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MANAGING NITROGEN MINERALIZATION AND BIOLOGICALLY ACTIVE ORGANIC MATTER FRACTIONS IN AGRICULTURAL SOIL

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Thomas C. Willson

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Ph.D. Crop and degree in _ Soil Sciences

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MANAGING NITROGEN MINERALIZATION AND BIOLOGICALLY ACTIVE ORGANIC MATTER FRACTIONS IN AGRICULTURAL SOIL

By

Thomas C. Willson

A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Managing Nitrogen Mineralization and Biologically Active Organic Matter Fractions in Agricultural Soil

By

Thomas C. Willson

Integrated cropping systems use biological resources such as organic fertilizers, nitrogen fixing plants, and crop and cover crop residues to reduce the need for chemical inputs and improve sustainability. This research explores the effect of integrated management on nitrogen mineralization in a variety of corn-based agricultural rotations and early successional treatments at the Kellogg Biological Station (KBS) in Southwestern MI. Part 1 defines the effect of management on the seasonal dynamics of nitrogen mineralization potential (NMP), the intrinsic ability of a soil to mineralize N over time (ideally a growing season). Measurements of NMP, based on the accumulation of inorganic N in soils incubated under aerobic conditions for 10, 30, 70, 150 and 310 days at 25 °C, are evaluated using 1st order mineralization kinetics and repeated measures analysis of variance. Nitrogen mineralization potential was greatest in the spring and lowest in the fall in all treatments. It increased in response to compost additions, was greater in low-input rotations with cover crops than in conventional rotations, and was greater in successional treatments without tillage than in tilled succession or agronomic treatments. During a corn-corn-soybean-wheat rotation, NMP was lowest under 2nd

year corn and highest under wheat and 1st year corn, reflecting both legume inputs and the lack of spring tillage in wheat. Part 2 of the research found that macroorganic matter was correlated with NMP in most treatments, but that microbial biomass was not. The strongest correlation ($r^2=.41$) was between macroorganic C in the 53-1000 μ m size class and inorganic N accumulation in 150 day incubations. Macroorganic matter increased more rapidly after compost additions than NMP, but was less affected by residue inputs. Part 3 of the research found that conventional fertilization produced higher corn and wheat yields and greater leaching loss than additions of leaf and dairy compost. Net annual N mineralization (calculated from plant uptake and leaching loss) was greater in the compost treatments, but N availability was low because of lower than expected compost mineralization (9% yr¹). Nitrogen mineralization was greatest in years with corn production and lowest in years with soybean and wheat production, reflecting (in part) differences in residue input from the previous crop. Much of the variation in N mineralization could be predicted by laboratory measurements of NMP and macroorganic matter, particularly total macroorganic N (53 – 1000 μ m), potentially mineralizable organic matter (No), and N mineralization predicted in 70 and 150 day incubations based on the 1st order kinetics. Leaching loss (October through September) was best predicted by initial inorganic N and total inorganic N (initial plus mineralized) in incubations performed after sidedress fertilization in the preceding season. This research shows that N mineralization varies appreciably within and between growing seasons, and responds predictably to integrated agricultural management.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
Locations:	
LFL	Living Field Laboratory
LTER	Long Term Ecological Research Experiment
KBS	Kellogg Biological Station
LTER Treatments:	
СТ	High-input rotation with conventional tillage
NT	High-input rotation with no-till management
LI	Low chemical input rotation with cover crops
ZI	Zero chemical input rotation with cover crops
HTS	Historically tilled succession
NTS	Never-tilled succession
ATS	Annually tilled succession
Measurements: †	
NMP	Nitrogen mineralization potential
ММ	Macroorganic matter
MB	Microbial biomass

† See Table 3.1 (page 75) for additional abbreviations used in Chapter 3.

INTRODUCTION

Integrated cropping systems use biological resources such as organic fertilizers, nitrogen fixing plants, and cover crop residues to reduce the need for chemical inputs and improve sustainability. If we are to manage integrated cropping systems for high productivity and minimal NO₃-N loss, we must understand how our management affects the timing and extent of nitrogen mineralization.

This dissertation explores the effect of integrated management on N mineralization from three perspectives. Chapter 1, "Seasonal Changes in Nitrogen Mineralization Potential in Agricultural Soils: ANOVA and Non-Linear Regression Analysis of Management Effects," explores the effect of management on the seasonal dynamics of nitrogen mineralization potential (NMP), the intrinsic N supplying capacity of the soil. We evaluate NMP based on the accumulation of inorganic N in long term aerobic incubations at 25 °C, and compare two methods of assessing it statistically. In chapter 2, "Managing Biologically Active Soil Organic Matter Fractions for Sustainable Crop Production," we explore the effect of management on the C and N content of the soil microbial biomass (determined by the chloroform fumigation, incubation procedure) and of macroorganic matter, which we have defined as the organic material in the sand-sized (53-1000 µm) fraction of dispersed soil. Both of these "biologically active" organic matter fractions are involved in the supply of N in agroecosystems: microorganisms as the primary agents of decomposition, and macroorganic matter as an important storage pool of actively decomposing and potentially decomposable organic matter particles. The methods used to measure

these pools are much less time consuming than long term incubations, and might therefore be more practical as routine indicators of N mineralization potential. In chapter 3, "Biological Indicators of Crop Performance, Nitrogen Mineralization, and Leaching Loss in Integrated Cropping Systems," we evaluate laboratory measurements of NMP, microbial biomass and macroorganic matter for their ability to predict crop performance, N mineralization, and leaching loss in the field. Leaching loss is measured using subsurface leaching lysimeters, and net annual N mineralization is calculated based on an annual N budget derived for each plot.

The data presented in this dissertation are based on measurements taken over a three year period (1994 through 1996) at the Long Term Ecological Research (LTER) main-site experiment and the Living Field Laboratory (LFL) experiment at the Kellogg Biological Station in Hickory Corners, MI (42°24'N, 85°23'W). These experiments are located adjacent to one another on a gently rolling glacial outwash plain (2 to 4 % slope) consisting of Kalamazoo loam and Oshtemo sandy loam (Typic Hapludalfs). Precipitation averages 880 mm yr¹ at this site(1988-1997), with precipitation exceeding potential evapotranspiration from October through March. The actual precipitation during the years of this study was slightly lower than normal, 821, 820 and 651 mm based on the October through September crop production year. There was a short period of insufficient moisture in the late spring of 1994, and prolonged drought in the summer of 1996.

The KBS LTER site was established in 1988 to investigate the ecological interactions within and between the major managed and unmanaged ecosystems in

the US corn belt. The LTER main-site experiment (Figure I.1) consists of four cornbased field-crop rotations, high input conventional tillage (CT), high input no-till (NT), low input conventional tillage (LI), and zero input conventional tillage (ZI), two perennial monoculture productions systems, alfalfa and poplar, and two early successional systems, historically tilled (HTS), and never tilled (NTS). The CT and NT treatments were managed as conventional corn-soybean rotations from 1989 through 1994 and wheat was added to the rotation in 1995. The LI and ZI treatments are corn-soybean-wheat rotations receiving either reduced chemical inputs (LI) or zero chemical inputs (ZI), and featuring hairy vetch as a green manure cover crop frost seeded into wheat and interseeded into corn. Prior to 1993 the LI and ZI treatments had been ridge-tilled, but since that time they have been moldboard plowed, as is the CT treatment. The HTS was last tilled in 1989, while the NTS treatment has been maintained by annual (winter) mowing. The HTS treatment contains a micro-plot that is tilled annually with a moldboard plow (ATS). The NTS treatment is located just south of the other treatments on a remnant of the pre-modern-agricultural soil profile. We sampled the CT, NT, LI and ZI treatments in 1994, and the CT, HTS, ATS, and NTS treatments in 1995 and 1996.

The LFL experiment (Figure I.2) was established in 1993 to investigate the sustainability of corn production strategies ranging from a low diversity, conventionally managed, corn monoculture, to a high diversity, organically managed, corn-corn-soybean-wheat rotation with cover crops. The experiment is laid out in a split-plot, randomized complete block design, with main plots for each



Figure I.1 Plot map of the KBS LTER main-site experiment. Blocks 1 through 4 of treatments 1 (CT), 2 (NT), 3 (LI), 4 (ZI), 7 (HTS) and 8 (NTS) were sampled along with a tilled micro-plot in treatment 7 (ATS).

Living Field Lab. 1994



Figure I.2. Partial plot map of the LFL experiment. (Block 1 of 4 in 1994.) The Integrated Fertilizer (Fertilizer) and Integrated Compost (Compost) management types were sampled.

of four management types, Organic, Integrated Compost (Compost), Integrated Fertilizer (Fertilizer) and Conventional, split for each entry point in a corn cornsoybean-wheat rotation plus continuous corn so that each crop in the rotation is represented each year. The Compost and Fertilizer management types use a combination of banded herbicides and mechanical tillage for weed control, while the Conventional treatment uses broadcast herbicides and the Organic treatment uses no chemicals for weed or pest control. The Conventional and Fertilizer treatments utilize urea and ammonium nitrate to maintain N fertility, whereas the Compost and Organic management types use compost made from leaves and dairy manure. All treatments use a chisel plow for primary tillage. Each entry point subplot in the Fertilizer, Compost, and Organic treatments are further split based on the presence or absence of cover crops. During this project, cover crops included red clover frost seeded into wheat and interseeded into 1st year corn, annual ryegrass interseeded into 2nd year corn, and a mixture of red clover and annual ryegrass interseeded into continuous corn (Figure I.3). We sampled the 1st year corn soybean and wheat entry points of the integrated fertilizer and integrated compost management types (cover and no-cover) in 1994 and all of the integrated fertilizer and integrated compost treatments except soybean and 2nd year corn in integrated compost in 1995 and 1996.

Soil Samples were taken to a depth of 20 cm on April 11, June 5, August 8, October 4 and November 20 in 1994, and to a depth of 25 cm on April 29, July 3, September 17, and November 29 in 1995, and on April 14 in 1996. The timing of these



Figure I.3 Diagrammatic representation of the crop and cover crop sequences at the Living Field Laboratory. Bars represent the time in which living crops and cover crops are in the field. Field preparation for wheat occurs immediately after soybean harvest. samples relative to field operations in the agronomic treatments is shown in Table I.1. Each soil sample consisted of 12 or 15, 2 cm diameter cores, bulked together and passed through a 6 mm sieve to remove large roots and residue fragments. Soil samples were cooled immediately and stored at 4 °C before processing for NMP, microbial biomass and macroorganic matter measurements. Detailed descriptions of these procedures are provided in Chapters 1 and 2. The procedures used to measure yield, crop and cover crop biomass, soil inorganic N, and leached inorganic N are discussed in Chapter 3. With only a few exceptions, these field measurements were obtained by co-workers associated with the Mott Chair for Sustainable Agriculture.

The overall objective of this research is to identify specific management practices that influence the availability and loss of nitrogen in cropping systems, and to identify specific laboratory measurements at specific sample dates that can be used to estimate the supply of N from soil under diverse management conditions.

			1001					1005		1006
Field	April	June	August	October	November -	Mav	VINC	September	November	April
				Corn and	d Soybean					
Tillage		×	×	×	×		×	×	×	
Compost added		×	×	×	×		×	×	×	
Crop planted		×	×	×	×		×	×	×	
Sidedress (corn)			×	×	×		×	×	×	
Cover planted			×	×	×			×	×	
Peak biomass			×	×	×			×	×	
Harvest				×	×			×	×	
				3	heat					
Tillage	×	×	×		×	×	×	×	×	×
Compost added	×	×	×		×	×	×	×	×	×
Crop planted	×	×	×		×	×	×	×	×	×
Cover planted	×	×	×			×	×	×		×
Peak biomass			×				×	×		
Wheat harvest			X					X		
† Each "x" indicate	es a field	operat	ion that v	vas comp	leted before	sample	date			

Table I.1 Timing of sample dates relative to field operations. †

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CHAPTER 1

Seasonal Changes in Nitrogen Mineralization Potential in Agricultural Soils: ANOVA and Non-Linear Regression Analysis of Management Effects

ABSTRACT

A soil's nitrogen mineralization potential (NMP) changes over time in response to the addition and decomposition of organic material. We used non-linear regression and repeated measures analysis of variance to explore seasonal changes in NMP in field experiments in Southwestern MI. In the analysis of variance, NMP was based on the average accumulation of inorganic N in 10, 30, 70 and 150 day aerobic incubations at 25 °C. This was 29% greater in agronomic treatments receiving compost than in inorganically fertilized treatments after 3 years of management, and 32% greater in corn-soybean-wheat rotations with cover crops than in corn-soybean rotations without cover crops after 5 years. A 6th year old-field succession had 50% greater NMP than either an annually tilled succession or an agronomic rotation. There were strong seasonal changes in NMP in all treatments with 65% greater accumulation in June than October samples in 1994 and 39% greater accumulation in April than September samples in 1995. The kinetics of N accumulation generally fit the single-pool exponential model $N_t = Ni + No$ (e^{-t/MRT}), where N_t is the inorganic N at time t, Ni is the initial inorganic N, No is the pool of mineralizable organic N and MRT is the steady-state mean residence time of No. However, lower than expected mineralization at 10 and 30 days had the effect of inflating estimates

of No and MRT in some incubations. Although we present several improvements in non-linear regression analysis, neither No nor MRT were themselves reliable indicators of NMP.

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INTRODUCTION

One of the major challenges facing agriculturists is to develop management strategies that better synchronize mineral N availability with crop demand. The supply of inorganic N must be sufficient during the growing season to achieve high levels of productivity, but soil nitrate levels must remain low at all other times to avoid loss and environmental contamination through leaching and denitrification (Oberle and Keeney, 1990, Smith et al., 1990). Such losses are particularly likely when rainfall exceeds potential evapotranspiration, as occurs in the cooler months in temperate climates. Our ability to develop truly sustainable management systems -whether these are based on organic inputs or chemical fertilizers -- will depend on the ability to predict and manage the seasonal patterns of mineralization from soil organic matter and plant residues.

Nitrogen mineralization potential (NMP) refers to the intrinsic nitrogen supplying capacity of the soil. Measurements of NMP provide relative estimates of field N mineralization, and can be used to model N availability and fertilizer N requirements if environmental conditions are known (Smith et al., 1977, Campbell et al., 1997). Nitrogen mineralization potential can be useful as an indicator of soil quality (Doran et al. 1987, Gregorich et al., 1994), and has been correlated with microbial biomass, microbial respiration, total organic matter and macroorganic matter content, and various physical indices of soil quality.

The NMP of a soil changes over time in response to the addition and decomposition of organic materials. By repeatedly measuring NMP over time, we can generate a seasonal profile that reflects the storage and release of organic-N in a soil under field conditions.

Mineralization Kinetics as an indicator of NMP

Stanford and Smith (1972) used first order kinetics to define a pool of potentially mineralizable N, N_0 , which decomposes exponentially according to a rate constant, k based on the equation:

Eq. [1] Nmin=No $(1-e^{-kt})$

where Nmin is the cumulative amount of N mineralized at time t in the incubation.

In the original Stanford and Smith method, soil is mixed with sand and mineralization measurements are obtained by periodic leaching. Maynard et al. (1983) tested an alternative method in which inorganic N is allowed to accumulate in replicate soil samples, which are then destructively sampled at various times during the incubation. While this method produced slightly different kinetic parameters than the leaching method, the quantity of N mineralized over time was the same, a finding confirmed by Motavalli et al. (1995). We have preferred this method in our lab because it can be more easily paired to C mineralization studies.

