

These laboratory and *in situ* incubations are not intended to represent actual field mineralizations, but to provide evidence of how this process may be affected by substrate

diversity, since *in situ* mineralizations were done with plant roots being excluded. Further studies are strongly recommended to quantify N release in presence of living roots. Growing roots not only affect N mineralization by exposing organic materials to decomposition and uptake of N, but rhizodeposit production represents a significant energy input to the soil microbial community (Bakken, 1990; Texier and Billes, 1990). Sanchez et al. (2000) in a previous study found that the presence of corn roots can dramatically increase N mineralization in soils with a large active N pool.

CONCLUSIONS

Farming practices promoting diversity have the potential to increase dramatically the mineralizable N pool and decrease fertilizer use. Among such practices, we suggest the use of rotations, cover crops, and organic amendments from animal sources. The ability of soil organisms to break down additional substrate did not change as a result of diversity. This is essential for improving levels of soil organic matter because organic materials entering a "diverse soil" will not decompose at higher rates. Maintaining desirable levels of soil organic matter is closely related to the overall long-term productivity and sustainability. The question of whether there is an additional gain in N mineralization by adding clover and compost combined was not definitely answered by this research. A possible synergistic effect was shown in the laboratory incubations but was not found *in situ*. Laboratory incubation is a reliable tool to estimate N mineralization in the field as long as plant roots are excluded. Further studies are needed to determine how mineralization of added substrates are affected by the presence of roots.

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Table 1. Selected chemical properties of soil from the "diverse" and monoculture cropping systems, and the organic materials added in the laboratory and *in situ* incubations.

Material	C/N	Total C	Total N	Lignin	Cellulose	Hemi-cellulose
				g kg^{-1}		
Diverse soil	11.5	15.0	1.3	-	-	-
Monoculture soil	11.0	11.0	1.0	-	-	-
Young red clover	10.6	433.0	41.0	106.0	100.0	94.0
Composted manure†	13.1	340.0	26.0	90.0	692.0	53.0

† Chemical properties based on weight of non-sand materials. Sand had been added as bedding in the dairy, and was present in the compost at approximately 50% as determined by water column separation.

Table 2. Net mineralized N from added substrates for two cropping systems at two incubation dates, expressed as percentage of added N.

	Diverse system		Monoculture	
	%			
	Laboratory incubation			
Treatments	70 days	150 days	70 days	150 days
Com	0	0	2	2
Clo	30	30	31	31
2Com	0	6	3	4
2Clo	30	34	32	32
Com + Clo	11	15	13	16
Com + 2Clo	21	30	19	29
2Com + Clo	9	12	11	12
2Com + 2Clo	14	18	14	17
	***In situ* incubation**			
2Com	6	-	1	-
2Clo	29	-	24	-
2Com + 2Clo	13	-	12	-

Table 3. Selected climatic data and amount of water added during the time period when *in situ* incubations were performed.

Year	Average of soil temperature at three depths (cm)			Accumulated precipitation	Water added during incubation
	1	4	11		
	°C			cm	
1998	25.1	27.7	24.0	19.2	13.3
1999	25.6	26.8	23.5	21.1	13.0
Average	25.4	27.3	23.8	20.2	13.2

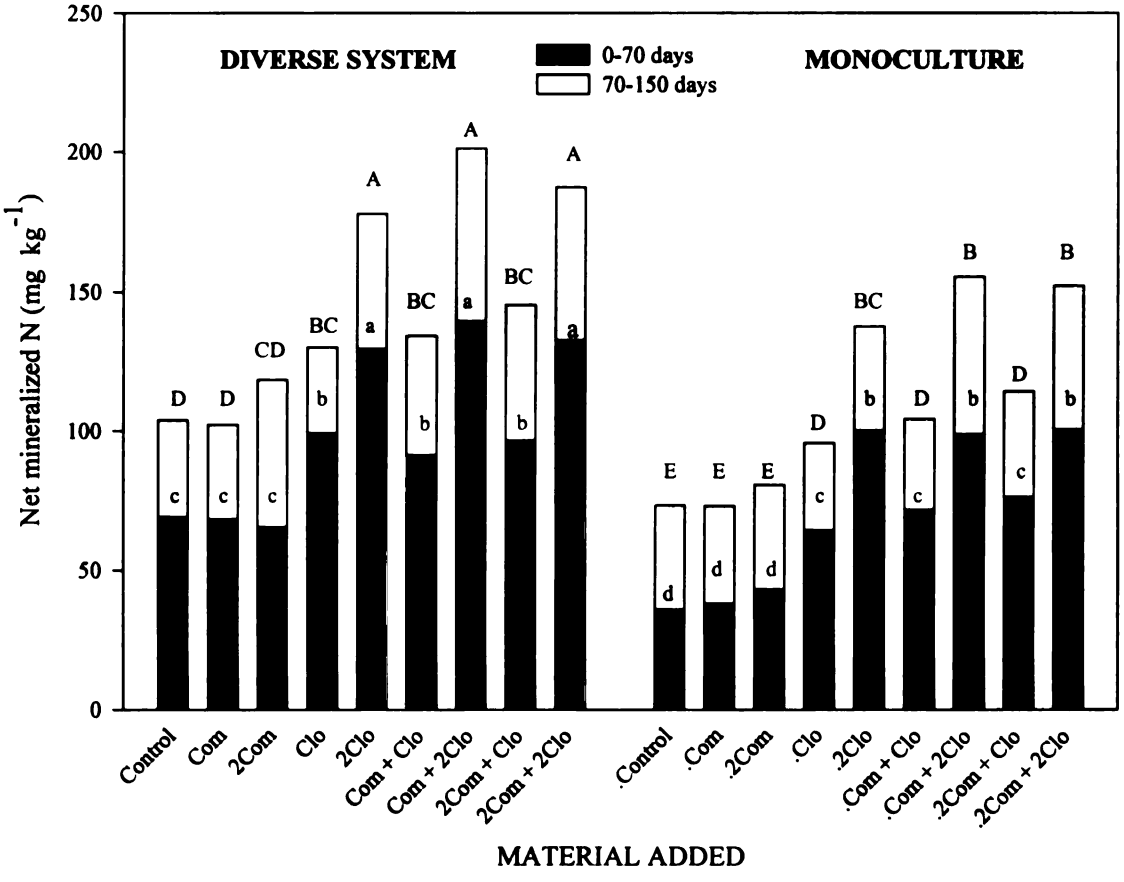

Figure 1. Net mineralized N after 70 and 150 days of laboratory incubations for soil sampled from for the "diverse" and monoculture cropping systems. Lower and upper case letters indicate significant differences in mineral-N at day 70 and 150 respectively ($P < 0.05$, Tukey-Kramer test).

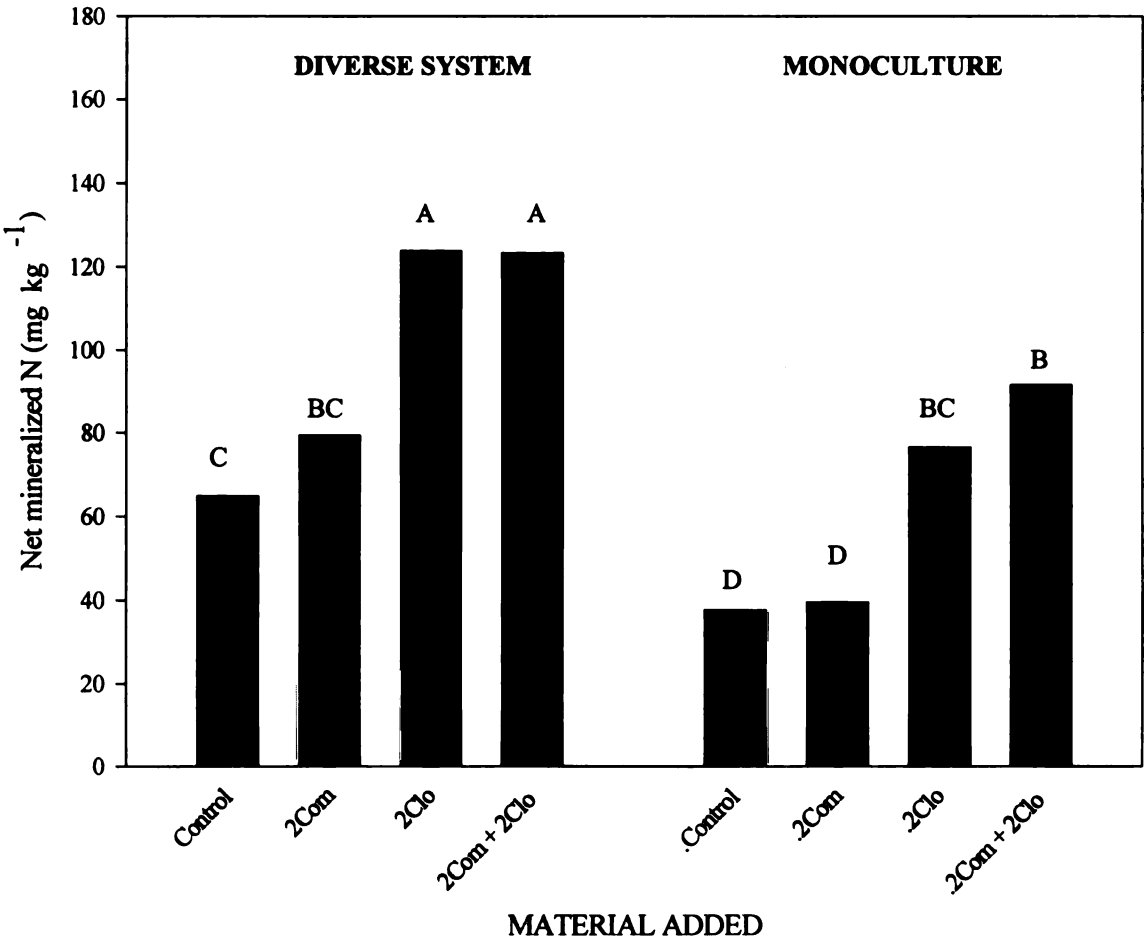

Figure 2. Net mineralized N after 70 days of *in situ* incubations for the "diverse" and monoculture cropping systems. Bars followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer test).