Statistical Analysis of N Mineralization Kinetics

Statistical methods for choosing between regression models are well developed (e.g. Robinson, 1985). However, methods for identifying significant treatment effects on

mineralization kinetics have received less attention. Most non-linear regression procedures provide asymptotic standard error estimates for each parameter estimated, and some, such as the NLIN procedure of SAS (SAS Institute, 1988), also provide asymptotic standard error estimates for the predicted values of the kinetic equation. One can determine whether two treatments produce different estimates for a parameter or predicted value by fitting a regression model with separate parameter estimates for each treatment, and using the asymptotic SE estimates to construct independent sample t-tests between the treatments. Alternatively, one can determine whether a given treatment or set of treatments has a significant effect on the regression as a whole by comparing the residual sum of squares of a model with unique parameter estimates for those treatments to a simplified model that does not include them. The appropriate test for the hypothesis that the simplified model is sufficient to describe the system is the sum of squares reduction test (Feber and Wild, 1989):

Eq. [2]
$$[SSRes_2 - SSRes_1)/(dfRes_2 - dfRes_1)]/MSRes_1$$

$$\sim F(\alpha, (dfRes_2 - dfRes_1), dfRes_1)$$

where $SSRes_1$ and $SSRes_2$ refer to the residual sum of squares of the full and reduced models, respectively, $dfRes_1$ and $dfRes_2$ refer to the residual degrees of freedom of the two models, and $MSRes_1$ denotes the residual mean square error for the full model. This test is also used to compare alternative kinetic models where one model can be derived from the other by simplification.

Inferences based on NLR statistics are only valid if the same kinetic equation provides a reasonably good fit to all treatments, and if each mineralization

measurement can be considered an independent estimate of the overall curve for that treatment. This independence criterion is met in the N accumulation method because each measurement is made on a unique subsample, but it is not met in the leaching method because repeated measurements are made on the same soil sample. The inherent autocorrelation in that method can be overcome by using mixed-model NLR techniques (Littell et al., 1997, Gregoire and Schabenberger 1996a, 1996b) such as the SAS NLINMIX macro (SAS Inst., 1996).

Repeated Measures ANOVA as an alternative to Kinetic Analysis

Non-linear regression techniques do not require a specific strategy for sampling inorganic N during an incubation. If incubations are sampled at discrete intervals, it may be preferable to define NMP based on repeated measures analysis of variance (ANOVA). Using this approach, each measurement is considered a repeated measure of the mineralization potential of the original soil sample, and the effect of a given treatment (i.e. its NMP) is based on the average inorganic N measurement for that treatment across all replications and incubation times. The ANOVA approach has two important advantages over non-linear regression. First, it does not assume that mineralization follows a particular pattern with time, and treatment comparisons can be made regardless of whether all treatments exhibit similar kinetics. Second, it facilitates the explicit specification of an experimental design, and greatly simplifies the interpretation of main effects and interactions in a factorial setting.

Objectives:

The objectives of this study are:

- 1. to determine the seasonality of N mineralization potential and the factors that affect it,
- 2. to quantify the effect of agricultural management on N mineralization potential,
- to compare repeated measures ANOVA with non-linear regression for its ability to interpret treatment effects on both the overall level of mineralization and the kinetics of mineralization curves.

MATERIALS AND METHODS:

The soils used in this study were obtained from the Long Term Ecological Research Site (LTER) and the Living Field Laboratory (LFL) at Kellogg Biological Station (KBS) in Hickory Corners, MI. These experiments are located adjacent to one another on a gently rolling glacial outwash plain (2 to 4 % slope) consisting of Kalamazoo loam and Oshtemo sandy loam (Typic Hapludalfs). The Ap horizon reaches a depth of 20 to 30 cm and has an organic C content of 10 g kg⁻¹ to 20 cm. (Robertson et al., 1997). Rainfall has averaged 880 mm per year from 1988-1997. Precipitation exceeds potential evapotranspiration from October through March. The never-tilled treatment at the LTER has an organic C content of approximately 17 g kg⁻¹.

The KBS-LTER main site experiment, initiated in 1989, consists of 7 treatments in a randomized complete block design with the never tilled treatment located just offsite. In April, June, and November 1994 we sampled two corn-soybean rotations with conventional fertilization; conventional till (CT) and no-till (NT), and two cornsoybean-wheat rotations with cover crops; low chemical input (LI) and zero chemical input (ZI). All plots were planted to soybean in June, 1994 and to wheat in November, 1994. (This was the first wheat crop in CT and NT.) The LI and ZI treatments were interseeded with hairy vetch when in corn and frost seeded with either hairy vetch or red clover when in wheat. In April, July, September and November 1995, and April 1996, we sampled the CT treatment and three

successional treatments, historically tilled succession (HTS), a 6th year old field, never tilled succession (NTS), a mowed grass treatment on the original soil profile, and annually tilled succession (ATS), a tilled micro-plot within the HTS treatment. Additional information is available on the internet (KBS-LTER, 1998 [online]).

The Living Field Laboratory was established in 1992 to explore the range of corn production alternatives from a conventionally managed corn monoculture, to an organically managed corn-corn-soybean-wheat rotation with cover crops. The factorial design of the LFL facilitates the separation of each component of these systems: rotation vs. monoculture, cover vs. no-cover, chemical vs. organic fertilization, and chemical vs. organic pest and weed management (c.f. Jones, et al., 1998).

In 1994, we sampled 1st year corn, soybean and wheat, in the Integrated Fertilizer (Fertilizer) and Integrated Compost (Compost) treatments with and without cover crops, and in 1995-96 we sampled 1st year corn, wheat and continuous corn (monoculture) in the same treatments. In addition to the April, June, and November 1994 sample dates described for the LTER treatments, we sampled 1st year corn and soybean in August and October 1994. In 1995 we also sampled 2nd year corn and soybeans were in the Fertilizer treatment. The Fertilizer and Compost rotations are identical except for the use of urea and ammonium nitrate fertilizer or dairy manure –leaf compost to maintain fertility.

Sample Handling and N mineralization

One bulk soil sample consisting of 12, or more, 2 cm diameter cores was obtained from each plot to a depth of 25 cm in 1994, and 20 cm in 1995-96. After sampling, the soils were cooled immediately and stored at 4 °C until processing. Each soil sample was thoroughly mixed in the lab and passed through a 6 mm sieve at field moisture, and 20 g dry-weight-equivalent sub-samples were produced and adjusted to a gravimetric moisture content of 0.19. Sub-samples were pre-incubated for five days.

Extractable NO₃ and NH₄ were measured at the start of each incubation and at day 10, 30, 70, and 150. An additional incubation to approximately 310 days was performed for the 1995-96 samples. All of the sub-samples for a given plot and date were placed in a single, glass, quart canning jar with a small pool of free water to prevent dehydration. Base traps containing 2 ml of 3M NaOH were used to absorb CO2-C for measurement during the incubation period. The inorganic N content was determined by 5:1 extraction with 1N KCl. The filtrate was analyzed at the Michigan State University Soil Testing lab using a Lachat automated analyzer (Lachat Instruments Inc. Milwaukee, WI).

Data Analysis

The data are divided into 5 discrete sets for analysis: 1) the 1994 LTER data set consists of four agronomic treatments examined at three sample dates, 2) the 1995-96 LTER data set consists of one agronomic and three successional treatments

sampled at 5 dates, 3) the 1994 LFL consists of a 2 x 3 x 2 factorial for fertilizer source, crop, and cover over 3 dates, but will also be analyzed as a 2 x 2 x 2 factorial over 5 dates, 4) the 1995-96 LFL factorial data, is a 2 x 3 x 2 factorial at five dates, and 5) the 1995-96 Fertilizer rotation, which is a 5 x 2 factorial consisting of all entry points in Fertilizer rotation plus continuous corn, split by cover, over 5 dates. Each field treatment consists of four replications (blocks), and inorganic N was measured six or seven times during each incubation.

We performed an ANOVA for each data set, using the SAS MIXED procedure (SAS Inst., 1997) in which each sample date and incubation time was treated as a repeated measurement of the overall NMP of the corresponding field plot. The SAS MIXED procedure estimates the correlation structure within experimental units and uses it to construct an accurate univariate analysis of treatment means. This technique overcomes many of the inherent limitations of multivariate repeated measures analyses (Cole and Gissel, 1966). The optimal covariance structure was determined using Akaike's Information (Littell et al., 1997).

Having used ANOVA to identify effects that produce significantly different mineralization curves, we used the NLIN procedure of SAS (SAS Inst., 1988) to parameterize these curves and identify significant differences in parameter estimates and predicted values. We used the SSRT (Eq. [2]) to compare the overall fit of three models: a single pool 1st order exponential model, Eq. [3], a linear model, Eq. [4], and a two-pool 1st order exponential model Eq. [5]. In these models MRT, MRT1 and MRT2 are the steady state mean residence times of the mineralizable organic N

pools No, N1 and N2 respectively. The parameter Ni represents the initial inorganic N content of the soil, and R is a constant mineralization rate.

- Eq. [3] $Nt = Ni + No(1 e^{-t/MRT})$
- Eq. [4] $Nt_i = Ni + Rt$
- Eq. [5] $Nt = Ni + N_1 (1 e^{-t / MRT_1}) + (N_2) (1 e^{-t / MRT_2})$
RESULTS

LTER 1994

The results of the ANOVA of the 1994 LTER data set (4 treatments x 3 dates x 5 incubation times) are summarized in Table 1.1. We observed less mineralization in the CT and NT treatments (49.7 and 53 kg N ha⁻¹) than in either the Low-input,(66.3 kg N ha⁻¹) or Zero Input (69.9 kg N ha⁻¹) treatment. There was also a significant date effect, with the April and November sample dates resulting in significantly greater overall mineralization (64 and 67.6 kg N ha⁻¹) than the June sample (47.5 kg N ha⁻¹). None of the interaction terms was statistically significant.

The key factor in increasing NMP in the LI and ZI treatments may have been the use of leguminous cover crops as green manure. The use of wheat in the rotation provides excellent conditions for the establishment and growth of the cover crop. The production of hairy vetch following wheat in the LI and ZI treatments averaged 421 g m⁻² in 1990 whereas only 50 g m⁻² of hairy vetch was produced following corn in 1991 (Robertson et al., 1997 [online]).

The accumulation of inorganic N generally fit the single-pool, 1st order exponential model (Eq. [3]), with estimates of 145 kg N Mg⁻¹ soil for No, 17 kg N Mg⁻¹ soil for Ni, and a mean residence time of 96 days across all treatments and sample dates (Table 1.2). The predicted values for the date and treatment effects generally

I ADIA I.I				1 1010 001. I			
		ANOVA F-T	ests		2	Aean Separation T	ests
				P-value for		Mean (SE) of	Significance
	ıĻ			Interaction with		Inorganic N	Group based on
Factor	Statistic	DF	P-value	Incubation Days	Factor Level	Accumulation	Protected LSD
	MST /	Numerator,				kg N ha ⁻¹	Same letter if
	MSE	Denominator				(25 cm)	p>0.05
Treatment	4.8	3, 9	0.029	0.259	CT ‡	49.7 (4.6)	U
	2				NT	53.0 (4.9)	ğ
					⊐	66.3 (4.9)	ab
					ZI	69.9 (4.6)	в
	A 53	2.24	0.021	0.160	April	64.0 (4.6)	Ø
Date	201	Ī			June	47.5 (4.9)	p
					November	67.6 (4.9)	n
on board of	AS MIXED	procedure (see to	ext). All signi	ficant main effects and in	nteractions are show	wn.	

Summary of ANOVA for the 1994 LTER data set. t Table 1.1

Kinetic parameters, predicted values and their asymptotic standard errors (in parentheses)	for treatment and date effects in the 1994.1 TER data set +
Table 1.2	

								ž						
		Ž	letic Pa	rameter	ş			Predict	ed Acc	umula	tion (A	bove N)	++	
•	z		ટ		MRT		¥	Я	ğ			150d		
	kg N	ha ⁻¹ §	kg N	- E	φ		¥	JNha ¹	kg N	Ē		kg N	Та Г	
Treatment acros	is date	SSR (SSR	Tp < 0.	(1000										
CT P	7	(4)	121	(9	144	(8 3)	80	(3)	8	(4)	с#	82	(2)	م
L	1	(4)	<u>8</u>	(16)	87	(Z)	5	<u>(</u> 2)	83	(7	q	8	(2)	q
	4	(4)	117	(14)	8	(<u>8</u>	13	(2)	67	(7	B	8	(2)	a
	17	(4)	8	(17	8	(9 2)	13	<u>(</u> 2)	2	(4	9	1 8	(2)	Ø
Date across tree	atments	s (SSRI	not sig	nificant)	_									
And	କ୍ଷ	(10)	135	(24)	113	(38)	=	<u>(</u> 2)	ଷ	€	B	8	(2)	B
and	5	(0)	8	(13)	81	(3 8)	1	(C)	ន	4	q	2	2	م
November	8	(01)	<u>8</u>	(17)	8	ଝି	5	(C)	8	(4)	Ø	97	(2)	v
All Data	13	3	116	(10)	8	(18)	Ŧ	(2)	ଞ	3		હ	ଚ	

+ Based on SAS NLIN procedure.

* Predicted inorganic N content on day 10, 70 and 150 of incubation minus predicted inorganic N content on day 0. § To a sample depth of 25 cm

Significance group within column and effect. Values with the same letter are not significantly different at $\alpha = 0.05$ P Abbreviations: CT = Conventional Tillage NT = No-Till U = Low Chemical Input Z = Zero Chemical Input

correspond to the data presented in Table 1.1, but there are no significant differences in treatment or data effects for any of the kinetic parameters. There was substantial immobilization during the April CT incubations and this had the effect of inflating the estimates of No and MRT in April across treatments and in the CT treatment across dates. An SSRT analysis shows that there is a significant improvement in overall fit when the treatments are fit individually, but little improvement when parameters are fit for each date.