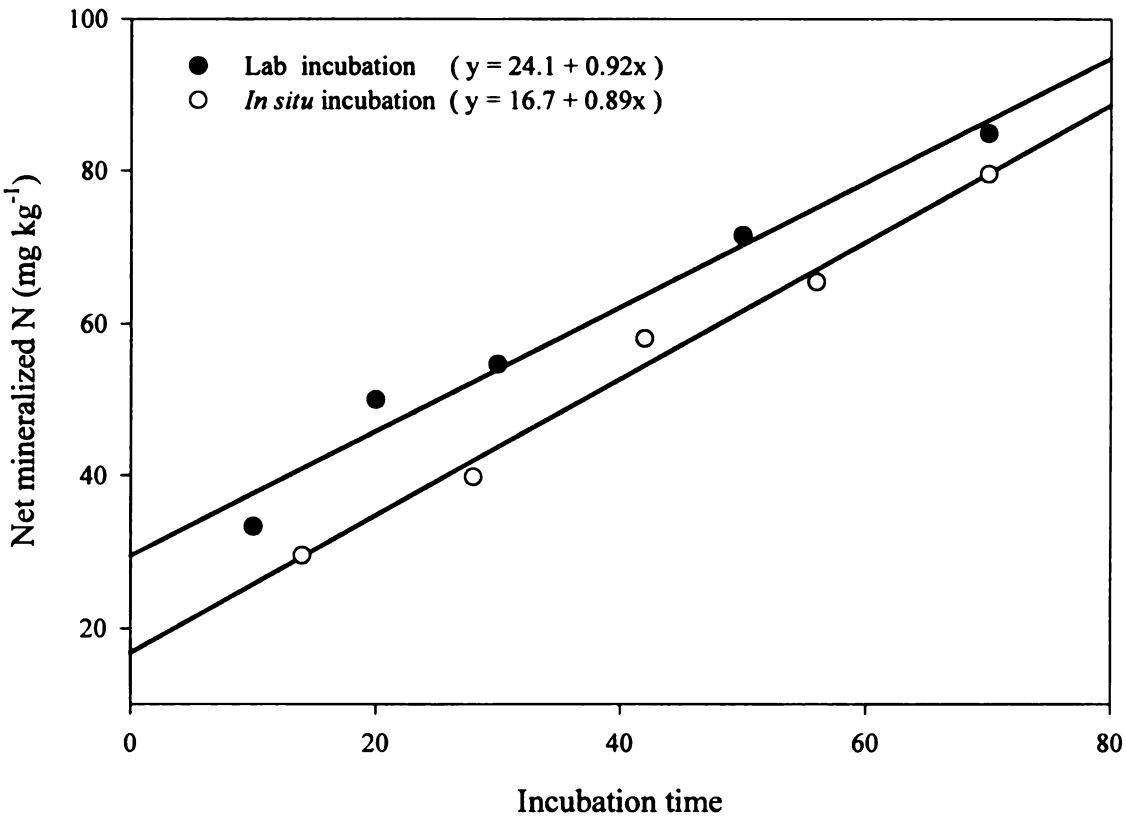

Figure 3. Level of agreement between laboratory and *in situ* incubation across cropping systems and addition of organic materials. Individual data points are the LS-means of 32 observations.

CHAPTER 2

Cropping Effects on Nitrogen Mineralization

ABSTRACT

The main objective of this study was to determine the effect of corn (*Zea mays* L.) roots on nitrogen (N) mineralization. Rotations of corn-corn-soybean (*Glycine max* L.)-wheat (*Triticum aestivum* L.) in combination with cover crops, and the application of composted manure were used as "conditioners" to increase the active N-pool. Polyvinyl chloride (PVC) cylinders were placed in the 1st year corn plots before planting to confine or exclude roots of corn and wheat. Soil moisture was controlled to prevent extreme drying-wetting periods and to minimize N leaching and denitrification. The moisture control regime also promoted shallower root growth, primarily in the Ap horizon. *In situ* N balance was calculated, and changes in N- and carbon (C)-pool sizes were determined by laboratory incubations. Living corn roots increased the mineral-N supplying capacity of conditioned soil by more than 50%. We suggest that this increase is caused by a stimulation of N mineralization but that soil conditioning is necessary before significant stimulation can be realized. No significant increase in available N was observed when wheat was planted, indicating the possibility that this effect may vary dramatically among plant species. The possible effect of specific plant species in maintaining soil organic carbon levels is discussed. This information is vital for the management of N in sustainable cropping systems.

INTRODUCTION

Nitrogen mineralization is a microbiologically mediated process consisting of the transformation of organic N to ammonium, and is a major contributor to the amount of soil N available for plant uptake. Corn may derive up to 70% of its required N from the mineralization of soil N (Parkin et al., 1998). The presence of plant roots has significant effects on the soil microbial population since conditions for microbial growth are especially favorable in the rhizosphere (Barber and Lynch, 1977; Newman, 1985; Foster, 1988; Bazin et al., 1990). The soil microflora is heavily influenced by carbon sources derived from rhizodeposition (Curl and Harper, 1990). These easily-decomposable substrates are translocated from the above-ground parts of the plant to the roots and subsequently into the surrounding soil as root respiration and root-derived material in the form of root exudates, mucilage, and sloughed cells and tissues (Qian et al., 1997). The role of rhizodeposits in the turnover of soil organic matter (SOM) has been controversial for many years. A number of studies reported an increase in the decomposition rate (Billes and Bottner, 1981; Fisher and Gosz, 1986b; Cheng and Coleman, 1990). Others reported no change (Cuenca et al., 1983; Harmer and Alexander, 1985), or even a reduction by the presence of plant roots (Fuehr and Sauerbeck, 1968; Gadgil and Gadgil, 1975; Jenkinson, 1977, Sparling et al., 1982; Fisher and Gosz, 1986a; Staaf, 1988; Faber and Verhoef, 1991).

Contradictory effects have also been reported for N mineralization. A stimulatory effect of plant roots has been observed in some studies (Bartholomew and Clark, 1950; Molina and Rovira, 1964; Clarholm, 1985a; Berg and Rosswall, 1987; Bottner et al., 1991; Haider et al., 1993). Others have found that the net effect of plant roots is to

increase N immobilization (Huntjens, 1971; Texier and Billes, 1990; Qian et al., 1997). The discrepancy of these results reflects the diversity of soils, plant species, experimental methods, and the complexity of the plant-soil interaction on C and N dynamics. Previous workers did not attempt to identify field conditions that enhance the stimulatory effect of plant roots on N mineralization. However, the addition of N-rich plant residues (Bhattacharyya et al., 1986; Wang and Bakken, 1989) or N fertilizer (Liljeroth et al., 1990; Swinnen et al., 1995) may increase N mineralization in the presence of plant roots.

In this work we propose a strategy where previous soil conditioning could be used to enhance root stimulation of N mineralization. Soil conditioning, as we define it, is the process of improving the quantity and quality of the active organic N-pool. We use practices such as long-term crop rotation, cover crops, and the addition of composted manure to build up the active pool of organic N. The main objective of this study was to determine the possible effect of corn roots in stimulating N mineralization. *In situ* N balances were calculated for the bare soil, corn, and wheat treatments at 70 days after planting. Mineralization potentials determined by laboratory incubations were used to quantify changes in the organic-N and -C pools after cropping. According to the results of our study, corn roots growing in a conditioned soil increase soil mineral-N supply by more than 50%. Strategies aimed at developing sustainable cropping systems and N management should consider this possible rhizosphere effect on SOM turnover.

MATERIALS AND METHODS

Site Description

This research is part of the Living Field Laboratory (LFL), a long-term experiment established in 1993 at the Kellogg Biological Station in Hickory Corners, Michigan. The soil is a mixture of Kalamazoo and Oshtemo sandy loam (Typic Hapludalfs). The depth of the Ap horizon is 20 to 25 cm, and pH ranges from 6.3-6.8. Average precipitation has been 860 mm y^{-1} (1988-1999), and potential evapotranspiration typically exceeds precipitation from May through September. The LFL is designed to test various combinations of rotation and cover crops under several agronomic management regimes (Jones, 1996). The main goal is to test alternative strategies for achieving nutrient cycling efficiency and reducing chemical input requirements. The experimental design is a four-replication split-split-plot, randomized complete block, with main plots for each management type. The plots used for this study were in the Integrated Compost management type. This type includes minimal application of pesticides, banded herbicide plus cultivation for weed control, and composted dairy manure as the nutrient source. Subplots represent each year in a corn-corn-soybean-wheat rotation, plus continuous corn. All crops except soybean are grown with and without a cover crop. Cover crops include red clover (*Trifolium pratense*) frost-seeded into wheat, crimson clover (*Trifolium incarnatum*) interseeded into 1st year and continuous corn, and annual ryegrass (*Lolium multiflorum*) interseeded into 2nd year corn. Before planting, approximately 4 Mg ha^{-1} of composted manure is added annually to all crops except soybean. Reduced tillage (chisel plow) is used throughout the LFL.