LTER 1995-96

Nitrogen mineralization potential was an average of 47% greater in the HTS and NTS treatments than the ATS and CT treatments, in the 1995-96 LTER data set (Table 1.3) despite greater net primary productivity and residue return in the tilled systems (Robertson, et al., 1997 [online]). Mineralization potential was significantly greater in April 1994 than in any of the other sample dates. There were significant interactions between incubation time and each of the other terms. This indicates that the relative mineralization at each point of the incubation curve varies with both treatment and date. All of the curves were well described by single pool, 1st order kinetics (Table 1.4). The CT and ATS treatments produced nearly identical parameter estimates (N₀ = 112 kg N ha⁻¹ soil, MRT=86 days for CT, and No = 110 kg N ha⁻¹ and MRT = 77 days for ATS). The HTS treatment produced a similar MRT (84 days), but a higher No (183 kg N ha⁻¹), while the NTS treatment had the highest estimates for

Table 1.3	Summary of	f ANOVA for the	1995-96 LTER	data set.†				
	ANOV	A F-Tests				Mean S	Separat	on Tests
				P-value for		Mean (S	SE) of	
				Interaction with		Inorgani	ic N	Significance
Factor	F-Statistic	DF	P-value	Incubation Days	Factor Level	Accumu	Ilation	Group
	MST /	Numerator,				kg N	ha -1	Same letter if
	MSE	Denominator				(20	(L)	p>0.05
Treatment	12.73	3,9	0.001	0.000	CT ‡	51	(4)	q
					HTS	62	(4)	Ø
					ATS	53	(4)	q
					NTS	74	(4)	a
Date	4.85	4.48	0.002	0.003	April 95	77	(4)	Ø
		-			July	63	(4)	bc
					September	55	(4)	υ
					November	62	(4)	bc
					April 96	65	(4)	q
Treatment x								
Date	1.26	12,48	0.275	0.008				
+ Based on S	AS MIXED pn	ocedure (see tex	đ).			(-	

\$ Abbreviations: CT = Conventional Tillage HTS = Historically Tilled Succession ATS = Anually Tilled Succession NTS = Never Tilled Succession

Table 1.4	Kinetic para	meters, I	predict	ed valu	es and	asym	ptotic si	tandaro	d errors ((in paren	theses) for th	e 1995	-96 LTI	ER date	a set †		
			N.	netic Pa	ramete	S						Pred	icted V	alues (above	Ni)	++	
		Ī			ę		MRT		}	9	σ		704			150	q	
	kg	N ha		₽ ₽	Nha		days			kg N	ha -		kg	Nha .	-	kg	N ha	_
Treatments	across date:	s (SSRT	p < 0.	0001)														
CT	11.4	(4.4)	8	112	(8)	ပ	86	(16)	q	12	(3)	م	62	(4)	q	92	(4)	U
HTS	2.9	(4.4)	م	183	6	م	84	(6)	م	21	(3)	ъ	103	(4)	0	152	(4	q
ATS	9.4	(4.4)	ab	110	6	ပ	77	(14)	q	13	(9)	Ą	66	(4)	٩	95	(4)	υ
NTS	9.8	(4.0)	ab	290	(19)	B	181	(25)	ø	16	(3)	ab	93	(7	9	163	(4)	B
Dates acro	ss treatments	s (SSRT	p < 0.0)5)														
Anril. 19:	95 4.2	(1.2)	þ	159	(14)	م	61	(14)	م	24	(9)	ø	108	(8)	ŋ	145	(11)	ø
vlut	5.3	(6.6)	٩	159	(12)	م	100	(21)	ab	15	(9)	ab	80	(8)	٩	124	6	ab
Sentemb	Ar 8.8	(9.9)	_	143	(12)	Ą	<u>98</u>	(22)	ab	14	(9)	ab	73	6	q	112	(8)	م
Novembr	Br 5.5	(6.2)	ם	220	(23)	ŋ	170	(41)	ß	13	(9)	٩	74	6	q	129	(8)	ab
April, 19	96 18.8	(6.3)	ß	180	(18)	ab	144	(36)	ŋ	12	(9)	٩	69	6	م	116	(8)	٩
All Data	8.9	(3.0)		167	(9)		107	(11)		15	(2)		80	(3)		126	Ξ	
+ Based or	SAS NLIN P	procedure	e. Soil	depthi	is 20cm	n. Valu	les with	the sa	me lette	r are not	signific	cantly o	differen	t (withi	u group	(s)		
+ Dradicter	linomanic N	content	on dav	10.70	and 1	50 of ir	ncubatic	on minu	us predic	ted inor	janic N	conte		ay u.				

F Predicted Inorganic N CONTENT OF Lay 10, 10 and 100 and

both parameters (181 days and 290 kg N ha⁻¹). The predicted values follow the same pattern as the ANOVA means, with NTS and HTS predicted to mineralize at a greater rate than CT and ATS.

Among sample dates, there was more consistency in No than in MRT. The April 1995 samples had the lowest MRT (61 days) and a relatively low No (159 kg ha⁻¹ soil), while November and April 1996 have the highest (220 kg ha⁻¹ and 170 days for November and 180 kg ha⁻¹ and 144 days for April 1996). The relatively high MRT values in November 1995 and April 1996 can be explained in part by greater immobilization at those dates. Treatments with high predicted mineralization have high values for No and MRT whereas dates with high mineralization have low values of No and MRT. This suggests that No is relatively more important in establishing treatment effects, while MRT is relatively more important in establishing date effects. Management may have a greater effect on the quantity of mineralizable N (No), whereas its quality and the availability (MRT) may be more strongly controlled by seasonal differences.

LFL 1994

Figure 1.1 shows the accumulation of NO₃ at 10, 30, 70, and 150 days in the 1994 LFL incubations. The top of each bar segment represents the accumulation of inorganic N from the start of the incubation to the corresponding incubation time. The error bars represent the mean N accumulation across incubation times (the mean used in the ANOVA) plus and minus one standard error.



Figure 1.1 Accumulation of inorganic N during laboratory incubations at 25C in the 1994 LFL data set. Error bars represent standard errors for the mean of the 10, 30, 70, and 150 day sub-samples. Units are based on a depth of 25 cm.

Both ANOVA models (3 crop x 3 date and 2 crop x 5 date) detected significant fertilization, crop, and date effects and significant fertilization x date and crop x date interactions. There was 20% greater NMP in the Compost treatment (86 kg N ha⁻¹) than in the Fertilizer treatment (72 kg N ha⁻¹), and there was 13% greater NMP in the 1st year corn plots (83 kg N ha⁻¹) and 9% greater NMP in the wheat plots (80 kg N ha⁻¹) than in the soybean plots (72 kg N ha⁻¹) based on the April, June, and November samples. The increased mineralization associated with the Compost treatment was greatest immediately following compost applications. Compost was not applied in the Soybean plots until November (before wheat), and this accounts for part of the difference between crops. However, differences in residue quality were also important. Soybean follows corn or annual ryegrass, whereas wheat follows soybean, and 1st year corn follows either red clover (cover) or weedy fallow (no cover), both of which have a high N content when incorporated in the spring.

The mineralization curves for the 1994 LFL data set fit a single-pool, 1st order exponential model and produced reasonable parameter estimates, except in April 1994, when immobilization tended to produce inflated estimates of No and MRT. As with the LTER data, these regression models produced 70 and 150 day predicted values (not tabulated) that would provide the same interpretation of treatment and date effects as the ANOVA means.



Figure 1.2 Accumulation of inorganic N during laboratory incubations at 25C for the 1995-96 LFL data set. Error bars represent standard errors for the mean of the 10, 30, 70, and 150 day sub-samples. Units are based on a 20 cm sample depth.

LFL 1995-96

In the 1995-96 data set (Figure 1.2) there was 25% greater NMP in the Compost treatments (82 kg N ha⁻¹) than in the Fertilizer treatments (74 kg N ha⁻¹), and 29% greater N accumulation in the two April samples (89 and 80 kg N ha⁻¹) than in July, September, and November 1995 samples (68, 66, and 64 kg N ha⁻¹, respectively). There was greater NMP in the plots with cover crops (75 kg N ha⁻¹) than in the plots without cover crops (72 kg N ha⁻¹) and greater NMP in the wheat plots (77 kg N ha⁻¹) than in the continuous corn plots (71 kg N ha⁻¹), although 1st year corn (73 kg N ha⁻¹) was not significantly different from either. The highest mineralization potentials were achieved in wheat following soybean (97.5 kg N Ha⁻¹ in April 1995) and in 1st year corn with cover following wheat (93 kg N Ha⁻¹ in April 1995, 90.5 in April 1996). These figures compare to an average of 78.3 kg N ha⁻¹for the other treatments in April 1995 and 78.8 in April 1996.

The first order model provided a good fit to the data except in November, when immobilization resulted in inflated parameter estimates. The Compost treatment produced a significantly greater No estimate (165 kg N ha-1) and significantly lower Ni and MRT estimates (20 kg N ha⁻¹ and 70 days) then the Fertilizer treatment (No = 146 kg N ha⁻¹, Ni = 23 kg N ha⁻¹, and MRT = 90 days). Wheat was associated with a lower Ni estimate (15 kg N ha⁻¹) than 1st year (31 kg N ha-1) or continuous corn (27 kg N ha-1), but there were no significant differences between the crop or cover treatments in No or MRT. The strong immobilization experienced in November may have been the result of root decomposition. Measurements of root

biomass taken in a related study (Willson et al., 1997) showed a dramatic decrease in identifiable roots between September and November.

LFL 1995-96 Rotation Effect

When we examine the Fertilizer rotation as a whole (Table 1.5), we find that 2nd year corn, has a significantly lower mineralization rate than 1st year corn, or wheat on average (58.9 kg ha⁻¹ compared to 65.3 and 67.3 kg ha⁻¹ respectively). The average mineralization potential associated with the continuous corn treatment (62.4 kg ha⁻¹) is approximately equal to the average mineralization potential across the rotation as a whole (62.4 kg ha⁻¹). The use of cover crops significantly increased NMP overall, but not in any particular crop. There is a significant crop x date interaction in the rotations (not tabulated) that seems to be related to the high November NMP relative to April 1996 in the soybean treatments. These plots were tilled and planted to wheat in November, which may have stimulated the early decomposition of the soybean residue.

Table 1.5Average accumalation of inorganic N in 10, 30, 70, and 150 day
incubations for the LFL Integrated Fertilizer rotation in the
1995-96 data set.

1995-90 0	iata set.						
	Cov	er	No	Cover	Con	nbined	
Rotation	kg ha	-1	kg ha	•1	kg ha	-1	
soybean	64.3	ab †	59.4	ab	61.9	ab	
wheat	69.1	а	65.5	а	67.3	а	
1st year corn	68.4	а	63.2	ab	65.8	а	
2nd year corn	59.5	Ь	58.2	b	58.9	b	
Rotaiton Average	63.9	Α	60.7	В	62.3		
Corntinuous corn	64.7	ab	60.0	ab	62.4	ab	

† Based on a 20 cm sample depth

‡ Values with the same letter are not significantly different at $\alpha = 0.05$. Capital letters designate differences within rows, non-caps designate differences within columns

DISCUSSION:

The Seasonality of N Mineralization Potential

Nitrogen mineralization potential was greatest in spring and smallest in the early fall in almost all of the treatments observed in this study. Although Bonde and Rosswall (1987), El-Harris et al. (1983), and Franzluebbers et al. (1994) examined different cropping systems at different sample dates, they also found that NMP was highest before planting and after harvest and lowest near physiological maturity. Thus as a general rule we can say that NMP increases when plant residues and other material is made available through tillage or senescence, but decreases as N is mineralized and sequestered in crop tissue.

Treatment effects on N mineralization potential

The 1994 LTER data set presents a contrast between two reduced chemical input treatments with comparatively high mineralization potentials and two conventional treatments with low mineralization potential. We identified several factors that could have contributed to this difference, including the recent use of leguminous cover crops and the historical presence of wheat followed by hairy vetch in the rotation. The reduced levels of chemical fertilization and weed control in these treatments may also have had some effect on mineralization potential by selecting for more N efficient plant and microbial communities. This explanation has been proposed to describe the greater N-efficiency of a low input cash grain cropping system relative to a conventional cash grain cropping system at the Rodale Institute (Doran et al., 1987, Harris 1993, and Wander et al., 1994)..

The HTS and ATS treatments in the 1995-96 LTER data set demonstrate that a dramatic increase in mineralization potential can be achieved after only a few years of undisturbed fallow in this climate, and that this increase can be eliminated just as quickly after the resumption of tillage. These changes in NMP appear to involve both the direct effect of disturbance in promoting decomposition, and its indirect effect on the plant community. The diverse, perennial, plant community found in the HTS treatment seems to enhance the storage of labile sources of organic N, probably in the form of fine roots and rhizosphere organisms, which increases the potential of this soil to mineralize N when disturbed.

A similar effect could be involved in increasing the supply of N following wheat production. There is a 20 month period between tillage for wheat and tillage for corn in the corn (corn) soybean wheat rotations that we examined. During this time there is nearly continuous plant production, even in systems without cover crops. Additional study will be required to determine how important this fallow period is in increasing N availability to corn, although it has the obvious effect of increasing the time for red clover production in systems with cover crops.

We recorded an average increase in inorganic N accumulation of 22% after two years of compost addition and 29% after three years. The amount of compost N applied differed each year. In 1993, all of the plots received 43 kg N ha⁻¹. In 1994,

the wheat plots received 209 kg N ha⁻¹ and the corn plots received 697 kg N ha⁻¹. In 1995, the wheat plots received 489 kg N ha⁻¹ but the corn plots received only 67 kg N ha⁻¹. The total input of N in the Compost plots was approximately double the input in the fertilized plots over the first three years of the experiment.

The only significant cover crop effect was a temporary increase in NMP associated with the red clover following wheat. It is possible that there will be greater divergence between the cover crop and no cover treatments over time.

The Fertilizer rotation provides a useful window on the impact of crop sequence and rotation on mineralization potential. Mineralization potential is highest in 1st year corn and wheat and lowest in 2nd year corn and soybean, with continuous corn somewhere in the middle. While small, these differences may be important in explaining the functioning of the corn-corn-soybean-wheat rotation. Seasonal levels of inorganic N are at their highest in 1st year corn and decrease each year until they reach their lowest point under wheat. First year corn provides the greatest yield, and the yield of 1st year corn following red clover is equally high in the fertilized and compost amended plots (Jones et al, 1998).

The LFL and LTER data presented in this study strongly suggests that the type of vegetation and the time without disturbance are important determinants of NMP

Evaluation of the Methods used in this study

The repeated measures analysis of variance provided a more robust analysis of management effects than the linear regression. Because they are based on the average accumulation in 10, 30, 70 and 150 day incubations, the least square means are inherently weighted toward long term results, but also provide some information about the extent of immobilization. It would be easy to re-weight the analysis mathematically by choosing different incubation lengths.

The non linear regression analysis was weakened by immobilization, the effect of which were most apparent in the April, 1994 and November 1995 sample dates. Immobilization can be a problem in any kinetic analysis of Net N mineralization, and we echo the suggestion of Smith (Ref.) that samples early in the incubation should be avoided. We observed immobilization well past the 4th week in these incubations. Although kinetic models have been proposed that allow for a lag in mineralization (Bond and Lindberg, 1988 White and Marinakas, 1991), use of the more conventional exponential models to study short term N mineralization will require the measurement of gross N mineralization.

The regression parameters N_0 and MRT were extremely sensitive to immobilization, and were often difficult to interpret. The 70 and 150 day predicted values were less effected by immobilization and produced a similar separation of the treatments to that provided by the ANOVA least square means even when the parameter estimates were distorted. It is not clear, however, what advantage can be gained by

performing regression analyses when the parameter estimates themselves are not interpretable.

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CHAPTER 2

Managing Biologically Active Soil Organic Matter Fractions for Sustainable Crop Production

ABSTRACT

We sampled agricultural and plant successional experiments in Southwest MI over a two year period to study the effects of alternative management practices on microbial biomass and macroorganic matter. Microbial biomass was determined by the chloroform fumigation, incubation method, and macroorganic matter was defined as the C and N content in sand-size soil separates. Microbial C decreased during a drought in 1994, and was greater in treatments receiving compost than fertilizer in 1995. Microbial C:N ratio was lower in July and November (6.0) than in April and September (7.3), and lower in successional treatments without tillage (5.2) than in agronomic treatments (6.7). Microbial biomass accounted for 2.6% of soil C and 4.9% of soil N in 1995. Macroorganic matter accounted for 19.7 % of C and 14.8% of N. Its C:N ratio was 20.8 in a never tilled successional treatment and 16.0 in other soils, 17.0, on average, in the $250 - 1000 \,\mu$ m fraction and 15.5 in the 53 - 250 μm fraction. Macroorganic C increased after compost additions, and was greater in successional plots without tillage than in tilled treatments. It was strongly correlated with N mineralized in 70 and 150 day aerobic incubations. Microbial biomass was not. There was greater N mineralization per unit macroorganic matter in April and November than in September and October. This suggests that

macroorganic matter could be used to estimate N mineralization if combined with information about recently deposited plant residues.