This study includes a field experiment using root confining or excluding cylinders in the 1st year corn plots with cover crops. The C content of this soil is 13 g kg^{-1} with a C/N ratio of 10. Plots in 1st year corn (immediately following wheat + clover) were selected because their soil has a larger short-term (based on 70 days incubation) organic-N pool compared to other crops in the rotation or to continuous corn (Figure 1). The 70-day N-pool closely represents the portion of organic-N available for mineralization during a growing season. In early May 1998, six PVC cylinders (70-cm long and 30-cm i.d.) were pressed with minimal soil disturbance 60 cm into the soil immediately after corn (cv. Pioneer 3751) planting. Two corn plants were allowed to grow in three of the cylinders of each plot and the corn seeds were removed from the other three rings (Bare treatment). Using new 1st year corn plots, the experiment was repeated in 1999 with the addition of three cylinders planted with spring wheat (cv. Russ). Four grams (approx. 100 seeds) of wheat were planted in a radial distribution from the center of the rings.

Field Measurements

Immediately after cylinder insertion, soil samples were taken at 0-10, 10-25, 25-50, and 50-60 cm depths from each plot. Six extra cores were taken adjacent to each cylinder at 0-10 cm depth and combined for additional laboratory testing. Soil moisture was controlled in the cylinder, using temporary rain shelters and periodic addition of water, to prevent extreme wetting-drying events, and to minimize nitrate leaching and denitrification. We intended that controlling the soil moisture would also concentrate root growth in the Ap horizon. Soil moisture was determined weekly using time domain reflectometry (Tektronix 1502B) measurements. The amount of water to be added was calculated by subtracting the actual soil moisture from the estimated water holding

capacity for the 0-10 cm depth. The calculated amount was divided in half and each part was added at two different days of the week. The process was repeated every week. Removable plastic shelters were used as needed to exclude rain from entering the cylinders.

The soil surface of each cylinder was mapped into 6 sections in a pie shaped configuration for sampling. Every 14 days, 3 soil cores (1.9 cm dia.) were taken at 0-10 cm depth from one section of each cylinder. Sections were randomly selected and samples were taken along an imaginary radius. After each sampling, the open holes were plugged using small pieces of ¾-inch PVC pipes (15-cm long). The objective of this sampling was to monitor changes in N throughout the experimental period and to confirm soil moisture gravimetrically.

After 70 days, each cylinder and its intact soil core was removed using a front-end loader. The PVC pipe was separated from the soil core by sawing longitudinally and splitting the pipe, exposing the intact core. Soil samples at 0-10, 10-25, 25-50, 50-60 cm depth were taken from the cores, and deep samples were taken from beneath the tubes (60-70 cm depth) to determine N movement throughout the profile. Bulk densities were determined for the 0-10, 10-25 and 25-60 cm depths for nutrient concentration corrections. Corn and wheat biomass were separated from the soil cores and analyzed for total C and N using a Carlo Erba N A 1500 Series 2 N/C/S analyzer (CE Instruments Milan, Italy).

Laboratory Measurements

Pre-planting and final $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ concentrations were determined using the extraction technique described by Kenney and Nelson (1982) and a Lachat automated colorimetric analyzer (Lachat Instruments Inc. Milwaukee, WI). For clarity we will use the terms "pre-planting" to describe the soil at day 0, and "final" to describe the soil at termination 70 days after planting, including the bare, corn, and wheat treatments. During the preparation of final soil, most remaining roots were removed using a 6 mm sieve. Wheat roots were difficult to separate due to their small size and senescence at the time of sampling. The pre-planting and final soil samples from the 0-10 cm depth of each plot were composited by treatments and incubated to determine cumulative mineralizable N and C in the laboratory. Three (20 g dry weight equivalent) aliquots of each sample were weighed into 100 ml plastic specimen cups and brought to 50 % of water holding capacity. The specimen cups were stored in plastic storage containers that had a thin layer of water on the bottom to maintain humidity. These containers were then placed in a controlled temperature room at 25 °C for 30, and 70 days. At the end of each incubation date, the corresponding samples were removed and frozen temporarily to stop microbial activity. Inorganic N was determined using the previously described method. Net N mineralization potential was calculated as the difference between inorganic N content at the end of the incubation period and at day 0. Separate laboratory incubations were used to determine cumulative C mineralization at 20, 30, 50, 70, 99, and 148 days of incubation (Paul et al., 2000).

Nitrogen Balance Calculation

The mineral-N supplying capacity of soil was calculated using the equation:

$$N_{SC}=N_{Harvested} + \Delta N_{Inorganic\ (soil)}$$

where N_{SC} is the cumulative mineral-N supplied by soil during the 70 days in the field, $N_{Harvested}$ is the harvested plant biomass N, $\Delta N_{Inorganic}$ is the N result of subtracting initial from final soil N ($N\text{-}NO_3^-$ + $N\text{-}NH_4^+$).

This simplified equation assumes that deep N leaching was prevented and gaseous N loss minimized. One can argue against our assumption concerning the lack of gaseous N loss since no measurement was attempted. Accurate data for denitrification and volatilization are rarely available for upland farming systems (Robertson, 1997). But in a different location within Southwest Michigan, a short-term field experiment using ^{15}N on a poorly drained soil showed that denitrification amounted to only 1-2% of the immobilization plus nitrification rates (Christensen et al., 1990). Linn and Doran (1984) reported that in a well-drained soil the relative activity of anaerobic denitrification is negligible. Also, a recent study in an adjacent field indicated that N loss due to denitrification ranged from 1.3 to 0.4 kg ha^{-1} per year (Robertson et al., 2000). Our agronomic treatments were nearly identical to that study.

All data were analyzed using the Proc Mixed of SAS (SAS Institute, 1999). Analyses of variance and the Tukey-Kramer test at the 95% confidence level were performed.

RESULTS

Nitrogen Mineralization

Data of *in situ* N balance is shown in Table 1. Mineral-N supplying capacity in soil planted with corn was significantly greater than bare (unplanted) soil for both years. In 1999, N supplying capacity of soil with corn was significantly greater than soil with wheat. No difference was found between bare and wheat soils. Table 2 shows the distribution of mineral-N throughout the profile. The high concentration in the Ap and the small amount in the C-horizon indicate that deep N leaching was insignificant. A more detailed sampling in 1999 shows that the concentration of N in the 60-70 cm depth was less than 3 mg kg^{-1} in all treatments. As expected, more than 90% of the total corn and wheat roots were collected from the 0-25 cm depth (Table 3). The information provided in Tables 2 and 3 supports the effectiveness of the experimental method in preventing leaching and concentrating root growth in the Ap horizon.

Mineralized N for the pre-planting soil and final soil of each treatment at 30, and 70 day of laboratory incubation is shown in Figure 2. The 70-day N-pool size for the soil planted with corn was 70% smaller than that of the bare soil. This provides additional evidence suggesting that the increased N supply in the presence of corn roots was obtained primarily from the potentially mineralizable organic N pool. There was also a significant reduction in the N-pool size with wheat.

Carbon Mineralization

Cumulative CO_2-C released from pre-planting soil and the three treatments at 20, 30, 50, 70, 99, and 148 days of laboratory incubation is shown in Figure 3. There is no significant difference in CO_2 evolution between soil planted with corn and the pre-planting soil at any point in the incubation. This implies that the 148-day C-pool was unchanged by the presence of corn roots. In contrast, the bare soil treatment produced significantly less CO_2 than all other treatments at all dates. The reduction of the short-term carbon pool in bare soil compared to the pre-planting soil was over 20%. After 30 days, CO_2-C evolved from wheat soil was significantly lower than that of corn and pre-planting soils. Thus, wheat roots also reduced the 148-day C-pool size, but this reduction was smaller than that of bare soil.

DISCUSSION

The mineral-N supplying capacity of soil in the field was increased over 50% in the presence of corn roots compared to bare soil. We suggest that this increase is caused by a stimulation of N mineralization. Although the strategy (soil conditioning prior to crop stimulation) used in this study has rarely been describe in previous literature, previous works provide a great deal of understanding of the processes governing N mineralization. The effects of roots on soil include uptake of nutrients and water, movement of soil caused by root penetration, and release of rhizodeposits (Priha et al., 1999). Growing roots expose new organic material to decomposition and plant uptake creates a nutrient gradient from the bulk soil toward the roots. A more direct root control of N-mineralization is related to root rhizodeposit production (Clarholm, 1985b). The plant's release of these organic materials represents a significant energy input to the soil microbial community (Bakken, 1990; Texier and Billes, 1990). Whipps (1985) reported that in young corn, 47-69% of the total C transferred to the roots could be lost in the form of rhizodeposition. Helal and Sauerbeck (1987) estimated that the amount of C released by corn roots from 7-30 days after planting was equivalent to more than 1000 kg ha^{-1} of organic matter. This root-derived C acts as a "fuel" for a sequence of events that would not occur in the absence of roots (Clarholm, 1985b). As a result, microbial biomass will increase, since soil microorganisms are typically carbon-limited (Paul and Clark, 1996). Under these circumstances two major events will occur. First, N-mineralization is stimulated because of the abundance of low C/N ratio substrate provided by the soil "conditioning". Secondly, N-immobilization is enhanced due mainly to the increase in bacterial biomass, but it is counter-balanced by much greater mineralization. The

increased bacterial biomass is subject to predation by nematodes and protozoa, causing an increase in microbial turnover (Elliott et al., 1979; Clarholm, 1985 a, b; Robinson et al., 1989; Kuikman et al., 1990). During these simultaneous processes, corn roots continue releasing organic compounds and removing any surplus of NO_3^- and NH_4^+, continuously affecting N dynamics.

Martin and Puckridge (1982) estimated that wheat roots release about 1000 kg ha^{-1} of C during the growing season. While corn and wheat plants release considerable amounts of C into the rhizosphere, no significant increase in the available N for wheat was observed. This difference may be related to the amount and quality of rhizodeposits released into the soil. Merckx et al. (1987) reported that materials released from corn roots were incorporated in the microbial biomass to a higher degree than materials from wheat roots. In addition, corn roots may possess a more vigorous growth habits than wheat, allowing them to expose more organic material to decomposition and compete more efficiently with microbes. Wedin and Tilman (1990) found dramatic divergences of net N-mineralization rates for five different grass species growing in initially identical soils, confirming the potential for strong interactions between plant species and N cycling.

The 70-day N-pool size for the corn treatment was a third of the size that of the bare soil treatment. This implies that the additional mineral-N, supplied by the soil in presence of corn roots, was obtained primarily from that particular pool. The significant reduction in the size of the 70-day pool for the soil planted with wheat was probably caused by greater N mineralization during root growth. It is possible that the additional N was not available for wheat plants due to microbial competition and lesser bacterial predation by protozoa and nematodes compared to corn. The increase of the 70-day N-

pool for the bare soil when compared to the pre-planting soil may be due to the transformation of a portion of the less active N-pool into more readily-available forms during the May-June period with optimal soil moisture and temperature.

It is important to recognize the role of soil conditioning as prerequisite to enhance the stimulatory effect of living roots on N mineralization. Soil conditioning practices such as rotations, cover crops, addition of organic amendments, and conservation tillage, not only increase the active N-pool size but provide a beneficial environment for plant development and microbial activity (Willson et al., 2000).

The greater mineralization of soil N in the presence of growing corn is undoubtedly linked to an increase in the mineralization of soil C. However, the laboratory incubation data indicate that there was no decrease in mineralizable C after corn growth, whereas mineralizable C decreased in wheat and bare soil treatments. Rhizodeposition was sufficient to compensate for the C mineralization in the soil planted with corn, but not in the soil planted with wheat. It is likely that root-derived C is a relatively more important energy source for microbial activity under corn than under wheat. Our data agree with those of Shields and Paul (1973); Reid and Goss (1982, and 1983); and Haider et al. (1989, 1991, and 1993) showing that growing plants tend to maintain soil C levels whereas bare soil tend to decrease it.

CONCLUSIONS

We conclude that corn roots stimulate N-mineralization in the presence of a large active organic N-pool, and this effect can vary dramatically among plant species. This could represent a very important step in designing sustainable cropping systems. The combination of soil conditioning practices including rotations, cover crops, organic amendments, and conservation tillage with a cash crop specially designed to stimulate N mineralization have the potential to increase yields and decrease fertilizer requirements. Further studies are needed to classify plant species and cultivars according to their level of stimulation in presence of varying N-pool sizes

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Table 1. N content of soils and plants immediately before and 70 days after planting, and calculation of N supplied by each soil.

Treatments	pre-planting mineral soil N (A)	shoot N (B)	root N (C)	final mineral soil N (D)	N supplied by soil (E)
			kg ha^{-1}		
			1998		
Bare	35.7 †	-	-	148.0	112.3b
Corn	35.7	159.0	30.3	18.7	172.3a
			1999		
Bare	32.9 †	-	-	138.4	105.5c
Corn	32.9	149.1	26.9	21.0	164.1a
Wheat	32.9	82.4	10.2	56.7	116.4bc

E=B+C+D-A
Same letter within each year are not significantly different in mineral-N at day 70.
† A single bulk sample was used for all treatments.

Table 2. Mineral-N concentration in the soil profile at the end of the experiment (70 days after planting).

Soil depth	Treatments		
	Bare	Corn	Wheat
(cm)	mg kg^{-1}		
		1998	
0-25	35.6	3.5	-
25-60	5.9	1.3	-
		1999	
0-10	60.6	8.0	19.3
10-25	19.2	3.2	9.1
25-50	7.1	2.0	4.8
50-60	3.7	2.1	3.2
60-70	2.6	1.7	2.1

Table 3. Distribution of root biomass dry-weight and root N in the soil profile based on recovered roots at the end of the experiment (70 days after planting).

Soil depth	Corn		Wheat	
(cm)	kg ha^{-1}			
	Biomass	N	Biomass	N
			1998	
0-60	3236	30.3	-	-
			1999	
0-25	3333	25.2	625	10.0
25-50	205	1.4	13	0.2
50-60	41	0.3	2	0.1
0-60	3579	26.9	640	10.3

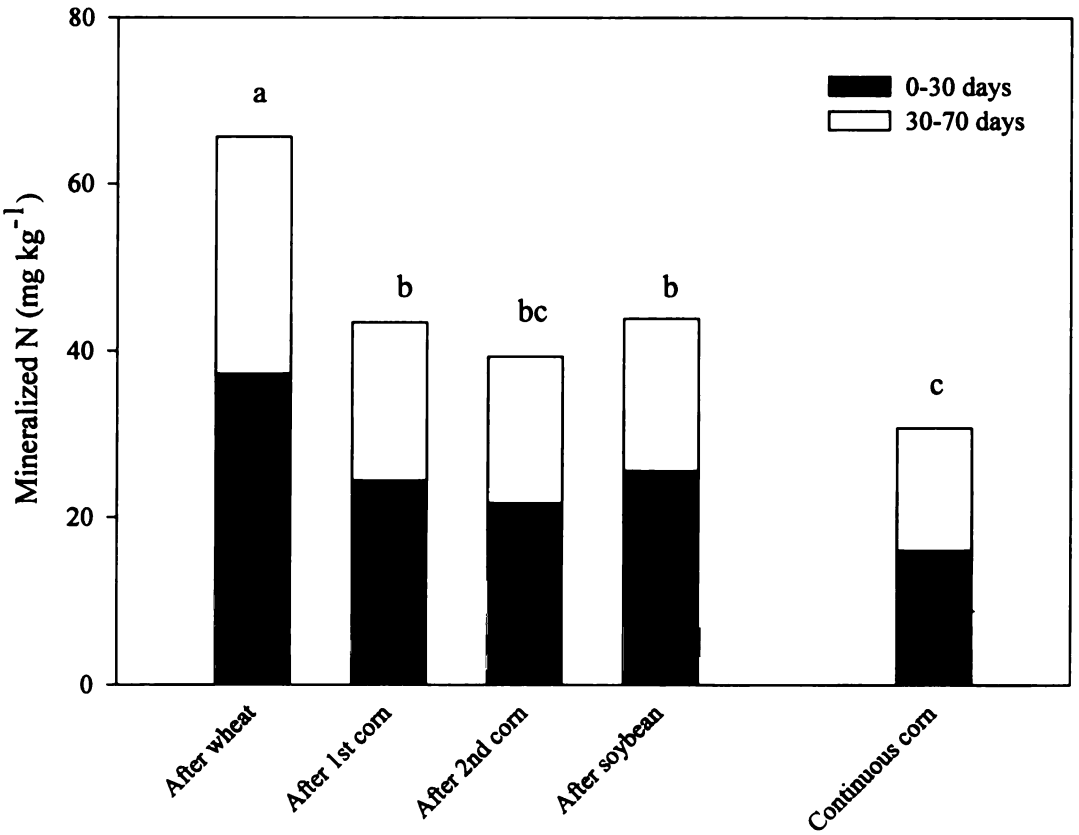

Figure 1. Cumulative mineralized N using laboratory incubations in soils sampled before planting (May 1999) from a 4-year rotation and continuous corn at the LFL. Units are based on a 10 cm sample depth. Bars with the same letter are not significantly different in mineral-N at day 70. The After-wheat soil was defined as "conditioned" soil in this study.