INTRODUCTION

The need to develop cropping systems that better synchronize the availability of inorganic N with crop demand is a major challenge facing farmers and agricultural researchers. Sufficient inorganic N must be available during the growing season to facilitate high yields, but an accumulation of excess soil NO₃⁻ can lead to increased N-loss and environmental contamination, particularly when precipitation exceeds evapotranspiration.

The use of inorganic N fertilizer provides a partial solution to the problem of synchrony in that the amount and timing of fertilizer applications can be controlled. Logistical and economic constraints often limit farmers to a single application, and even when split applications are possible, losses are inevitable. Jokela and Randall (1997) applied ¹⁵N labeled fertilizers at the time of planting and at the V8-stage in continuous corn in the Northern US Corn belt and found that losses from the plant soil system were 13 - 27 % (depending on application rate) regardless of the time of application. Similar losses have been reported across a range of crops and conditions (Russelle et al., 1981, Kitur et al., 1984, Meisinger et al. 1985, Reddy and Reddy, 1993, Timmons and Baker, 1992).

Fertilizer N is often applied at unnecessarily high rates, leading to increased N loss. Keeney (1987) and Meisinger (1984) note that fertilizer recommendations are often made on the basis of insufficient information about the supply of N from plant and soil resources. Libra et al. (1987a, 1987b) and Hallberg (1986) estimate that 50-80 kg N ha⁻¹ arable land is leached into groundwater each year in the Big Springs watershed in Iowa, and attribute much of this flux to a failure to account for N mineralized from legumes and animal wastes in estimating crop needs. Existing recommendation systems are often ignored or misused. In a study of irrigated corn production in Nebraska, Schepers et al. (1986) observed that farmers overestimated their yield by an average of 32 bu acre⁻¹, resulting in an average over-fertilization of 50 kg N ha⁻¹. Although Hallberg (1987) notes that farmers are becoming increasingly aware of the issue of groundwater contamination, there is usually much more incentive to prevent under- than over-fertilization.

Biologically based cropping systems use a combination of organic amendments and biological N₂ fixation to increase the N supplying capacity of the soil. Such systems have the potential to provide better synchronization with crop demand than conventionally fertilized systems, if only because inorganic N is released more gradually over the growing season. Mineralization is affected by temperature and moisture availability, so the supply of inorganic N can be expected to decrease under conditions not favorable to plant growth. However, management practices that increase mineralization during periods of high demand may also increase mineralization when demand is low and the potential for loss is high. Dou et al. (1995) found that the incorporation of hairy vetch as a green manure before corn production resulted in elevated NO₃ levels throughout the season, and a higher NO₃ level at the last fall sample date than was observed for fertilized corn following a non-legume fallow. In a similar study, Brown et al. (1993) found that late fall NO₃

levels can increase in both tilled and no-till systems if the decomposition of vetch is delayed by lack of soil moisture.

There is a great potential to use crop and cover crop sequences to enhance the synchrony of both biologically and chemically fertilized systems (McGill and Myers, 1987, Pierce and Rice, 1988, Myers et al. 1994). Winter cover crops have been shown to reduce late fall NO₃ levels in a variety of systems (Smith et al., 1987, Solberg, 1995, Philips and Stopes, 1995), while the incorporation of these residues may either increase or decrease spring NO₃ levels depending on the extent of immobilization (Wegger and Mengal, 1988). Crop sequences also have consequences on N availability, although increased N availability may account for only a small portion of the increased yields commonly associated with crop rotations that include legumes (Baldock et al., 1981, Hesterman et al., 1987).

We define nitrogen mineralization potential (NMP) as the intrinsic ability of the soil to supply inorganic N through mineralization over time. Nitrogen mineralization potential is constantly changing in response to the accumulation and decomposition of organic materials, and can be altered by managing the inputs of organic material, and by changing the conditions under which decomposition occurs.

In a previous paper, (Willson et al., 1999) we measured NMP based on the accumulation of inorganic N in 150 and 310 day aerobic incubations of soil from agricultural and extended fallow treatments at the Kellogg Biological Station in Hickory Corners, MI. We found that NMP was highest in the spring and lowest in

fall, greater in untilled perennial systems than in tilled annual systems, increased with the addition of compost, and was greater in rotations including wheat and legume cover crops than in corn-soybean rotations without cover crops. These findings confirm that NMP is sensitive to short term changes in agricultural management.

In this paper, we use data from the same field experiments to examine the relationship between NMP and the C and N content of microbial biomass and macroorganic matter. Both of these pools are closely associated with N mineralization: microbial biomass because mineralization is a microbially-mediated process (Paul and Voroney 1984, Smith and Paul 1989), and macroorganic matter because it contains much of the partially decomposed plant material that fuels mineralization (Gregorich et al., 1994, Hassink, 1995a, 1995b). The objective of this paper is to determine how alternative management practices alter the size and composition of these fractions and to determine whether these fractions can be managed, or used to predict changes in NMP. Measurements of organic matter fractions are relatively simple, and might be useful as a basis for fertilizer recommendations.

MATERIALS AND METHODS

Soil samples were obtained from experimental treatments at the Living Field Laboratory (LFL) and the Long Term Ecological Research (LTER) experiment at Kellogg Biological Station in Hickory Corners, MI (42°24'N, 85°23'W). These experiments are located adjacent to one another on a mixture of Kalamazoo loam and Oshtemo sandy loam (Typic Hapludalfs). The climate is temperate, with warm humid summers and cool moist winters. Precipitation on site has averaged 880 mm yr¹ (1988-1997) evenly distributed throughout the year.

Composite soil samples consisting of 12-15 two cm diameter cores were obtained to a depth of 25 cm in April, June, August, October and November, 1994 and to a depth of 20 cm in April, July, September and November, 1995 and in April 1996. The samples were cooled immediately, sieved through a 4mm screen and stored at 4°C during processing. Because of the change in depth, and because different treatments were sampled, the 1994 and 1995-96 data sets are analyzed separately.

The KBS LTER site was established in 1989 to facilitate cross-disciplinary research on the ecological interactions inherent in row-crop agriculture and related ecosystems in the Midwestern United States. Treatments examined in this study include four corn-based rotations and three successional treatments. The agronomic treatments included conventionally fertilized, corn-soybean rotations with and without tillage (conventional tillage (CT) and no-till (NT)), and corn-soybean-wheat rotations with hairy vetch and red clover as green manure and either reduced

fertilization (low-input (LI)) or no chemical inputs of any kind (zero input (ZI)). The successional treatments include a mowed, but never-tilled, successional meadow (NTS), a historically tilled, old-field succession (HTS), and an annually tilled successional treatment (ATS). All four corn-based treatments were sampled in 1994, while CT and the three successional treatments were sampled in 1995 and April 1996. The agronomic treatments were planted to soybean in June, 1994 and wheat in October 1994 after having been in corn with or without hairy vetch in 1993. The CT and NT treatments had not been planted to wheat prior to 1994. Management protocols for the LTER main site experiment can be found on the internet (KBS LTER, 1998 [online])

The LFL was established in 1993 to provide additional information about the effects of alternative management strategies in sustainable agriculture. The LFL has a factorial design that allows the separation of important management alternatives such as rotation vs. monoculture, inorganic vs. organic fertilizer (compost), broadcast vs. banded vs. non-chemical weed control, and the presence or absence of cover crops. Each crop in the corn-corn-soybean-wheat rotation is grown each year. This prevents the confounding of crop sequence effects with year to year variations and facilitates the comparison of rotation vs. continuous corn(Jones et al., 1998).

We sampled 1st year corn, soybean and wheat in 1994, from the Integrated Compost (Compost) and Integrated Fertilizer (Fertilizer) management types with and without cover crops. In 1995-96 we sampled the complete corn-corn-soybean-wheat rotation along with continuous corn in the integrated fertilizer management type but only 1st year corn, continuous corn, and wheat in the Compost management type. The integrated fertilizer and Compost management types differ only in N source; ammonium nitrate fertilizer and dairy manure - leaf compost. Both management types utilize reduced tillage (chisel plow) and a combination of banded herbicides and mechanical cultivation for weed control. Cover crops include red clover frost seeded into wheat, red clover interseeded into 1st year corn and continuous corn, and annual ryegrass interseeded into second year corn.

Microbial biomass was determined using the chloroform fumigation, incubation method described by Harris et al. (1997), including the partial control subtraction equation for microbial C (equation [1]) developed empirically by Horwath et al. (1996),

[1] MBC = 1.73 Cf - 0.53 Cc

and the corresponding microbial N equation (equation [2]) of Harris et al. (1997),

[2] MBN = MBC (0.56 (Nf-qNc)/(Cf-pCc) + .095)

where Cf and Nf refer to the net CO_2 -C and NH₄-N respectively produced in fumigated soil over a 10 day incubation at 25°C, Cc and Nc refer to the net C and N mineralization in a non-fumigated control under the same conditions and p and q are the proportion of the non-microbial C and N in the control incubation that is consumed in the fumigated sample. The value of q and p is assumed to be equal for the purpose of this analysis, and is derived using equation [3] from Horwath et al. (1996).

$$[3] \quad p = q = 0.29 (Cf/Cc) + 0.23$$

All CO₂-C measurements were made by titration of NaOH traps, and NO₃-N and NH₄-N measurements were made on 1M KCl extracts using a Lachat automated colorometric analyzer (Lachat Instruments Inc. Milwaukee, WI).

Macroorganic matter is defined as the total C and N content of sand-sized (53 – 1000 μ m) soil separates. Air dried soil was dispersed in 5% sodium polyphosphate solution, shaken for 8 hours, and passed through nested sieves of 1000, 250, and 53 μ m using a flow of distilled water to ensure separation. The material remaining on the 250 and 53 μ m sieves was analyzed for total C and N using a Carlo Erba N A 1500 Series 2 N/C/S analyzer (CE Instruments Milan, Italy) and defined as coarse and fine macroorganic matter respectively.

Treatment and date effects on both microbial biomass and macroorganic matter were analyzed using Proc Mixed of SAS (SAS Institute, 1997) given a randomized complete block design with 4 blocks at the LTER and a split-split-plot randomized complete block design at the LFL with management type (compost or fertilizer) as the main plot, each crop as a subplot and the presence of cover crops as a sub subplot treatment. The location of the cover and no cover sub-subplots in the LFL is not randomized within each crop subplot, so cover crop effects could be confounded

by positional effects. The correlations between treatment means for microbial biomass C and N and each macroorganic matter fraction, and between these indices and the N mineralization data of Willson et al. (1999) are generated using SAS procedures (SAS Institute, 1988).

RESULTS

There were no significant differences in microbial biomass C due to management in the 1994 data set, but there was a pronounced decrease in biomass in the June sample relative to other sample dates (Figure 2.1). The relatively low June biomass C coincides with a prolonged drought: the LTER weather station recorded only 1.4 cm of precipitation between May 1 and June 6. The greatest decrease in biomass C from April to June (963 kg ha⁻¹ or 70%) occurred in the LTER NT treatment. Additional data taken at the time of sampling (not shown) shows that this treatment retained more soil moisture to 25cm than the other agronomic treatments, but also had the highest proportion of microbial biomass near the surface, where it is more likely to encounter temperature and moisture stress.



Figure 2.1 Microbial biomass C in the top 25 cm in the 1995 LTER Soybean Treatments. Bars represent the standard error of the mean.

The lack of a significant difference in microbial C between the compost (833 kg ha⁻¹) and fertilizer (754 kg ha⁻¹) treatments is surprising given the large amounts of compost applied. The 1994 wheat plots received 3150 kg C ha⁻¹ in October 1993, the corn plots received 9000 kg C ha⁻¹ in May 1994, and the soybean plots received 6500 kg C ha⁻¹ in October 1994. (All plots also received 500 kg C ha⁻¹ of compost in April, 1993.). These additions are larger than the above ground net primary productivity in these treatments, and contain 2 to 7 times as much N as was applied in the corresponding fertilizer treatment.

The effect of compost was much more pronounced in the 1995 data set (Figure 2.2). There was an average of 728 kg C ha⁻¹ for the compost plots and 598 kg ha⁻¹ in the fertilizer plots for the1st year corn, continuous corn and wheat treatments. The difference was greatest in the 1st year corn plots (789 kg C ha⁻¹ for compost, 504 kg ha⁻¹ for fertilizer) even though these plots received the least compost over the life of the experiment (4950 kg C ha⁻¹, compared to 6960 kg C ha⁻¹ for wheat and 10800 kg C ha⁻¹ for continuous corn). Microbial C tended to increase in the warmer months. It was significantly greater in July or September 1995 than in April 1995 or April 1996 in this data set.

Microbial C and N were significantly greater in the never tilled succession (NTS) than in the other LTER treatments in the 1995-96 data set, but this treatment also had a greater total C content (1.7%) than the others (1.0%). Expressed per unit C, there are no significant differences in microbial C or N. The HTS and NTS



Figure 2.2 Microbial biomass C and N content in the top 20 cm in the 1995-96 LFL Samples. Bars represent the standard error of the mean.

treatments have lower microbial C:N ratios (5.0 and 5.4) than any of the other treatments (6.3 to 7.2) in the 1995-96 data set (Table 2.1). The C:N ratios in the HTS and NTS treatments were relatively stable whereas those in the tilled treatments were significantly lower after tillage in July and after senescence in November than in the other sample dates. These C:N ratios suggest that the microbial community is shifted toward bacterial production when fresh residues are available (July and November), whereas there are relatively more fungi at other sample dates.

The greatest changes in macroorganic matter in the 1995-96 data set (Figure 2.3) are associated with the large compost inputs following soybean (October 1994), wheat (October 1993) and 1st year corn (May 1994). Macroorganic C increased by 1300kg ha⁻¹ and macroorganic N increased by 50 kg ha⁻¹ in the November LFL soybean

							Month						
Treatm	ent	Apr	il 1995	5 Jul	y	Se	otember	No	/ember	Ap	ril 1996	Av	erage
LTER	NTS †	6.4	b‡	5.2	2	4.8	С	3.8	b	4.5	Ь	5.0) b
	HTS	5.4	b	5.4		6.0	Ь	5.3	ab	4.9	ab	5.4	b
	ATS	7.0	ab	5.6		7.7	а	6.4	ab	5.0	ab	6.3	ab
	СТ	8.5	а	5.6		7.4	а	6.8	а	7.6	а	7.2	а
LFL	Compost	7.4		6.6		6.5	Ь	6.0		5.9	Ь	6.5	
	Fertilizer	7.9		5.7		7. 9	а	6.0		7.3	а	6.9	
All Data		7.4	Α§	5.9	в	7.0	A	5.9 E	3	6.3	В	6.6	
+ Abbres	distinger AlT	0 - M-	T			alon Ll'	TC - Llie	torigall	Tilled	Cuesa	alan		

Table 2.1 Date and treatment effects on microbial C:N ratios in the 1995-96 data set

† Abbreviations: NTS = Never Tilled Succession HTS = Historically Tilled Succession

ATS = Annually Tilled Succession CT = Conventionally Tilled Wheat

‡ Values with the same letter are not significantly different (α =0.05).

§ Capital letters indicate comparisons between dates.