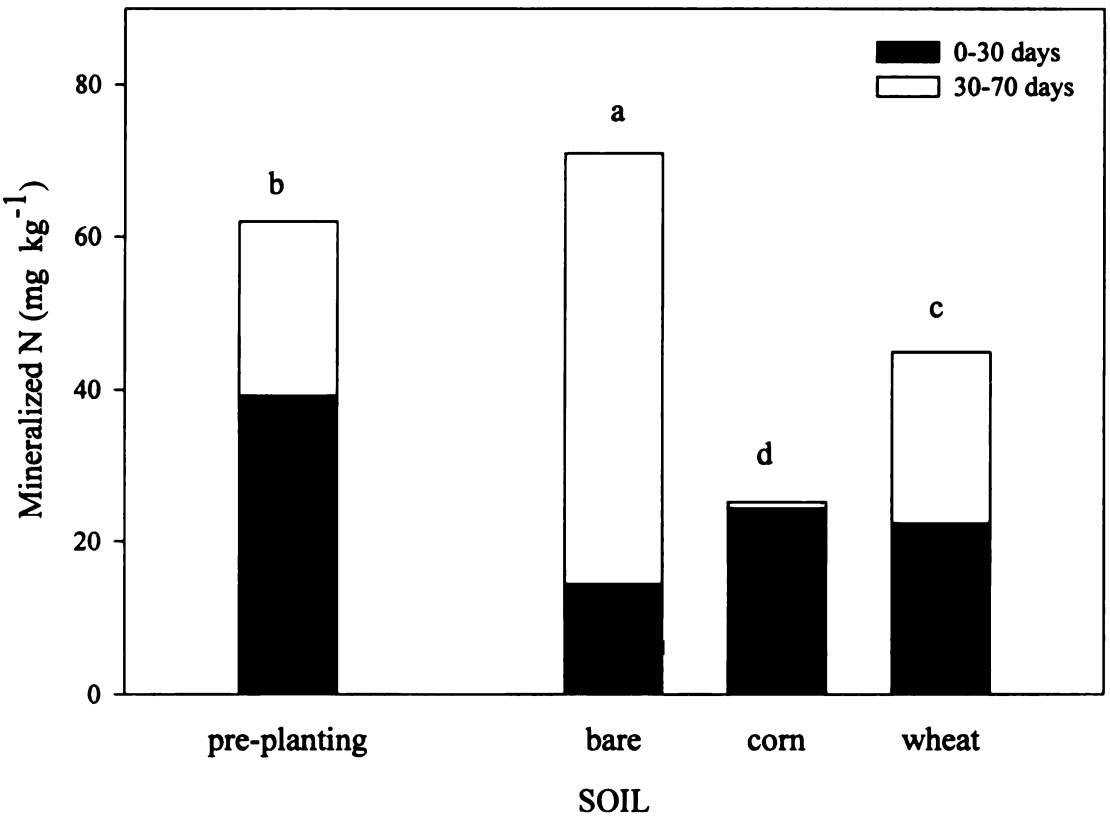

Figure 2. Cumulative mineralized N during laboratory incubation for soil sampled at planting and 70 days after planting. Field plots used in this study were from the "conditioned" soil (After-wheat) at the LFL. Units are based on a 10 cm sample depth. Letters indicate significant differences in mineralized N after 70 days of incubation.

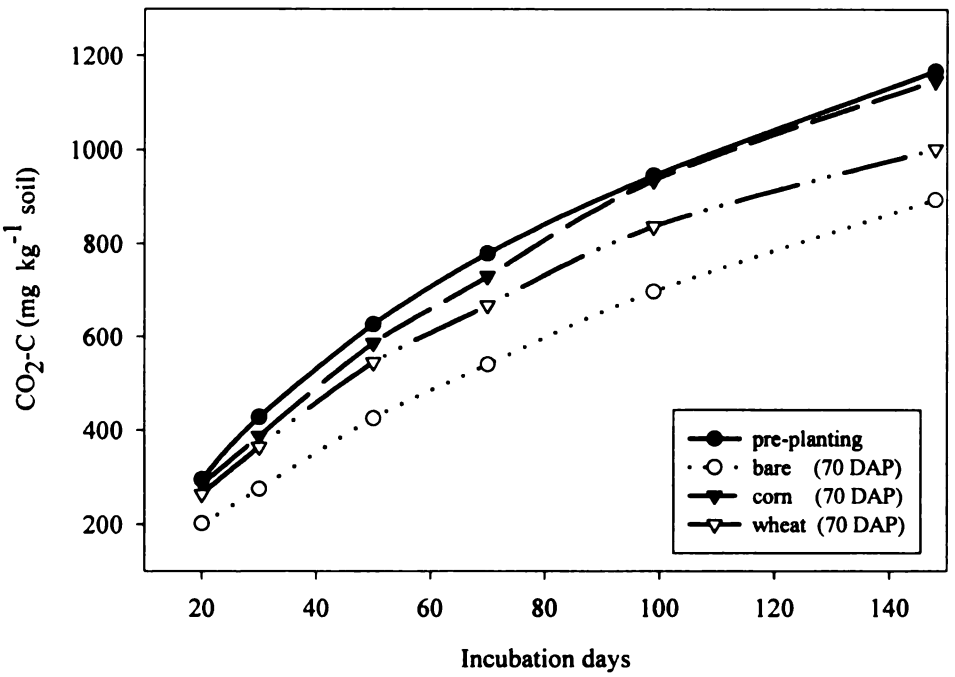

Figure 3. CO_2-C released during laboratory incubations for pre-planting and 70 days after planting (70 DAP) soils. Individual data points are the least squares means of 4 replications.

CHAPTER 3

The Impact of Management on Patterns of Soil Nitrate Fluctuation in Long-Term Cropping Systems

ABSTRACT

This study was done to assess how management practices influence the seasonal nitrate (NO_3) fluctuation patterns. The specific objectives were 1) to determine if fluctuation patterns could be used as a suitable indicator of soil fertility changes within or between seasons, and 2) to determine the leaching potential of several management strategies. Composted dairy manure or fertilizer was applied to a rotation of corn (*Zea mays* L.)-corn-soybean (*Glycine max* L.)-wheat (*Triticum aestivum* L.), and to continuous corn, both with and without cover crops. For six years soil NO_3 levels were measured from April through December. Nitrate levels and fluctuation patterns were very sensitive to management. In corn and wheat receiving fertilizer, NO_3 levels were generally higher than those receiving compost. The only exception was the cover cropped 1st year corn. Nitrate levels responded not only to fertilizer applications but also to an increased mineralizable N pool. The leaching potential in fertilized corn was always significantly higher than in corn receiving compost. In soybean and wheat, no difference between fertility sources was observed. A comparison between leaching potential and NO_3 levels in the following spring revealed that fertilized corn may be susceptible to higher leaching losses. No significant differences were observed in the fertilized wheat and in all crops receiving compost. Seasonal NO_3 fluctuation provides useful information of soil N fertility status and has the potential to be used in testing management practices that enhance biological fertility and reduce leaching losses.

Efficient N management implies an optimum crop uptake for maximum yields while minimizing N losses. This is essential for enhancing the economics of crop production and decreasing the risk of environmental degradation. Conventional cropping systems that use high rates of N fertilizer have achieved high yields but have also affected water quality (Keeney, 1989; Hassink et al., 1991). Increased N losses to the groundwater may have originated from excessive application of fertilizer and were exacerbated by a reduction in the soil's ability to hold water and residual N (Addiscott et al., 1991). This decreased ability is related to the declining levels of soil organic matter (McKenney et al., 1993). Thus, to reduce NO_3 leaching and contamination of groundwater, it is necessary to incorporate farming practices that enhance soil organic matter while reducing the fall NO_3 levels (Kuo and Sainju, 1998). These practices may include the use of biological resources such as crop residues, N_2-fixing plants, cover crops, organic fertilizers, (Willson, 1998) and a finely tuned fertilizer management program (Keeney, 1983). A variety of agronomic practices can be successfully integrated to achieve desirable environmental benefits (Russelle and Hargrove, 1989).

In this study, composted dairy manure or fertilizer was applied to a rotation of corn-corn-soybean-wheat, and to continuous corn both with and without cover crops. Our main purpose was to obtain a better understanding of how agricultural practices influence the seasonal fluctuation pattern of soil NO_3 levels. The first specific objective was to determine if a fluctuation pattern could be used as a suitable indicator of changes in soil N fertility within or between seasons. When fertilizer was used, NO_3 fluctuation was expected to closely follow fertilizer applications. Under compost with cover crops, this fluctuation was likely to be related to changes in N pool sizes. The second objective

was to use fall NO_3 levels in assessing the nitrogen leaching potential. Management practices that reduce NO_3 leaching potential can play an important role in minimizing groundwater contamination (Khakural and Robert, 1993). The estimated leaching potential was then compared to NO_3 levels of the following spring. Any significant reduction in NO_3 levels was expected to explain a possible leaching loss. To accomplish the described objectives, measurements of soil NO_3 were obtained monthly from 1994 to 1999 and used to generate curves describing the fluctuation patterns. Analysis of variance and covariance, and test for multiple comparisons were performed.