Figure 2.3 Two size classes of macroorganic Matter C and N in the top 25 cm of the 1994 LFL Treatments. Bars represent the standard error of the mean for each treatment

with compost samples relative to the April through October samples. This difference is equivalent to 23% of the applied compost C and 26% of the applied compost N. Similarly, there is an increase of 1400 kg ha⁻¹ C and 115 kg ha⁻¹ N between the April and June samples in the 1st year corn with compost plots, which represents17% and 19% of the applied compost C and N in those treatments. Much of this increase can be explained by the macroorganic matter content of the compost itself. We performed a size fractionation procedure directly on a sample of compost from the same source as applied in 1994 and found that 46% of compost C and 33% of compost N separated in the 250-1000 μ m fraction and an additional 10% of compost C and 8% of compost N separated in the 53-250 μ m fraction. The treatments that did not receive compost in 1994 averaged 4900 kg ha⁻¹ macroorganic matter C and showed only small changes during the season. There were no treatment or date effects on macroorganic matter C or N in the 1994 LTER data set.

Among the four LTER treatments sampled in 1995, NTS had the highest amount of macroorganic C, and the widest C:N ratio (Figure 2.4). It had about the same amount of macroorganic N as the HTS treatment. The HTS treatment had significantly greater macroorganic C and N than either of the tilled treatments (ATS and CT), but the difference was much greater in the coarse fraction (48% more C and 53% more N) than in the fine fraction (15% and 18% respectively). The difference in macroorganic C between the NTS and HTS treatments is primarily in the fine fraction. The coarse fraction may be more sensitive to recent changes in management, such as the conversion from tilled agriculture to succession without
tillage in the HTS treatment, whereas the fine fraction accumulates more slowly and is thus more indicative of long term management.



Figure 2.4 Macroorganic C and N in two size fractions in the top 25 cm of LTER wheat and successional treatments in 1995-96. Bars represent standard errors of the mean.

The NTS treatment had significantly wider C:N ratios for both coarse (23.4) and fine (19.0) macroorganic matter than the other LTER treatments. The NT soybean treatment and the other successional treatments (HTS and ATS) also had higher coarse fraction C:N ratios (17.9, 17.2, 18.5) than the tilled agronomic treatments (16.3 for CT soybean, 16.9 for CT wheat, 15.7 for LI, and 16.8 for ZI). The two low-input treatments, LI and ZI had the lowest fine fraction C:N ratios (15.9, 15.6) and the lowest total macroorganic matter C:N ratios (15.8, 16.1) at the LTER. It would appear that the C:N ratio of plant residues play a role in generating the observed treatment differences. The ATS and NTS treatments are dominated by

relatively low diversity of grass species whereas vegetation in the HTS treatment is more diverse and has a higher proportion of legumes.

The LFL treatments (Figure 2.5) continued to show significant effects of compost on macroorganic matter in 1995-1996. The compost treatments contained an average of 24% more macroorganic C and N than the fertilizer treatments. The coarse fraction accounts for 2/3 of this difference. All of the treatments in the 1995-96 data set show a sharp decrease in macroorganic matter in November relative to the other sample dates. While macroorganic matter is expected to reach a minimum in the fall, it is possible that some of this decrease may have been caused by the necessity of removing snow from the surface of certain treatments before sampling, which may have removed some of the surface soil.

The C:N ratios at the living field laboratory were extremely consistent throughout the study. While there were no significant differences between treatments or dates, there were significant differences between the size fractions. The C:N ratio of the fine fraction (15.28) was significantly lower than that of the coarse fraction (16.52) and was less variable (SD= 0.82 for fine macroorganic matter compared to SD=1.73 for the coarse fraction). These differences are consistent with the idea that newer macroorganic matter fragments may be larger, and have a higher C:N ratio, than older, finer, macroorganic matter.

Table 2.2 shows the coefficient, r, for the correlations between the organic matter pools discussed in this paper and the N mineralization measurements performed in



Figure 2.5 Macroorganic Matter C and N in two size fractions in the top 25 cm of LFL treatments in 1995-96. Bars represent standard errors of the mean.

Willson et al. 1999. The strongest correlations are those between macroorganic matter C (coarse and total) and the accumulation of inorganic N after 150 days of incubation. Macroorganic N is also a good predictor of NMP, but microbial biomass is not. The 10 and 30day N mineralization measurements are poorly correlated with all other indices, due to the influence of immobilization early in the incubation. The relationship between macroorganic C and 150 day N mineralization changes over the season as the availability of residue N changes (data not shown). Nitrogen mineralization per unit macroorganic matter was lowest in October and September when most plants are near peak biomass and highest in November and April when most plants have senesced. This is similar to the overall pattern found for NMP in Willson et al. (1999), and is consistent with the idea that macroorganic matter and fresh plant residues are the two most important sources of mineralizable N in these soils.

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	Inorganic N	accumulation	n
10 days	30 days	70 days	150 days
0.12 NS	0.39 ***	0.58 ***	0.64 ***
0.17 *	0.41 ***	0.59 ***	0.64 ***
0.03 NS	0.30 ***	0.47 ***	0.54 ***
0.16 *	0.44 ***	0.57 ***	0.59 ***
0.19 *	0.45 ***	0.57 ***	0.57 ***
0.08 NS	0.36 ***	0.47 ***	0.51 ***
-0.13 NS	0.12 NS	0.21 **	0.28 ***
-0.17 *	-0.12 NS	0.14 NS	0.41 ***
	10 days 0.12 NS 0.17 * 0.03 NS 0.16 * 0.19 * 0.08 NS -0.13 NS -0.17 *	Inorganic N Inorganic N 10 days 30 days 0.12 NS 0.39 *** 0.17 * 0.41 *** 0.03 NS 0.30 *** 0.16 * 0.44 *** 0.19 * 0.45 *** 0.08 NS 0.36 *** -0.13 NS 0.12 NS -0.17 * -0.12 NS	Inorganic N accumulation 10 days 30 days 70 days 0.12 NS 0.39 *** 0.58 *** 0.17 * 0.41 *** 0.59 *** 0.03 NS 0.30 *** 0.47 *** 0.16 * 0.44 *** 0.57 *** 0.19 * 0.45 *** 0.57 *** 0.08 NS 0.36 *** 0.47 *** -0.13 NS 0.12 NS 0.21 ** -0.17 * -0.12 NS 0.14 NS

Table 2.2 Correlations (r) between biologically active organic matter fractions and cumulative mineralization measurements across all treatments and dates.

*, **, and *** represent significance at the .05, .01 and .001 probability levels.

DISCUSSION

Pool sizes in relation to plant needs.

The N contents of the microbial biomass and macroorganic matter fractions relative to above ground plant biomass, yield, and residue N is calculated in Table 2.3 The minimum inorganic N throughput is an estimate of the quantity of inorganic N that must be made available during the growing season to supply plant needs. It is based on an overall uptake efficiency of 50% of available inorganic N (50% of fertilizer plus mineralized N is captured in plant tissue). This is near the upper end of the range of recovery efficiencies reported in fertilization studies (Keeney, 1982, Jokela and Randall, 1997).

Microbial biomass accounts for only 4% of Soil N in the fertilized 1st year corn treatment and 8% in the compost amended and a native succession soils. The data presented in Figure 3 shows that microbial N changed substantially between dates in each treatment, with a maximum increase of 120 kg N ha⁻¹ between April and July in the C1 compost- plots, more than doubling the initial N content. Given that the inorganic N pool never exceeded 45 PPM (117 kg N ha⁻¹) in the top 20 cm during this study, microbial immobilization would seem to be very important in modifying inorganic N availability. The change in microbial N from April to July was smaller following the incorporation of red clover in the 1st year corn, cover plots in 1995 than following the incorporation of a higher C:N ratio "weedy fallow" in the no cover plots. This suggests that low C:N ratio cover crops could be used to

		kg N ha-1		% of Soil N		
	LFL 1 st withou	year com ut cover	LTER	LFL 1 st year corn I without cover		LTER
	Fertilizer	Compost	NTS	Fertilizer	Compost	NTS
SOM †	2340	2470	2990	100	100	100
Microbial biomass	95	136	239	4.1	5.5	8.0
macroorganic matter 53-250	135	239	225	5.8	9.7	7.5
macroorganic matter 250-2000	154	265	170	6.6	10.7	5.7
macroorganic matter Total	289	504	395	12.4	20.4	13.2
Above ground biomass ‡	248	209	68	10.6	8.5	2.3
Yield Removed	137	125		5.9	5.0	
Residue Returned (1995)	111	85	68	4.7	3.4	2.3
Fertilizer / Compost	134	67		5.7	2.7	
Residue Input (from 1994)	51	46	79	2.2	1.8	2.6
Minimum N Required §	496	418	136	21.2	16.9	4.5

Table 2.3. Selected N pools in 1995-96 relative to one another and to soil N.

† Soil parameters are averaged across sample dates.

‡ Plant parameters were measured at time of peak biomass (late summer) except yield (late fall).

§ The minimum amount of inorganic N required for uptake, assuming 50% efficiency.

decrease the extent of early season immobilization, thereby freeing more inorganic N for plant uptake. A microbial turnover rate of 2-3 generations per year (Harris and Paul, 1994) would result in an average of 250 - 370 kg N ha⁻¹ cycled through the microbial biomass in the LFL agronomic treatments, which is similar to the minimum throughput of 240-500kg N ha⁻¹ estimated to be needed to produce the observed crop biomass.

Macroorganic matter represents 20% of soil N in the 1st year corn, compost treatment, and 12 and 13% in the fertilizer and NTS treatments respectively. As discussed previously, there were large increases in Macroorganic N associated with the large compost additions in 1994, and there was a temporary decrease in most treatments in November 1995. Date to date changes were relatively small, otherwise, indicating that the decomposition of macroorganic matter is balanced by the formation of new macroorganic matter for much of the season. The decrease in Macroorganic N in November 1995 was equivalent to 100 kg N ha⁻¹ on average and a maximum of 214 kg N ha⁻¹ in the 1st year corn treatment with compost and cover. It has been estimated that the steady state turnover time for macroorganic matter is 20-40 years. This would imply an annual turnover of 7-25 kg N ha⁻¹ in the treatments described in Table 2.3, which is 6-30% of the total amount of plant and compost N added to the soil in these treatments.

Pool sizes in relation to N mineralization Potential

Willson et al. (1999) found that nitrogen mineralization potential (NMP) was enhanced by the addition of compost, the incorporation of legume residues, and the conversion from annual to perennial vegetation as in the HTS and NTS treatments. Nitrogen mineralization potential was greatest in the late spring (April - June), and lowest in the early fall (September - October), although it often recovered by November.

Like NMP, macroorganic C and N are enhanced by compost additions and are much greater in the NTS and HTS treatments than in the tilled treatments. However, the incorporation of residues did not significantly change macroorganic matter C and N during in this study, whereas the incorporation of legume residues increased spring and fall NMP in Willson et al. (1999), particularly in contrast to the low NMP observed in September and October.

The correlation between macroorganic C and NMP found in this study, and in Hassink (1995), suggests that macroorganic C may be useful as an aid to fertilizer recommendations, particularly if combined with information about recently incorporated residues. The measurement of macroorganic C should be sufficiently simple to be included in routine testing, and would provide an objective method for modifying fertilizer recommendations in cases where past management has resulted in unusually high or low N supplying capacity. Macroorganic matter measurements that combine size and density fractionation (Meijbloom et al., 1995, Gadisch et al., 1996, Barios et al., 1996) and the anaerobic incubation of Waring and Bremner (1964) have also proven to be excellent indicators of N mineralization, but would be more expensive to implement in routine testing.

Microbial biomass was a less reliable indicator of changes in substrate availability than either macroorganic matter or NMP. Microbial biomass not enhanced by cover crops, increased with compost only in the 1995-1996 season and experienced a decline in June 1994 that was not related to substrate availability.

Seasonal Changes in Microbial Biomass

There was a trend toward greater microbial biomass in the warmer months (July-October) than the cooler months (April and November). This is consistent with the data at other moist temperate sites (Kaiser and Heinmeyer, 1993, Kirchner et al., 1993, Jorgensen et al., 1994, and Paul and Harris, 1997 [online]). The opposite pattern was found for cool-season production systems in semi arid environments(Van Gestel and Ladd, 1992, Bremer and van Kessel, 1992, Collins et al., 1992, and Franzluebbers et al.,1994). Maxwell and Coleman (1995) also report lower microbial biomass in the summer in a moist riparian habitat, but in that study summer microbial biomass seems to have been limited by microfaunal predation. While substrate availability is usually assumed to be the most important determinant of microbial biomass, it is clear from these studies that other factors have a greater influence on patterns of seasonal change.

We detected significant differences in microbial C:N in the 1995-96 data set, with lower C:N ratios after tillage and plant senescence (July and November, 1995) in the tilled treatments and lower C:N ratios at all dates in the untilled successional treatments. The C:N ratio of the microbial biomass should reflect the species composition, particularly the ratio of bacteria:fungi (Jenkinson, 1976, Shen et al., 1984, Sparling and Zhu, 1993). The C:N ratios of Fungi range from 4.5:1 to 15:1 while the C:N ratios of bacteria tend to be between 3:1 and 5:1 (Paul and Clark, 1996). If one assumes a C:N ratio of 10 for fungi and 4 for bacteria, the bacteria:fungi ratio of the July and November samples overall would be approximately 2:1 whereas the ratio in April and September would be 1:1 and the ratio in the NTS treatment would be approximately 6:1 across all dates. Direct microscopy of LTER treatments in 1991 and 1993 and of the LFL in 1994 resulted in bacteria:fungi ratios ranging from 0.3:1 to 5:1 (Paul and Harris, 1997 [online]). The high relative abundance of bacteria at this site contradicts the assumption of Anderson and Domsch (1975) that agricultural soils are dominated by fungi.

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CHAPTER 3

Biological Indicators of Crop Performance, Nitrogen Mineralization, and Leaching Loss in Integrated Cropping Systems

ABSTRACT

Integrated cropping systems use biological resources such as organic fertilizers, nitrogen fixing plants, and crop and cover crop residues to reduce the need for chemical inputs and improve sustainability. We present crop performance, and N cycling data for corn (Zea mays L.) monoculture and corn corn soybean (Glycine max L.) wheat (Triticum aestivum L.) rotations receiving compost or inorganic fertilizer, with and without cover crops. Laboratory measurements of microbial biomass, macroorganic matter, and N mineralization potential (NMP) were evaluated for their ability to predict yield, leaching loss, and N mineralization under field conditions. Nitrogen mineralization was calculated using the equation: N_{min}* = $N_{harvested}$ + $N_{residue}$ + $N_{leached}$ - $N_{fertilizer}$ - N_{fixed} , which does not include losses due to denitrification or volatilization. Leaching was measured using 0.3 m diameter, intact core lysimeters installed 0.3 m below the surface to facilitate tillage. The rotation produced about the same mineralization and leaching loss as the corn monoculture. The use of compost increased mineralization and decreased leaching loss. It produced equal yields in 1st year corn and soybean, but lower yields in 2nd year corn, continuous corn and wheat. Field N mineralization was correlated with predicted N mineralization in 70 and 150 day aerobic incubations and with

macroorganic N in the 53-1000 μ m size class across all dates. Correlations between NMP and both leaching loss and yield were often negative because of lower N availability in the compost treatment. However leaching loss was positively correlated with inorganic N and 10 day N mineralization in the previous summer.