MATERIALS AND METHODS

Site Description

This field study was conducted from 1994 to 1999 as part of the Living Field Laboratory (LFL). The LFL is a long-term experiment established in 1993 at the W. K. Kellogg Biological Station located in Southwest Michigan. Climate is described as cold moist winters and warm humid summers. Average precipitation has been 860 mm y^{-1} (1988-1999). Evapotranspiration potential typically exceeds precipitation from May through September. The soil is a mixture of Kalamazoo and Oshtemo sandy loams (Typic Hapludalfs). The depth of the Ap horizon is 20 to 25 cm, and pH ranges from 6.3-6.8. The LFL was designed to test combinations of rotation and cover crops under several agronomic management regimes (Jones, 1996). The main goal is to test alternative strategies for achieving nutrient cycling efficiency and reducing chemical input requirements. The experimental design is a four-replication split-split-plot, randomized complete block, with main plots for each management type. Integrated Compost and Integrated Fertilizer management type plots were used for this study. Both management types include minimal application of pesticides, and banded herbicide plus cultivation for weed control. The main difference is the nitrogen source: Integrated Compost uses composted dairy manure and Integrated Fertilizer uses commercial fertilizer. For clarity we will use the terms "Compost" and "Fertilizer" to identify these two types throughout the paper. Subplots represent each year in a corn-corn-soybean-wheat rotation, plus continuous corn. All crops except soybean are grown with and without a cover crop. Cover crops include red clover (*Trifolium pratense*) frost-seeded into wheat, crimson clover (*Trifolium incarnatum*) interseeded into 1st year and

continuous corn, and annual ryegrass (*Lolium multiflorum*) interseeded into 2nd year corn. Corn plots treated with fertilizer received 10 kg ha^{-1} of N at planting. In late June, sidedress N rates were determined according to the pre-sidedress nitrate test (PSNT) (Magdoff et al., 1984). Fifty kg ha^{-1} of urea-N was applied in mid March to the fertilized wheat. Soybeans received no fertilizer. In the compost plots, approximately 4 Mg ha^{-1} of composted dairy manure was added annually before planting to all crops except soybean. Reduced tillage (chisel plow) was used throughout the experiment.

Sampling and Laboratory Procedures

During the six years, soils were sampled once in April, twice in May and June, and once monthly from July to December. Sampling was generally performed at the middle of each sampled month, except for May and June when they were carried out early and late in the month. Occasionally, dry soil conditions did not allow sampling. Five soil cores were taken at 0-25 cm depth using a 1.9 cm dia. probe, composited, and dried at 35 °C for 72-96 h. Soils were then ground and the $N\text{-}NO_3^-$ concentrations were determined using the extraction technique described by Kenney and Nelson (1982) using a Lachat automated colorimetric analyzer (Lachat Instruments Inc. Milwaukee, WI).

Data Analysis

The factors used in the statistical model were: year, replication, nitrogen source, crop, cover crop, and month of the year. Both data points of May and June respectively were averaged. We performed analysis of variance (ANOVA) and covariance, using the SAS Mixed procedure (SAS Institute, 1999) in which the data from each month of the year was treated as a repeated measurement of the corresponding experimental unit. The optimal covariance structure was determined using Schwarz's Bayesian Criterion (Littell

et at., 1997). Compound-symmetry (CS) was the covariance structure that best explained our data set. CS assumes a constant variance and covariance (SAS Institute, 1999). After using ANOVA to identify effects that produce significantly different NO_3 levels, the Tukey-Kramer test was used to compare overall LS-means. In addition, curves were constructed for each crop using monthly means and their corresponding standard errors. The interpretation of results focused on the analysis of combined years since our purpose was to identify overall trends and/or patterns.

Nitrate leaching potential, as we define it for our sandy loam soil, is the fall (early or late) NO_3 concentration that may be susceptible to leaching loss. The estimation of leaching potentials required some additional adjustment of values. "Early fall" values were generated by combining the NO_3 levels from September and October. Meanwhile for the "late fall", the November and December data were used. September-October will be referred to as "early fall", and "late fall" will represent November-December. The leaching potential was assigned based upon the presence or absence of cover crops. In cover cropped soils, the late fall NO_3 level was assumed as the leaching potential. It was expected that the presence of plant cover would partially uptake water and residual NO_3 during most part of the fall, therefore reducing the NO_3 loading and downward movement of water. Some authors have reported that leaching loss is closely related to soil NO_3 levels of late in the fall (Brown et al., 1993; Solberg, 1995). In the absence of cover crops, the early fall NO_3 was assumed as the leaching potential. Water and NO_3 was expected to gradually leave the Ap horizon from early fall when the soil begins to recharge. Previous works agrees that NO_3 leaching from the root zone is likely to start in that period and continue until early spring (Steenvoorden, 1989). This period in

Michigan is characterized by precipitation exceeding evapotranspiration (Cavigelli et al., 1998). Using early fall values as leaching potential in uncovered soils therefore appears to be more appropriate especially when using shallow soil sampling. The estimated leaching potentials were contrasted to those values of the following spring (April). It was assumed that any reduction in NO_3 levels observed in early spring was primarily due to loss by leaching.

RESULTS AND DISCUSSION

Nitrate Levels Influenced by Management

An analysis of variance (Table 1) indicated that time and the farming practices evaluated in this study influence soil NO_3 levels. Timing, explained by the effect of year and month, is influenced by the temporal variation of temperature and precipitation predominant in Southwest Michigan. These climatic factors play a fundamental role on microbial mediated N transformation processes (Swift et al., 1979; Tian et al., 1993) and possible leaching losses (Paul and Clark, 1996). Timing may also be influenced by the time of fertilizer application. As expected, two- and three-order interactions were frequently statistically significant, indicating that factors were not independent of one another (e.g. sidedress of N is a not a random occurrence). The April-December NO_3 levels (Table 2) for crops with compost were significantly lower than those with fertilizer. The only exceptions were soybean and the cover cropped 1st year corn. Cover crops substantially enhanced NO_3 levels in 1st year corn regardless of the nitrogen source. This increase was related to the N contribution by legume cover crops. Other researchers have reported the beneficial effect of legume cover crops in providing N to main crops (Hesterman et al., 1986; Power, 1987; Oberle and Keeney, 1990; Rasiah, 1999). In unfertilized soils, the N contribution from mineralization of soil organic materials is essential because it is the primary source of mineral-N to non-legumes crops. In the 1st year corn compost plots, the interacting action of cover crops with varied residues from rotation and the compost itself may also be responsible for this increase. Sanchez et al. (2000b) reported that the use of rotation, cover crop, and addition of composted manure can dramatically increase the mineralizable N pool size. Rotation appears to play an

important role in soil fertility by maintaining or enhancing soil organic matter levels (Campbell et al., 1992; Mitchell et al., 1991; Friedel et al., 1996; Franco-Vizcaino, 1997). N contributions of composted manure may not be significant in the short-term since most of the N exists in stable organic forms (Paul and Beauchamp, 1993), but the long-term effects of annual additions are likely to improve soil fertility. The presence of corn roots may be another possible interacting element in enhancing N mineralization. Sanchez et al. (2000a) found that the presence of corn roots can dramatically increase N mineralization in soils with a large active N pool.

The monthly fluctuations (Fig. 1 to 5) revealed striking differences between NO_3 responses to nitrogen sources. Soil NO_3 levels in fertilized corn (Fig. 1, 2, and 5) dramatically increased by late June as a result of sidedress fertilizer application. The cover cropped 1st year corn (Fig. 1) showed a lower NO_3 level than corn without cover. This was expected since the cover cropped 1st year corn received considerably less fertilizer based on PSNT recommendations. Soil NO_3 levels remained high until mid August and then plummeted by mid September. This decrease coincides with the period of maximum N uptake needed for grain filling. The NO_3 fluctuation in the cover cropped 1st year corn under compost appears to follow the course of N mineralization. Silgram and Shepherd (1999) indicated that denitrification and volatilization are negligible, so that the N released by mineralization will be reflected in the NO_3 levels. The mineralizable N pool size is highest in spring and is smallest near physiological maturity (EL-Haris et al., 1983; Willson, 1998). Other authors found that N mineralization can be considerable after harvest (Bonde and Rosswall, 1987; Franzluebbers et al., 1994). This may explain why most soils experience an additional increase in NO_3 level after crop

harvest. In corn using compost, this late increase was not pronounced and probably can be attributed to a drastic reduction in the N pool size during corn growth. In soybean (Figure 3), no differentiating patterns were observed between fertility sources. Even though neither compost nor fertilizer and cover crops were applied during the soybean growing season, we expected higher NO_3 levels in those plots historically receiving compost and cover crops. The contribution of compost and legume cover crops in providing N to succeeding crops has been well documented throughout the literature but it appears that soybean does not stimulate N mineralization as does corn. This may have an enormous significance in providing evidence that an appropriate selection of crop rotation in combination with cover crops and addition of organic amendments may lead to a build up of the mineralizable N pool. The application of fertilizer in March dramatically influenced the April NO_3 levels in fertilized wheat (Fig. 4) following soybeans.

Nitrate Leaching Potential

Significant differences in NO_3 levels during the growing season nearly always lead to significance in early and late fall leaching potentials. This strongly suggests that the potential for NO_3 leaching loss increases with certain agricultural practices such as fertilizer applications and continuous corn monoculture. Soil NO_3 levels for early fall, late fall, and the following spring, are shown in Table 2. The leaching potential in fertilized corn was always significantly higher than corn using compost. In soybeans and wheat, no difference between fertility sources was seen. The same management practices applied to all soybean plots may explain the similarity of leaching potentials. This may indicate that leaching potential was not influenced by the previous cropping history. The

wheat's ability to remove much of the soil NO_3 during its growing season may be responsible for the lowest leaching potential of the rotation. Microbial immobilization may also influence those low levels because of decomposition of the high C/N ratio residue after wheat harvest.