INTRODUCTION

Nitrate contamination of groundwater is becoming prevalent in many agricultural regions of North America (Hallberg 1987, Power and Schepers, 1989, Hamilton and Helsel, 1995, Goss and Goorahoo, 1995 Richards et al., 1995, Franco and Cady, 1997). As this contamination spreads, there will be increasing pressure to develop cropping systems that minimize leaching loss while producing high economic returns. A key strategy in the development of sustainable crop production systems has been to increase the use of biological resources such as crop residues, N₂-fixing plants, and organic fertilizers to reduce the need for conventional N fertilization. While these innovations can occasionally be implemented without decreasing profitability, (c.f. Dobbs et al., 1988, for small grains, Kelly et al., 1995, and Creamer et al., 1996, for tomatoes, Chase and Duffy, 1991, and Lichtenberg et al., 1994, for corn rotations), it has yet to be shown that biologically fertilized systems are inherently better for the environment.

To manage biological N resources successfully, we must be able to predict their effect on inorganic N availability and loss. Specifically, we need to understand how management changes the timing and extent of N mineralization relative to plant uptake, and we must identify accurate predictors of N mineralization that can be included in our calculation of fertilizer N requirements (Keeney, 1982).

In a previous paper (Willson et al., 1999b), we presented measurements of N mineralization potential (NMP) based on the accumulation of inorganic N in

aerobic laboratory incubations of soils obtained from field experiments in Southwestern, MI. At the Living Field Laboratory (LFL) site we sampled continuous corn and selected crops in a corn-corn-soybean-wheat rotation, with and without cover crops, and receiving either inorganic N fertilization or leaf-dairy manure compost. We found that NMP was highest in the spring and lowest in the early fall in all treatments, that compost additions increased NMP throughout the season, and that legume cover crops increased NMP primarily in the early spring. In a companion study (Willson et al., 1999a) we measured the C and N content of the soil microbial biomass and two macroorganic matter size fractions in the same soil samples to determine whether these biologically active organic matter fractions respond to management in the same way as NMP. Microbial biomass increased slightly with compost additions, but was otherwise insensitive to management, and was not strongly correlated to NMP. All macroorganic matter fractions were correlated with NMP and followed a similar seasonal pattern. The strongest correlation was between the C content of macroorganic matter in the 250-1000 µm size class and change in inorganic N in the first 150 d of the incubation. The slope of this relationship was dependent on the time of sampling, with the greatest mineralization potential per unit macroorganic C in the spring and the lowest in the early fall. In general, macroorganic matter was more sensitive to compost additions and less sensitive to crop residue additions than was NMP.

In this paper we explore whether the laboratory measurements presented in Willson et al. (1999 a and b) can be used as predictors of N availability, crop performance and leaching loss in the field. We present yield, N uptake and N leaching data for

the Integrated Fertilizer and Integrated Compost treatments at the LFL and use these data to estimate net annual N mineralization. We then perform a correlation analysis to determine which laboratory measurements at which sampling dates provided the best predictions of yield, leached N and field N mineralization both in the current and in the following growing season.

MATERIALS AND METHODS:

Site Description and Treatments Sampled

The data used in this analysis is from the 2nd, 3rd and 4th year of the Living Field Laboratory (LFL) experiment in Hickory Corners, MI (42°24'N, 85°23'W). This experiment is located on a gently sloping glacial outwash plain (2 to 4 % slope) with underlying material consisting primarily of coarse sand. The soil is a mixture of Kalamazoo loam and Oshtemo sandy loam (Typic Hapludalfs). The depth of the Ap horizon is 20 to 25 cm at this site with an average organic C content of approximately 10 g kg⁻¹. Average precipitation is 880 mm yr⁻¹ (1988-1997), with precipitation exceeding potential evapotranspiration from October through March.

The LFL was established in 1993 to explore the sustainability of corn production strategies ranging from a low diversity, conventionally managed, corn monoculture, to a high diversity, organically managed, corn-corn-soybean-wheat rotation with cover crops. The experimental design is split-split-plot, randomized complete block, with main plots for each of four management types, subplots for each entry point in a corn-corn-soybean-wheat rotation plus continuous corn, and sub-subplots based on the presence or absence of cover crops. Cover crops included red clover frost seeded into wheat and interseeded into 1st year corn, annual ryegrass interseeded into 2nd year corn, and a mixture of red clover and annual ryegrass interseeded into continuous corn. Sub-subplots measure 4.6 x 15.2 m, corresponding to a single pass

with a 6-row equipment (0.76 m row spacing). Reduced tillage (chisel plow) is used throughout the experiment, and the design is replicated in four blocks.

We present data from the Integrated Fertilizer (Fertilizer) and Integrated Compost (Compost) management types at the LFL. These systems are managed identically except for the use of inorganic fertilizers or leaf - dairy manure compost to maintain N fertility. Fertilizer N inputs (Table 3.1) are based on soil test recommendations for wheat and the pre-sidedress nitrate test (PSNT) for corn, with a yield goal of 9.4 Mg ha⁻¹ (150 bu ac⁻¹). Compost additions were intended to provide adequate N for crop production based on an assumed mineralization rate of 25% per year. The small applications in 1993 (based on an estimated crop demand of only 12 kg N ha⁻¹) were judged insufficient, and starting in 1994, applications were based on an expected crop demand of 125 kg N ha⁻¹ at an assumed mineralization rate of 20%. The 1993 application of compost in the soybean plots was intended to provide differentiation between the compost and fertilizer treatments in the first year of the study. Over the first 4 years of the experiment, twice as much total N was added to the compost plots as to the fertilizer plots.

Field Measurements

Yield was determined in July for wheat, in September for soybean, and in the mid to late fall for corn, depending on the dryness of the grain. Corn was hand harvested in 1993-1995, but all other yield data was obtained using 2 or 3-row harvesters. Crop biomass samples were taken at physiological maturity for each

tyr	oes at th	he LFL							0
		1993	1	994	1	995	-	996	Cumulative
-	Cropt	kg N ha-1	Crop	kg N ha-1	Crop	kg N ha-1	Crop	kg N ha-1	kg N ha-1
Integrated	ប	43	с С	670	ົິ	0	S	67	781
Compost	C C	43	S	0	3	490	5	125	658
	လ	43	3	209	5	67	C C	502	821
	3	43	ប	670	3	59	S	0	772
	ပ္ပ	43	ပ္ပ	670	ပ္ပ	67	ပ္ပ	125	906
Integrated	ប	103	C2	06	S	0	3	78	272
Fertilizer	S	103	S	0	3	89	5 5	132	324
	ഗ	0	3	06	<u>5</u>	111	C C	146	347
	3	78	ប	06	C2 C2	136	S	0	305
	ဗ	103	с С	06	00	122	00	132	447
T Abbreviation:	s: C1 =	: 1st year corn	C2 = 2nc	d year com	S = soybea	an W = whea	t CC = co	ntinuous cor	

Table 3.1 History of N inputs in all entry points of the Integrated Compost and Integrated Fertilizer management

crop, and cover crop biomass was measured at approximately the same time as corn biomass.

Nitrogen leaching loss was measured using subsurface leaching lysimeters. These were installed in the cover crop sub-subplot of each entry point in the integrated management types and in two entry points in the Conventional and Organic treatments. (We have used the lysimeters in the Conventional treatment to estimate leaching loss in the Fertilizer, no cover treatment for the purpose of the correlation analysis. These are treatments are identical except for weed control methodology, banded herbicides in the Fertilizer treatment and broadcast in the Conventional.) Each lysimeter was constructed of a 0.6 m section of 0.3 m diameter PVC pipe buried 0.3 m below the surface to facilitate tillage, and contains an intact soil profile 0.3 - 0.9 m in depth. Leachate was pumped from a 201 carboy attached to the base of the lysimeter at approximately one month intervals or as needed to prevent overflow of the reservoir. Leachate was stored at 4 °C until analyzed for NO₃-N and NH₄-N using a Lachat automated analyzer(Lachat Instruments Inc. Milwaukee, WI).

Soil nitrate was sampled to a depth of 0.3 m, 7-12 times a year and to 0.9 m in November. The soil samples were dried at 35 °C, ground, shaken for 30 min. in a 5:1 dilution of 1MKCl, and gravity filtered using pre-leached filter paper. Plant tissue analysis was performed using a Carlo Erba NA 1500 Series 2 N/C/S analyzer (CE Instruments Milan, Italy) and NO₃-N was measured using a Lachat automated

analyzer. The reader is referred to Dehne (1995), Jones (1996), and Jones et al., (1998) for more a more detailed description of these protocols.

Calculation of Field N Mineralization

The problem of estimating the nutrient supplying capacity of the soil to prevent both over- and under-fertilization has been addressed by numerous authors (c.f. Cabrera et al., 1994, Rice and Havlin, 1994, Campbell et al., 1997). Meissinger (1984) suggested that fertilization recommendations should ultimately be based on an Nbalance approach. He proposed the equation:

[1] NFertilizer + N_{Misc.} – N_{Harvested} - N_{Leached} – N_{Gas} – N_{Erosion} = Δ N_{SOM} + Δ N_{Inorganic}.

to described the annual N budget of a given soil under agricultural management, where $N_{Misc.}$ includes all external inputs other than fertilization and ΔN_{SOM} includes all of the organic material in the soil. In systems without legumes, $N_{Misc.}$ consists primarily of atmospheric deposition, and both $N_{Misc.}$ and $N_{Erosion}$ are usually small. Atmospheric deposition at KBS was measured at 6.3 kg N ha⁻¹ yr⁻¹ from 1994-1996 (National Atmospheric Deposition Project, 1998 [online]), which is probably greater than erosion and much less than the other fluxes in equation [1]. Under these conditions, Meisinger suggests that $N_{Misc.}$ and $N_{Erosion}$ can be removed from the analysis leaving the equation:

[2] $N_{Fertilizer} - N_{Harvest} - N_{Leached} - N_{Gas} = \Delta N_{SOM} + \Delta N_{Inorganic}$.

We can generalize equation [2] for use with integrated cropping systems by adding specific input terms for N_2 -fixation (N_{Fixed}) and organic fertilization (N_{OF}), and by

redefining the system boundaries to include plant residues (N_{Res}) as a storage pool of

N. The resulting equation would be:

[3] NFert. + NOF + NFixed - NLeached - NHarvest - NGas = $\Delta N_{SOM} + \Delta N_{Inorganic} + \Delta N_{Res}$,

If we define N_{Res1} and N_{Res2} as the non-harvested plant biomass N at the beginning and the end of the year such that

$$[4] \qquad \Delta N_{Res} = N_{Res2} - N_{Res1},$$

and we time the endpoints of our crop production year such that all of the plant material added to the soil during the year comes from Nres1, we can say that

$$[5] \quad \Delta SOM = N_{Res1} + N_{OF} - N_{Min}$$

where N_{Min} is the annual net N mineralization in the field. By substituting [4] and [5] into [3] we can calculate Nmin as:

[6] $N_{Min} = N_{Harvested} + N_{Leached} + N_{Gas} + N_{Res2} + \Delta N_{Inorganic} - N_{Fertilizer} - N_{Fixed}$.

Equation [6] should be applicable whenever the assumptions of eq. [5] are valid. This can be achieved in annual cropping systems by setting the endpoint of the crop production year after the physiological maturity of summer crops, but before those crops are incorporated into the soil. We base our analysis on a crop production year of October 1 to September 30, which coincides with the transition from soybean to wheat in the corn-corn-soybean-wheat rotation.

In order to satisfy equation [5], measurements of N_{Res} should include root production. We assume that corn root N is 50% of corn stover N based on LFL data reported in Paul et al. (1998), and that wheat and soybean root N are 72% and 58% of wheat straw and corn stover N respectively (Bouyanovsky and Wagner, 1986). (These calculations assume that root C:N equals straw or stover C:N at physiological maturity.) Estimation of cover crop root biomass is complicated by the fact that these plants are in an intermediate stage of development when sampled. We base our analysis on direct measurements of root and shoot N provided by Kunelius et al. (1992) who reported a root to shoot ratio of 1.15 for red clover and 0.29 for annual ryegrass underseeded into barley. The "weedy fallow" biomass following wheat is assumed to have the same root:shoot N ratio as red clover.

We have calculated N_{fixed} based on the assumption that the proportion of soybean and red clover N derived from the atmosphere (Ndfa) is constant for each plant. Percent Ndfa in soybean depends on the variety and maturity group (Henderson and Zapata, 1984, Newhausen et al., 1998), and the type and abundance of bacterial symbionts (Vasilas and Fuhrman, 1993, Galal, 1997), but is particularly sensitive to soil NO₃-N content. Soil NO₃-N is generally low at the LFL, rarely exceeding 10 mg N kg⁻¹ soil during soybean or red clover production. We assume a value of 60% Ndfa for soybean, which is greater than the 33-49% reported for a similar soil N content by Herridge et al. (1990), but less than the 69% reported by George and Singleton (1992) or the 71-77% reported by Newhausen et. al. (1988). Values reported for red clover Ndfa (e.g. Heichel et al., 1985, Mallarino et al., 1990, Furnham and George, 1993) tend to be slightly higher but they are just as variable, and appear to be just as sensitive to soil NO₃-N levels. We assume a value of 70% Ndfa for red clover in this study.

Robertson (1997) notes that accurate data for denitrification and volatilization (N_{Gas}) are rarely available for upland farming systems. Ammonia volatilization is greatest after the application of ammonium and urea based fertilizers, and can range from <1% to >60% of applied N depending on the soil acidity, the method and rate of application, the moisture content of the soil, and the pattern of rainfall after application (Shankaracharya and Mehta, 1969, Gezgin and Bayrakil, 1995, Fox et al., 1996). Significant N-volatilization has been measured in maturing plants (Hoopker et al., 1980, Francis et al., 1993) and in decaying plant tissues (Janzen and McGinn, 1991), but the overall volatile-N balance for the plant - soil system is unknown due to the complexity of sources and sinks (Francis et al., 1997). Point measurements of denitrification rate can be made using either the C_2H_2 inhibition technique (Yoshinari et al., 1977) or the ¹⁵N enrichment technique (Buresh and Austin, 1988), but the extreme spatial and temporal variability of denitrification makes it difficult to derive accurate field scale values from these measurements (Robertson, 1977).

The best estimates of Ngas come from N balance studies in which gaseous loss is determined by difference. Robertson (1997) suggests that an average of 20-40 kg N ha⁻¹ is typical for upland agricultural soils, but data from the studies reviewed by Allison (1955), von Reinbaban (1990) and Peoples et al. (1995) range from near 0 to >80 kg N ha⁻¹. Faced with so much uncertainty, and in the absence of any direct data, we prefer to base our analysis on a simplified version of equation [6]

[7] $N_{Min}^* = N_{Harvest} + N_{Res2} + N_{Leached} + \Delta N_{Inorganic} - N_{Fertilizer} - N_{Fixed}$ where Nmin* underestimates N_{min} by the quantity of N_{gas.}

Laboratory Measurements

The laboratory measurements used in this paper (Table 3.2) are identical to those used in Willson et al. (1999a and b) except that separate kinetic analyses were performed for each combination of treatments (management type, entry point and cover crop) at each sample date. Soil samples were obtained in April, June, August, October and November 1994, in April, July, September and November 1995, and in April 1996. Each sample consists of a composite of 12, 2 cm diameter soil cores taken to a depth of 25 cm in 1994 and 20 cm in 1995-96. The 1st year corn, soybean and wheat entry points were sampled in the Compost and Fertilizer rotations in 1994 (cover and no cover), while in 1995, all of the Fertilizer plots were sampled along with 1st year corn, continuous corn and wheat in the Compost plots. Microbial biomass N was determined only in 1995-96 and data for N mineralization and macroorganic matter C and N were not obtained in the wheat plots in August or October 1994.