Comparing the estimated nitrate leaching potential with those values obtained in the following spring was expected to closely explain any possible leaching loss. A significant reduction in NO_3 levels was observed in all fertilized corn plots. These suspected losses strongly agree with the leaching values reported by Smeenk et al. (2000) measured from the same plots. These values for fertilized corn are within the range of leaching loss reported by Paul and Clark (1996). No significant differences between leaching potential and spring NO_3 levels were observed for fertilized wheat and all crops using compost. In cover cropped 1st year corn, minimal loss may have occurred despite enhanced spring NO_3 levels indicating that N use may be efficient. Furthermore, Jones (1996) reported that the use of rotation, cover crops, and composted manure can supply adequate N to a growing 1st year corn crop. This may suggest that using management practices that enhance the mineralizable N pool size have the potential to increase yields and reduce fertilizer requirements while reducing the risk for groundwater pollution. The lowest leaching potential in wheat may indicate an additional environmental benefit of this small grain. Smeenk et al. (2000) found that wheat leached the lowest amount of nitrate. The comparison was not feasible for the soybean plots in the fertilizer management since those plots received late winter (during wheat) application of fertilizer, and that explain the high NO_3 values in spring.

We conclude that seasonal NO_3 fluctuation patterns could be used as sensitive indicators of N fertility status. Nitrogen fertility can be increased by application of conventional fertilizer and also by soil conditioning practices that increases the mineralizable N pool size. The potential for NO_3 leaching loss increases with certain agricultural practices such as fertilizer applications and continuous corn monoculture. Monitoring NO_3 levels throughout the season has the potential to be used to test management practices that enhance biological fertility and reduce leaching losses.

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Table 1. Analysis of variance for soil NO_3 levels as affected by year (1994-1999), nitrogen source, crop, cover crop, and month of the year.

Source of variation	1994	1995	1996	1997	1998	1999	All years
				Pr > F			
Replication	NS	NS	NS	NS	NS	NS	NS
Year	NA†	NA	NA	NA	NA	NA	***
Month	***	***	***	***	***	***	***
Nitrogen source (N)	***	***	***	***	***	***	***
Crop (C)	**	***	***	***	***	***	***
Cover crop (CC)	**	*	**	**	NS	***	**
Interaction effects							
N x C	*	**	***	***	***	***	***
N x CC	NS	*	*	NS	NS	*	*
N x CC	NS	***	**	**	***	***	***
N x C x CC	NS	*	***	NS	***	NS	**

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† NA, not applicable.

Table 2. Soil NO_3 levels as affected by crop, nitrogen source, cover crop, and time of the year.

Crop	April-December	Early fall	Late fall	Following spring
	——— mg kg^{-1} ———			
1st year corn				
Fertilizer + cover	11.8a†	13.3Aa‡	9.0Ba	4.3Ca
Fertilizer only	9.8b	9.8Ab	7.2Aab	4.5Ba
Compost + cover	8.6b	5.5Ac	5.3Abc	3.4Aa
Compost only	5.8c	3.5Ac	3.3Ac	3.6Aa
2nd year corn				
Fertilizer + cover	9.9a	13.8Aa	7.4Ba	3.7Ca
Fertilizer only	10.6a	14.3Aa	8.3Ba	4.9Ca
Compost + cover	5.0b	3.7Ab	3.7Ab	3.3Aa
Compost only	4.9b	3.0Ab	3.4Ab	3.4Aa
Soybean				
Fertilizer + cover	5.6a	5.3Ba	5.2Ba	8.1Aa
Fertilizer only	5.9a	5.8Ba	4.8Ba	8.6Aa
Compost + cover	4.8a	5.8Ba	4.8Ba	3.7Bb
Compost only	4.6a	5.0Ba	5.5Ba	3.8Bb
Wheat				
Fertilizer + cover	5.0a	3.0Aa	2.9Aa	3.1Aa
Fertilizer only	5.9a	3.4Aa	2.9Aa	3.5Aa
Compost + cover	2.9b	3.7Aa	2.8Aa	3.3Aa
Compost only	2.9b	2.9Aa	2.5Aa	3.0Aa
Continuous corn				
Fertilizer + cover	11.1a	11.8Aa	11.4Aa	4.1Ba
Fertilizer only	8.8a	9.4Aa	7.6Ab	4.8Ba
Compost + cover	4.8b	3.7Ab	3.8Ac	3.4Aa
Compost only	4.6b	3.8Ab	3.6Ac	3.6Aa

† LS-means within a crop column (fertility source x cover crop) followed by a different lowercase letter differ at the 0.05 probability level.

‡ LS-means within a row (time of the year) followed by a different uppercase letter differ at the 0.05 probability level.

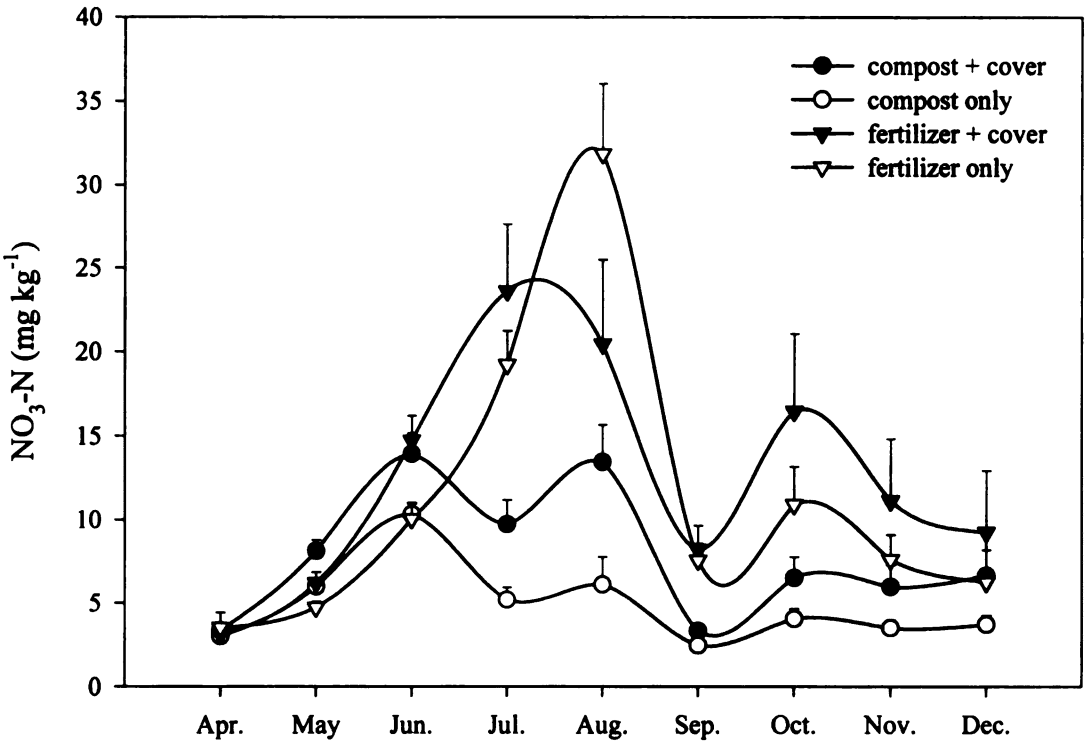

Figure 1. Soil NO_3-N concentrations in the 1st year corn plots of the rotation using data from 1994 to 1999. Monthly values have been adjusted to the middle of the month. Error bars represent that portion of the standard errors above the mean for each month.

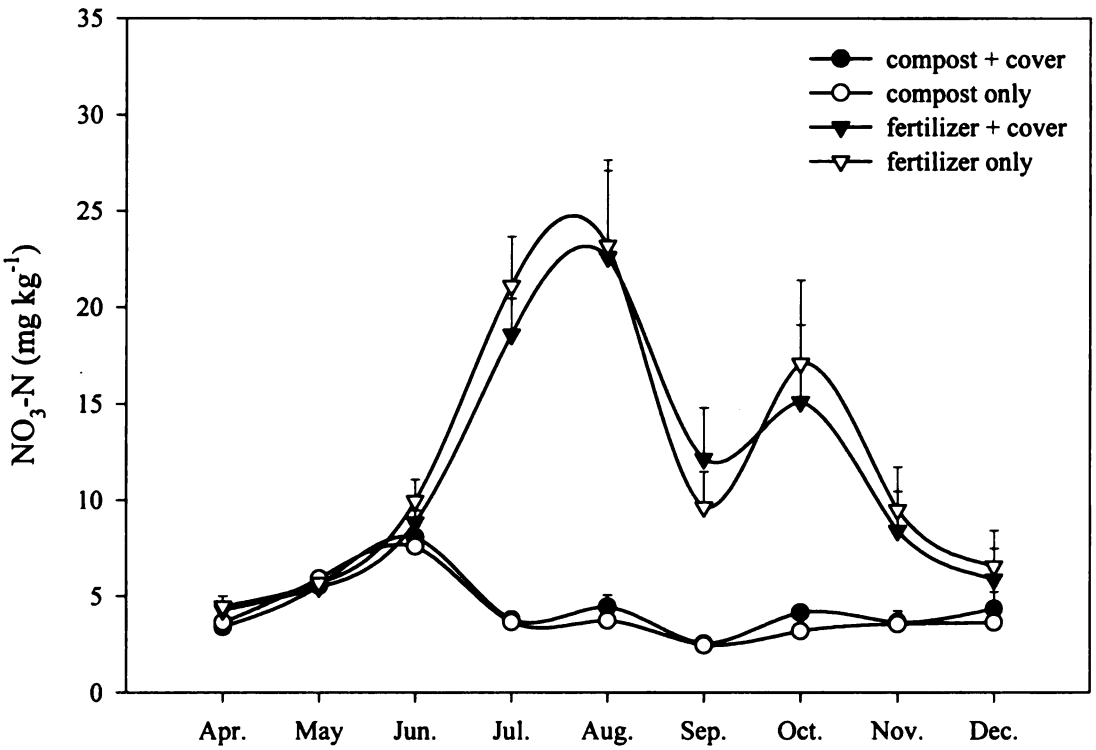

Figure 2. Soil NO_3-N concentrations in the 2nd year corn plots of the rotation using data from 1994 to 1999. Monthly values have been adjusted to the middle of the month. Error bars represent that portion of the standard errors above the mean for each month.

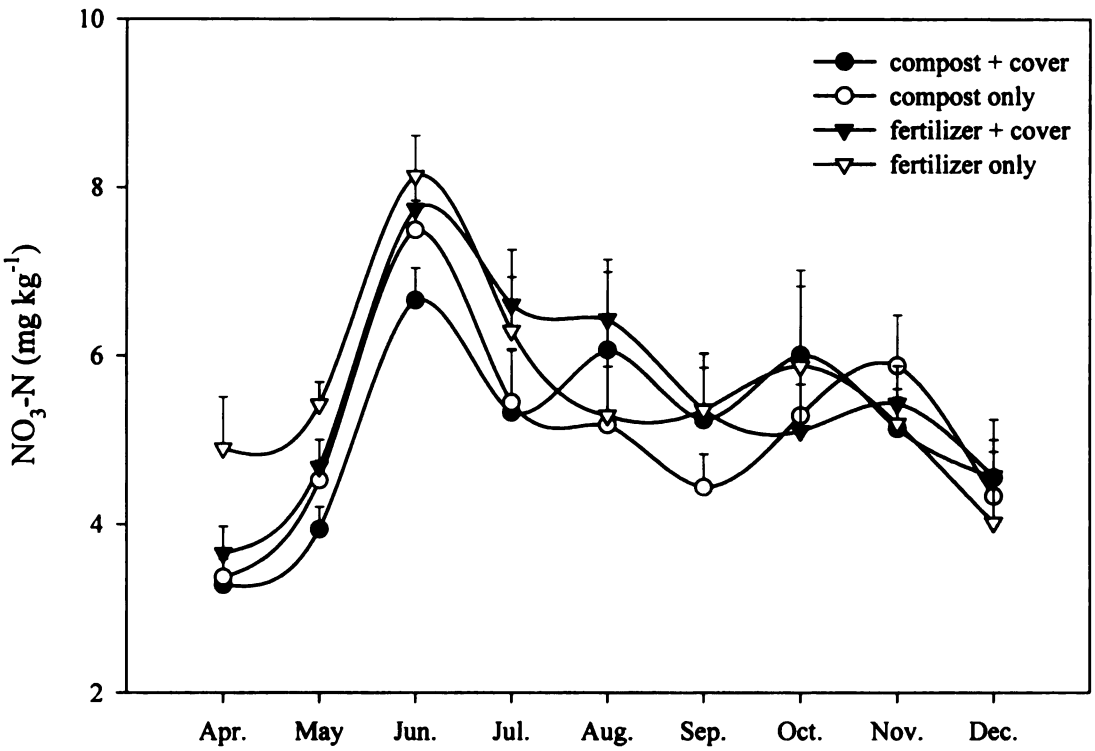

Figure 3. Soil NO_3-N concentrations in the soybean plots of the rotation using data from 1994 to 1999. Monthly values have been adjusted to the middle of the month. Error bars represent that portion of the standard errors above the mean for each month.

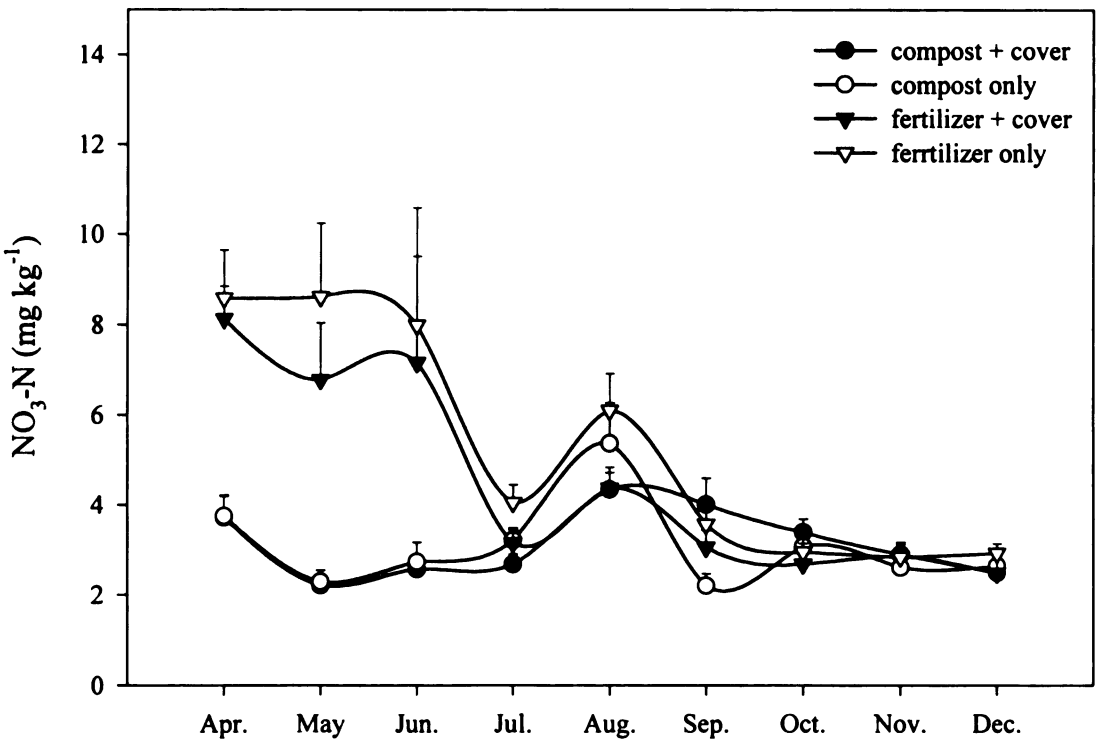

Figure 4. Soil NO_3-N concentrations in the wheat plots of the rotation using data from 1994 to 1999. Monthly values have been adjusted to the middle of the month. Error bars represent that portion of the standard errors above the mean for each month.

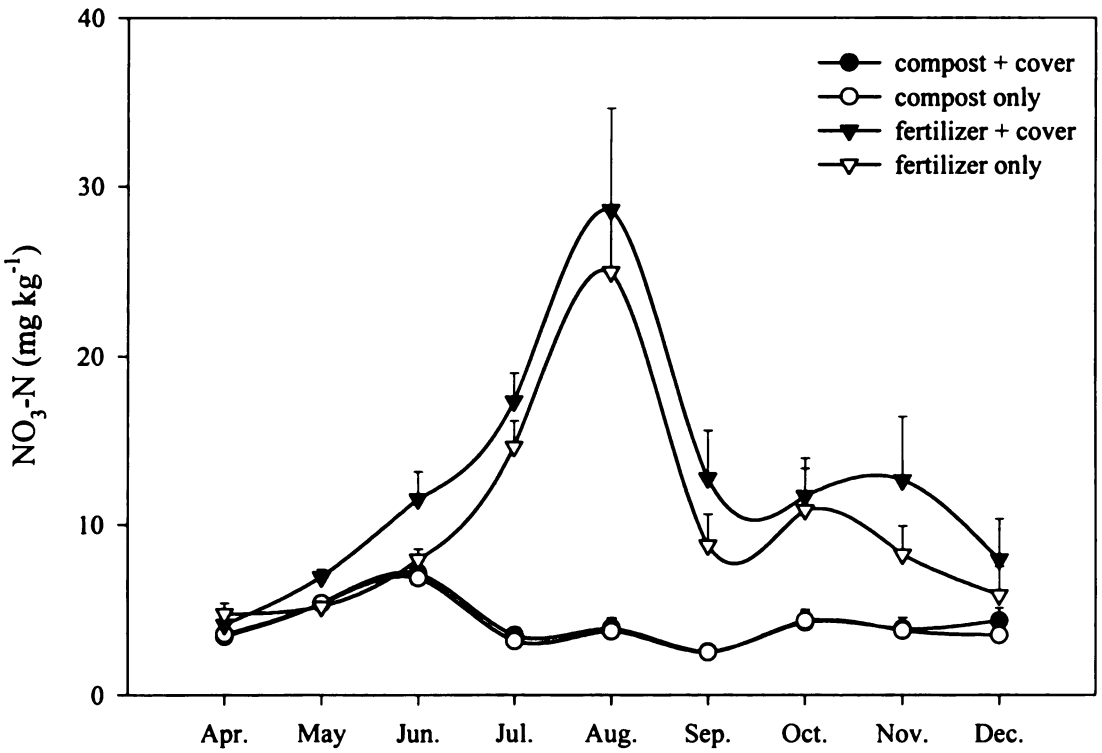

Figure 5. Soil NO_3-N concentrations in the continuous corn plots using data from 1994 to 1999. Monthly values have been adjusted to the middle of the month. Error bars represent that portion of the standard errors above the mean for each month.