The kinetic parameters No, MRT and Ni in Table 3.2 are based on the single pool exponential model $N_t = Ni + No (1-e^{-1/MRT})$ where N_t is the inorganic N content of a replicate soil sample at time t (days) in an aerobic incubation at ideal moisture and 25 °C. (The measurements of N_t are designated N_0, N10, N70, and N150 in Table 3.2.) The values p10, p30, p70, and p150 represent the predicted accumulation at time t, which is equivalent to No (1- $e^{-1/MRT}$). The measured increase in N_t above N_0 is designated d10, d30, d70, and d150. All of these parameters can be considered measurements of NMP except N_0 and Ni , which measure the inorganic N at the time of sampling. All should be positively correlated with

Table 3.2	Laboratory measurements and kinetic parameters used in the correlation analysis
Abbreviation	Definition
No MRT	Pool of mineralizable organic N as defined by 1st order kinetics. Steady state mean residence time as defined by 1st order kinetics. MRT = k ⁻¹ .
ž	Inorganic N at time 0 as defined by kinetic analysis.
N10, N30, N70, N150, ‡	measured inorganic N at time U. Measured inorganic N at day 10, 30, 70, 150 and 310 in incubation.
d10, d30, d70, d150	Measured increase in inorganic N after 10, 30, 70, 150, and 310 days.
p10, p30, p70, p150	Predicted increase in inorganic N after 10, 30, 70, 150, and 310 days.
MMC, MMN	Macroorganic matter C and N in 53 - 2000 um size class.
FMMC, FMMN	Macroorganic matter C and N in 53 - 250 um size class.
CMMC, CMMN	Macroorganic matter C and N in 250 - 2000 um size class.
C10	CO2-C respired in 10 day incubation. (Used in the calculation of MBN, MBC.)
MBC, MBN §	Microbial biomass C and N as determined by chloroform furnigation, incubation
	method.
1 Only the N_0, C10, and N	BC measurements were made on wheat plots in August and October 1995.

‡ 310 day incubations performed at the July, September and November 1995 and April 1995 sample dates only. § MBN measurements not obtained in 1994 only.

inorganic N availability except MRT, which is negatively correlated. All incubations were performed at a constant 25 °C and gravimetric moisture content of 19%, (about 2/3 of water holding capacity in KBS soils). Extractions of accumulated inorganic N (NH₄ plus NO₃) were performed as described above for soil nitrate.

Macroorganic matter C and N fractions are measured by shaking each soil sample overnight in 5% (NaPO₃)_n solution (3:1 dilution), passing the dispersed soil through nested 1mm, 250 μ m and 53 μ m sieves using a flow of water to transfer the particles, and measuring the C and N content of the material retained on the 250 and 53 μ m sieves (coarse and fine macroorganic matter respectively) on the Carlo Erba CNS analyzer. Total C and N were also measured using this instrument. Microbial biomass C and N were measured by the chloroform fumigation, incubation technique (Jenkinson and Pawlson, 1976) using the calculations for C and N content described by Horwath et al. (1996) and Harris et al. (1997) respectively.

Correlation Analysis

Each of the parameters in Table 3.2 was analyzed for its ability to predict yield (bu ac⁻¹), leaching loss (kg N ha⁻¹ yr⁻¹) and apparent field N mineralization (kg N ha⁻¹ yr⁻¹) using the SAS CORR procedure (SAS Inst., 1986). The correlation analyses were performed on a plot by plot basis within each month. Laboratory measurements taken in the 1994 season were analyzed for correlation with the 1994 and 1995 field data, and laboratory measurements taken in 1995-96 were analyzed for correlation with the 1995 and 1996 field seasons. Because of the inherent differences between

corn, soybean and wheat yield, separate analyses were performed for each crop.

Leached N and apparent N mineralization were analyzed across crops.

RESULTS

Crop Performance, N Leaching Loss and Apparent N Mineralization

Crop yields were strongly affected by moisture, crop sequence, and fertility source in the three years of this study (Table 3.3). Total precipitation (based on a crop production year of October 1 through September 30) was 821 mm in 1994, 820 mm in 1995, and 651 mm in 1996, compared to a 10 year average of 880 mm (1988-1997). A prolonged drought in the summer of 1996 dramatically reduced corn and soybean yields. Other than a late spring drought in 1994, moisture levels were generally adequate for wheat production. First year corn (following wheat) consistently outperformed 2nd year and continuous corn in all treatments. The use of compost rather than inorganic fertilizers decreased the yield of 2nd year corn, continuous corn, and wheat, but did not decrease the yield of 1st year corn or soybean. The use of annual ryegrass as an interseeded cover crop decreased 2nd year corn yield in 1994, and the use of red clover as a preceding as well as interseeded cover crop increased 1st year corn yield in 1995, but there were no other significant cover crop effects.

The annual N budget of the LFL cropping system (Table 3.4) suggests that many of the observed differences in yield can be explained by differences in inorganic N availability. In each case that yield is lower in the Compost treatment, plant N (harvested plus residue) and leached N were also significantly lower, indicating lower N availability. By contrast, leaching loss and plant N in 1st year corn with

cover were not significantly higher in the Fertilizer treatment, indicating that the supply of N from mineralization was nearly as great in the Compost treatment as the supply of N from mineralization and fertilization in the Fertilizer treatment. An average of 2.5 kg N was removed as yield for each kg N leached in the Fertilizer rotation, compared to 2.9 in the Fertilizer monoculture, 4.1 in the Compost rotation and 6.1 in the Compost monoculture. Although leaching loss was not significantly greater in the fertilized rotation than in the monoculture, leaching loss was equivalent to 60% of fertilizer N applied in the rotation, compared to only 30% in the monoculture. Because of greater N₂ fixation in the rotation, harvested yield accounted for 133% of fertilizer N applied (113% for 1st and 2nd year corn),

Table 3.3	LFL crop yields average	ged between c	over and no	-cover treatme	ents †
Сгор	Management	1994	1995	1996	Average
Monoculture		********	Mg h	a ⁻¹	
Corn	Fertilizer	8.7 *	8.7 *	4.1 *	7.2 *
	Compost	7.6	8.0	3.3	6.3
Rotation					
1st year corn	Fertilizer	11.8	9.7	4.5	8.7
	Compost	11.7	9.7 †	4.1	7.9
2nd year corr	n Fertilizer	10.5 *†	8.6	4.3	7.8 *
	Compost	7.4 †	8.0	4.2	6.1
Soybean	Fertilizer	2.4	3.4	1.2	2.3
·	Compost	2.9	3.4	1.2	2.5
Wheat	Fertilizer	3.1	4.1 *	4.4 *	3.9 *
	Compost	3.2	3.6	3.3	3.4

+ Cover crop effect was not significant except as noted

* Significant difference between the Compost and Fertilizer treatments (a=0.05) compared to 85% in the monoculture.

Table 3.4	Annual N bu	Idget for the LFL integr	ated fer	tilizer an	d comp	ost treatme	ents with	COVER CT	- PRAL UI SOO	1990.	
	Parenthese	s indicate the standard	error o	f the mea	Ľ.						
					~	Aeasured \	/alues			Calculate	A Values
Crop	Fertility	Cover	Harve	sted N	Residu	ue N ‡	Leac	hed N	Fertilizer N	N ₂ Fixation	S Nmin ⁻ IP
Monoculture							N gy	ha ⁻¹ yr ⁻¹			
Con	Fertilizer	clover + ryegrass	101	(13) *	95	(16)	35	• (6)	119	თ ი	136 166
	Compost	clover + ryegrass	85	(8)	83	(13)	14	(3)		ת	001
Rotation 1st vear corn	Fertilizer	red clover	133	(12)	118	(20)	40	(12)	104	16	168
		red clover	129	(13)	107	(19)	36	(6)		17	247
2nd vear com	Fartilizar	annial merrace	119	(13).	60	(16)*	68	(16) *	123	0	165
	Compost	annual ryegrass	202	(2)	52	(8)	25	(9)		0	145
Soybean	Fertilizer	none	8 6	(8)	88	(4)	55	. (6)		111	123
	Compost	none	102	(10)	87	(3)	19	(9)		114	93
Wheat	Fertilizer	red clover	86	• (6)	203	(11)	24	(4).	86	110	109
	Compost	red clover	59	(9)	178	(16)	13	(4)		110	131
Full Rotation	Fertilizer	various	112	(9)	125	(10)	47	(9)	78	59	141
	Compost	various	06	(9)	106	(6)	23	(3)		60	152
† October 1, 1993	to September	r 30, 1996									

IN budget for the LET interrated fertilitizer and composit freatments with cover crops in 1994 - 1996. à

Residue N includes cover crops and
N2 fixation calculated as 60% of Soybean N, 70% of Red Clover N. Fixation was negligible in corn
N 2 Mini* = d Inorganic N + Harvested N + Residue N + Leached N - Fixed N - Fertilizer
Significant difference between the Fertilizer and Compost treatments=0.05)

The estimates of Nmin* (net mineralization minus gaseous loss) range from 93 kg N ha⁻¹ yr¹ for soybean to 247 kg N ha⁻¹ yr¹ for 1st year corn in the Compost rotation, and from 109 kg N ha⁻¹ yr¹ in wheat to 168 kg N ha⁻¹ yr¹ in 1st year corn in the Fertilizer rotation. These differences correspond to a decrease in N mineralization potential from 1st to 2nd year corn reported in Willson et al. (1999a), and can be explained in part by the lack of compost additions in the soybean treatment, and in part by differences in residue input from the previous crop. The residue input before 1st year corn in the Compost treatment with cover crops was 178 kg N ha⁻¹ yr¹ while the input before soybean was only 52 kg N ha⁻¹ yr⁻¹. In the Fertilizer rotation, the input before 1st year corn was 203 kg N ha⁻¹ yr⁻¹ compared to 88 kg N ha⁻¹ yr⁻¹ before wheat. Nitrogen mineralization was an average of 11 kg N ha⁻¹ yr⁻¹ greater in the Compost rotation and 30 kg N ha⁻¹ yr⁻¹ in the Compost monoculture than the corresponding Fertilizer treatments. Given that residue inputs in the Fertilizer system were 29 kg N ha⁻¹ yr¹ greater in the rotation and 23 kg N ha⁻¹ yr¹ greater in the monoculture, the decomposition of compost must have accounted for 53 kg ha^{-1} yr^{1} in the monoculture and 40 kg N ha⁻¹ yr^{1} in the rotation. This implies an average mineralization rate for compost of only 9% per year based on the history of applications, which is about half of the expected mineralization rate.

Our analysis of the total inputs and outputs for the Fertilizer and Compost systems (Table 3.5), indicates a net loss of soil N in the Fertilizer system and a net gain in soil N in the Compost system. Including losses for denitrification and volatilization would decrease the balance in both systems (typically, by 20 - 40 kg N ha-1 yr-1) but would not alter this interpretation. Current LFL protocols call for an annual input of

120 - 140 kg N ha-1 yr-1 for each treatment, depending on the N content of the compost. At this rate, soil N will increase by another 180 - 200 kg N ha-1 yr-1 and mineralization by 15 - 20 kg N ha-1 yr-1 between 1996 to 2000.

Correlation analysis

Table 3.5	Average annua and Fertilizer t	IN balance reatments w	(not including g ith cover crops	jaseous loss) for the Con	npost		
			Inputs			Outputs		
Management	Rotation	External	N ₂ fixation	Total	Harvest	Leaching	Total	Change
				kg N ha	¹ yr ⁻¹			
Fertilizer	Monoculture	119	9	128	101	35	136	-8
	Rotation	78	59	138	112	47	159	-21
Compost	Monoculture	288	9	297	85	14	99	198
	Rotation	239	60	299	90	23	113	187

Laboratory measurements of N mineralization potential (NMP) and macroorganic matter taken in 1994 and 1995 were reasonably good predictors of field N mineralization (Nmin*) in 1994 and 1995 (Table 3.6). The correlations between laboratory measurements in1995 and 1996 Nmin* were weaker, probably because of the 1996 drought. The best indicators of Nmin* across the range of treatments tested were the predicted values for 70 and 150 day mineralizations (based on 1st order kinetics), and the C and N content of macroorganic matter in the 53-1000 and 250-1000 µm size classes. The kinetic parameters No (potentially mineralizable organic matter) and Ni (initial inorganic N) were also good predictors of Nmin* at certain dates. Measurements taken in August and October 1994 tended to have the highest correlation with Nmin* in 1994, although this may have been influenced by the fact that the wheat plots were not sampled at those dates. Measurements taken in November 1994 and April and September 1995 were the best predictors of Nmin*
•	Laboratory	Field	Field	Predict	ors of N Min	eralization	<u></u>		-
	Data Set	Season	Treatments †	Correlation ‡	Date	Parameter	n	•	
•	1994	1994	C1, S, W	0.83	Aug.	p70	20	***	
			(IC and IF)	0.77	Jun.	NI	28	***	
				0.76	Aug.	MMN	20	***	
				0.73	Oct.	No	20	***	
				0.72	Oct.	p150	20	***	
		1995	C2, W, C1	0.86	Nov.	p150	27	***	
			(IC and IF)	0.82	Nov.	p70	27	***	
				0.77	Nov.	No	27	***	
				0.76	Nov.	p30	27	***	
				0.73	Nov.	p10	27	***	
	1995-96	1995	C1, W, CC (IC)	0.72	Apr. 95	No	39	***	
			C1, C2, S, W, CC (IF)	0.71	Sep.	p70	39	***	
				0.70	Sep.	p 30	38	***	
				0.71	Apr. 95	p150	39	***	
				0.70	Sep.	p10	40	***	
		1996	C2, C1, CC (IC)	0.47	Sep.	NI	38	**	
			C1, C2, S, W, CC (IF)	0.41	Apr. 95	CMMN	38	*	
				0.40	Apr. 95	MMN	38	•	
				0.40	Sep.	No	38	•	
				0.40	Nov.	N10	38	•	

Table 3.6	Laborator	measurements with the highest correlation to field mineralization.
10010-0.0	Laborator	

† Abbreviations: C1- 1st year corn, C2 - 2nd year corn, CC - continuous corn, S - soybean,

W - wheat, IC - integreated compost, IF - integrated fertilizer.

‡ If there are more than 5 significant correlations, the 5 highest are shown.

in 1995 and 1996. The highest levels of NMP were found in the 1994 wheat plots with cover, which had developed an excellent stand of red clover in the winter of 1994-95. These plots produced the greatest Nmin* estimates in both 1994 (1st year corn) and 1996 (2nd year corn).

Laboratory measurements of NMP and macroorganic matter were also significantly correlated with corn yield (Table 3.7), although many of these correlations are negative because yields were higher in the Fertilizer treatment than in the Compost treatment. Yield tended to be positively correlated with NMP within each management type, which accounts for the positive correlations observed in 1995. As in the previous analysis, high NMP in the November 1994 wheat samples was correlated with high performance in 19951st year corn. Wheat yield (not shown) tended to be negatively correlated with NMP because of the N deficit in Compost wheat, whereas there was little correlation between NMP and soybean yield. The measurements N30, N70 and N150 include both initial inorganic N and N mineralized during laboratory incubations. In July 1995 they reflect the supply of inorganic N from fertilization and mineralization.

Leaching loss tended to be negatively correlated with NMP measurements taken during the same year, but positively correlated with measurements taken during the previous year (Table 3.8). The best predictors of leaching loss in the following year were inorganic N and short term N mineralization measurements taken after sidedress fertilization in the previous year (August 1994 and July 1995). These

Laboratory	Field	Field		Corn			-
Data Set	Season	Treatments †	Correlation ‡	Date §	Parameter	n	-
1994	1994	C1	-0.63	Oct.	C10	12	*
		(IC and IF)					
	1995	C1, C2	0.71	Nov.	d70	24	***
		(IC and IF)	0.64	Nov.	N70	26	***
			0.59	Nov.	d 30	28	***
			0.57	Nov.	p10	32	***
			0.56	Nov.	p30	32	***
1995-96	1995	C1, CC (IC)	0.58	Jul	N70	37	***
		C1, C2, CC (IF)	0.47	Jul	N30	40	**
			0.46	Jul.	N150	40	**
			0.46	Apr. 95	No	40	**
			0.43	Nov.	NÌ	40	**
	1996	C1, C2, CC	-0.52	Sep.	CMMC	48	***
		(IC and IF)	-0.50	Sep.	D70	47	***
		· · ·	-0.49	Sep.	CMMN	48	***
			-0.47	Sep.	N70	47	***
			-0.45	Nov.	p70	48	**

Table 3.7	Laboratory measurements with the highest correlation to corn viel	d.
1 abie 5.7	Laboratory measurements with the highest constation to com yiel	ч.

† Abbreviations: C1- 1st year corn, C2 - 2nd year corn, CC - continuous corn

IC - integreated compost, IF - integrated fertilizer.

‡ If there are more than 5 significant correlations, the 5 highest are shown.

§ This is the sample date for the laboratory measurement.

Laboratory	Field	Field	Pred	ictors of Le	eached N	
Data Set	Season	Treatments †	Correlation ‡	Date	Parameter	n
1994	1994	C1, S, W	-0.56	Nov.	d30	23 **
		(IC and IF)	-0.54	Aug.	C10	28 **
			-0.54	Nov.	p30	28 **
			0.54	Jun.	N_0	28 **
			-0.54	Nov.	p10	28 **
	1995	C2, W, C1	0.71	Aug.	N_0	27 ***
		(IC and IF)	0.57	Aug.	p10	20 **
			-0.57	Nov.	N_0	22 **
			0.49	Aug.	p30	20 *
			-0.48	Aug.	d30	18 *
1995-96	1995	C1, W, CC (IC)	-0.39	Sep.	p150	39 *
	C1,	C2, S, W, CC (IF)	-0.39	Sep.	p70	39 *
			-0.39	Nov.	CMMC	39 *
			-0.37	Sep.	p30	39 *
			-0.37	Sep.	d150	39 *
	1996	C2, C1, CC (IC)	0.68	Jul.	N10	38 ***
	C1,	C2, S, W, CC (IF)	0.66	Jul.	N_0	39 ***
			0.62	Jul.	N150	39 ***
			0.60	Jul.	N70	36 ***
			0.59	Sep.	N_0	39 ***

Table 3.8	Laborator	v measurements	with the highest	t correlation to N	l leaching loss
10010 0.0	Eaborator	11100001011101110	man and mgride		ricating rece

Abbreviations: C1- 1st year corn, C2 - 2nd year corn, CC - continuous corn, S - soybean,
 W - wheat, IC - integreated compost, IF - integrated fertilizer.

‡ If there are more than 5 significant correlations, the 5 highest are shown.

measurements would be expected to reflect the supply of inorganic N from fertilizers as well as from mineralization.

DISCUSSION:

In the first four years of the LFL experiment we have seen a gradual decrease in field N mineralization (Nmin*) in the Fertilizer treatment, which has been accompanied by increased fertilizer N requirements and decreased corn yields. Although the drought conditions in 1996 exaggerated this apparent decline, inorganic N levels early in the experiment may have been supplemented by the decomposition of root material from an 8-year stand of grassy alfalfa plowed down in October 1992.

Although twice as much total N was added to the Compost plots as the Fertilizer plots between 1993 and 1996, these additions were not sufficient to overcome the lower N status of the Compost treatments. The combination of compost and red clover has provided sufficient N for 1st year corn production, but there has been insufficient N to maintain high yields in 2nd year and continuous corn. The use of compost rather than inorganic fertilizers has resulted in lower leaching loss and greater production per unit leached, but it is difficult to determine whether the same results could have been obtained by simply reducing the amount of fertilizer applied in the Fertilizer system. Because the compost used at the LFL is a waste product of nearby dairy and leaf removal facilities, its cost is relatively low. An economic analysis performed by Jones (1996) found that that the economic return was greater for the compost system than the fertilizer system because of the lower cost of inputs. The same study found that there was no economic advantage in choosing the 4-year rotation, with or without cover crops, over the corn monoculture in the first 3 years

of the experiment. This conclusion may change if the productivity of corn in the monoculture continues to decline relative to corn in the rotation.

We can gain a reasonable estimate of inorganic N availability to each crop by adding the estimated N mineralization (Nmin*) to the amount of fertilizer N applied. This analysis confirms that N availability was nearly as high for 1st year corn with compost and cover (245 kg N ha⁻¹ yr⁻¹) as for fertilized corn (272, 288 and 245 kg N ha⁻¹ yr⁻¹ for 1st year, 2nd year, and continuous corn, respectively), but was much lower for 2nd year and continuous corn in the Compost treatment (145 and 166 kg N ha⁻¹ yr⁻¹ respectively). There was a good correlation between this availability index and corn yield in 1994 and 1995, but not in 1996 (Figure 3.1), and not for soybean or wheat (not shown). This supports the conclusion that corn yields have tended to be N limited, particularly in the Compost treatment.



Figure 3.1 Corn yield as a function of the supply of inorganic N from mineralization (Nmin*) and fertilization

The pre-sidedress nitrate test (PSNT) for corn uses the concentration of soil nitrate under V4-V6 stage corn to determine the additional fertilizer N required to achieve a given yield goal. It is recognized as an effective method of reducing overfertilization (Musser et al., 1995), and N-leaching (Durieu et al., 1996), in humid and sub-humid agricultural systems. One reason that PSNT is so effective is that late spring soil NO₃-N levels reflect both the residual inorganic N in the soil and the rate of early spring N mineralization. From this perspective, PSNT represents one of the few instances where an actual measurement of mineralization potential (albeit an indirect one) is used to make fertilizer recommendations (Keeney, 1982).

In this study there was no relationship between early June NO₃-N levels and estimated N mineralization in years with corn, or between PSNT-N and the yield of corn in the Compost treatment. It would appear that the factors that determine spring NO₃-N levels are too complex (or too variable) for this to be an adequate index of N mineralization.

The installation of leaching lysimeters at the LFL allowed us to measure the loss of soil NO₃-N directly, but similar measurements are rarely available in cropping system experiments. Many authors assume that leaching loss is closely related to the soil NO₃-N level in the late fall (e.g. Brown et al. (1993), Solberg, 1995, Philips and Stopes, 1995), which is sometimes referred to as nitrate leaching potential.

We found that there was no correlation between fall NO₃-N levels and leaching loss in three of the four years examined (Figure 3.2). Soil NO₃-N levels remained



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Figure 3.2 Late Fall whole profile NO₃-N as a predictor of leaching in the following field season.

relatively low at the end of the 1993, 1994, and 1995 field seasons, and leaching loss in the following years (1994-1996) was more closely related to N mineralization than inorganic N content. However, reduced crop N uptake and delayed N mineralization caused by the 1996 drought dramatically increased fall inorganic N levels and produced high leaching losses in 1997. Much of the observed variation in leaching loss in 1994-1996 may be an artifact of preferential flow patterns in the soil above the lysimeters. We have found much greater variation in leachate volume than in nitrate concentration between replicate lysimeters.

Limitations in the Analysis

Although the idea of using a correlation analysis to link laboratory measurements to field data is sound, its application in this study was hampered by the fact that not all treatments were sampled at each sample date, and by the influence of specific treatments on individual measurements, both in the laboratory and in the field. It would be more appropriate to use this technique to compare a larger number of treatments or a larger number of replicate data points within a smaller range of treatments. These limitations notwithstanding, it is clear from our analysis that laboratory measurements of NMP are correlated to N mineralization and leaching loss in the field, although the highest correlations to leaching loss are achieved by mid-season measurements that include both soil inorganic N content and NMP.

While the simplified formula for N mineralization was successful in demonstrating differences between treatments and years, our failure to measure Ngas, Nfixed, and the root component of Nres may have reduced the accuracy and integrity of the

analysis. The failure to measure Ngas probably resulted in N mineralization rates that are underestimated by 5-25% in all treatments – about the same as leaching loss. Gaseous losses should be greater in the Fertilizer treatment than the Compost treatment because of the generally higher soils NO₃-N content and the use of urea and ammonium nitrate in wheat and corn respectively. Accurate measurements of soil N would allow this loss to be calculated by difference in treatments without legumes or where inputs by N_2 fixation are also measured.

Indirect evidence that gaseous loss is no greater than leaching loss comes from a 15N fertilizer study performed on site in 1995-1996. At harvest in 1996, Willson et al., (1996) were able to account for 65% of the sidedress fertilizer N applied in 1995 in the top 20 cm of soil, in plant residues, and in the harvested components from 1995 and 1996. This total loss of 45% over 18 months (including loss to soil below 20 cm) compares to an average annual leaching rate of 22% of available N for the same treatments (fertilizer 1st year corn in 1995 and fertilizer 2nd year corn in 1996) in this study.

The generally lower NO₃-N content of soils in the Compost treatments may have increased the root to shoot ratio for all crops, and increased %Ndfa for soybean and red clover, relative to the Fertilizer treatments. Lower gaseous loss and greater %NDFA would decrease our calculation of Nmin* for the Compost treatments, while increased root to shoot ratio would have the opposite effect.

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SUMMARY

In these course textured soils potential and actual N mineralization are closely linked to the supply of organic materials from above and below ground plant residues. Nitrogen mineralization potential (NMP) was greatest following residue incorporation in the spring and fall and lowest at peak plant biomass (late summer). It significantly increased in systems with untilled fallow including the wheat-clover, wheat-fallow, and historically tilled succession (HTS) treatments. Nitrogen budget analysis suggests that much of the difference in N mineralization over the course of the corn-corn-soybean-wheat rotation at the LFL can be explained by differences in the return of residue-N from the previous crop.

The use of external organic inputs in the form of composted leaves and dairy manure increased mineralization (NMP and Nmin*) at the LFL. Application rates were sufficient to provide high productivity in 1st year corn when combined with inorganic N from the decomposition of red clover following wheat, but insufficient to produce the same productivity in 2nd year corn, continuous corn or wheat as was found in the fertilized system. Compost appears to have provided only about 50 kg N ha⁻¹ yr⁻¹ between 1994 and 1996, consistent with a mineralization rate of approximately 9% per year.

Tillage affects these systems directly by incorporating residues and disrupting soil aggregates and indirectly by altering the plant community. The incorporation of residues resulted in a temporary reduction in the C:N ratio of microbial biomass and

an increase in macroorganic matter and NMP. In unmanaged systems, the lack of tillage promotes the growth of perennial vegetation that appears to favor the storage of N below ground. In tilled systems, the longer period between tillage events in the wheat-fallow and wheat-clover systems promotes the robust growth of winter cover species and therefore greater supply of NMP within the system and a greater supply of N to the following corn crop. The lack of spring tillage in wheat increases the temporal continuity of the rhizosphere and reduces the disruption of soil aggregates, which may increase the storage of labile organic N in the soil.

Our attempts to find practical indicators of N mineralization in the field have met with mixed success. In general, mineralization in 70 and 150 day aerobic incubations provide the best indication of mineralization potential and are similar to the average of 10, 30, 70, and 150 day incubations used in the ANOVA in chapter one. However these measurements are time consuming and may be impractical in many cases. As defined in this paper, measurements of macroorganic matter are quick and easy and provide more information about NMP than do total C and N or microbial biomass. However, macroorganic matter measurements may underestimate the contribution of recently incorporated residues, particularly legume residues, which may release large amounts of N that does not contribute directly to macroorganic matter. Macroorganic matter measurements could be used to estimate N mineralization for the purposes of making fertilizer recommendations if they are combined with information about the productivity of the previous crop or cover crop. Additional testing will be required to determine whether macroorganic

matter provides a useful index of N mineralization potential across soils and indifferent climatic conditions.

APPENDIX

The following tables provide supplementary references for the nitrogen fixation estimates in Chapter 3.

Table 2a	Litterature values for %	N from atmosphere	(%Nfa) for soybean			
Crop	Soil NO3-N	Fertilizer N	Nfa	Treatment	Varieties	Source
	•	kg ha ¹	%			
Soybean	0.1 g kg ¹ 0.1 g kg	9 120	32 21	320 m elevation		George and Singleton, 1992
	0.01 g kg ¹	6	69	1050 m elevation		
	0.01 g kg	120	4			
	0.01 g kg ¹	006	13			
Soybean	Proposed world average	ð	52			Hardarson, 1994
Soybean	No Information	20	11-26	уг. 1	3 varieties	
		100	10-12	×	3 varieties	naruarson and zapata, 1984
		20	52-58	yr. 2	3 varieties	
		100	27-38		3 varieties	
Soybean	>260 kg ha ⁻¹ †	0	5-33			:
	140 kg ha ⁻¹	c	34-40			Herridge et al, 1990
	70 kg ha	00	33-65			
Soybean	"High fertility"		8	1997		
	"High fertility"		44	1998		
	"High fertility"		56	Corn residue added		
Soybean	13 kg ha ⁻¹ †		11	Recker Insmu soud		
	110 kg ha ⁻¹		59	St. Paul, sitt loam	u matunty group (averane)	Neuhausen et al., 1988
	13 kg ha'' 110 kg ha''		71	Becker, loamy sand	00 maturity group	
			52	St. Paul, silt loam	(average)	
Soybean	0.5 g kg ¹	20	81	control		
	0.5 g kg ⁻¹	06	83	in 0.8 m outdoor enclosure		Ursu-budu et al 1995
Soybean	No Information	10	17 - 35	vr 1	6 voriation	
		10	32 -48	yr 2	5 varieties	Vasias et al. 1990
Soybean	No Information	10	51-66	Neverth citt loom		
		10	62-89	Gennetown Sandu Loom		Vasilas et al, 1995
T based on 1.2	m depth			COGROMATIN COINT FOOTIN		

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Table 2b	Litterature values for	or %N from atmosp	ohere (%Nfa) for red	clover	
Crop	Soil Properties	% Nfa	Treatment	Comment	Source
		%			
Red Clover	Silt loarn	96-98	"N depleted"	4 varieties	Fahmam and George, 1993
Red Clover	Silt loam	38 70-85	yr. 1 yr. 2-4	Mixed with reed canarygrass	Heichel and Henjum, 1991
Red Clover	Silt loam	65 35-80	yr. 1 yr. 2-4		Heichel et al, 1985
Red Clover	2.6% C, pH 5.7	70-83 89-91	yr. 1 yr. 2	Mixed with tall fescue	Mallarino et al, 1990
Red Clover	pH 7.5 pH 6.3	86 43 86 83 85	yr. 1, Faribanks yr. 2 yr. 1, Delta yr. 2		Sparrow et al, 1995
Red Clover		75	0.3 m outdoor enc	losure	Warembourg et al., 1997

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