International Journal of Agronomy

N Mineralization in Production Agriculture

Guest Editors: H. Allen Torbert, Dexter B. Watts, and Mark Reiter

N Mineralization in Production Agriculture

International Journal of Agronomy

N Mineralization in Production Agriculture

Guest Editors: H. Allen Torbert, Dexter B. Watts, and Mark Reiter

Editorial Board

Contents

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 989365, 2 pages
doi:10.1155/2012/989365

Editorial

N Mineralization in Production Agriculture

H. Allen Torbert and Dexter B. Watts

National Soil Dynamics Laboratory, USDA-ARS, 411 South Donahue Drive, Auburn, AL 36832, USA

Correspondence should be addressed to H. Allen Torbert, allen.torbert@ars.usda.gov

Received 9 July 2012; Accepted 9 July 2012

1. Introduction

Nitrogen (N) plays a key role in the synthesis of aminoacids, proteins, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) needed to promote and sustain plant growth and is, therefore, the single most important nutrient needed for agricultural production. However, N availability is often limited or exists in forms that are unavailable for plant uptake. Within the soil-plant root zone, N continuously cycles among various organic (unavailable) and inorganic (available) forms. Thus, careful management is crucial to supply sufficient plant-available N from the continuous cycle for sustainable agricultural production.

Understanding the effects of N management (and how it relates to the N cycle) in soil ecosystems is essential to determining N availability. This special issue includes a broad range of articles addressing the impact of N mineralization from animal manures, soil organic matter (SOM) under elevated CO_2, tannings in forest and pasture soil, and horticultural potting media.

Understanding the dynamics of soil N mineralization is essential when developing N management practices for crop and forage production. While much research has been conducted on N mineralization, much more work is needed to understand the dynamics of the N cycle. As new agronomic practices are developed, new studies are needed to determine the impact on N fertilization. For example, due to increasing costs of traditional potting substrates, horticultural growers are expressing interest in alternative, lower-cost substrates. In this special issue, a study undertaken to determine the extent of N immobilization in the high wood-fiber content substrate—clean chip residual—is presented. Likewise, interest in bioenergy production has led to the development of cropping systems where cover crops are being harvested for biomass, but the impact on N mineralization is unknown. In another paper in this special issue, research on N a management in cotton (*Gossypium hirsutum* L.) is examined as impacted by cover crop harvest.

The advent of new crops and management systems into new areas also brings uncertainty into the N management. For example, Potatoes (*Solanum tuberosum*) are an important high-value commodity for producers in the Mid-Atlantic Region, but current production recommendations were based on white potatoes and practices for Russet potatoes have not been researched. A study presented in this issue examines the potential impact on N management for growing Russet potatoes in this region.

Improvements in scientific knowledge regarding soil N cycling have the potential to help develop improved N fertilizer management. For example, tannins are reactive secondary metabolites produced by plants and are believed to affect the N cycle through several direct and indirect mechanisms that reduce rates of net N mineralization or nitrification. A study on the impact of tannins and related phenolic compounds on the solubility of soil N and resulting management of N availability and retention in agricultural soils is presented.

Recent increases in commercial N fertilizer prices and high demand for organic production have generated renewed interest in animal manure as a nutrient source. Manure nutrients, as well as legumes, have been used to increase soil fertility since the beginning of agricultural production. However, use of animal manures depends on N mineralization process to provide plant-available N. Research is needed to understand the long-term impact of animal manure application and the impact on the soil environment. In this special issue, a study on N uptake by sugarbeet (*Beta vulgaris* L.) in years following manure application is presented.

Recent changes in the animal production systems and manure handling after it has been collected have led to uncertainty as to how N mineralization from animal manures may be impacted. In this special issue, studies addressing the potential impact on N mineralization from changes in the animal manure management are presented. For example, a study to examine the potential changes in N mineralization due to storage with and without animal urine is presented. In another paper, a study was conducted to determine how raw dairy slurry and anaerobically digested slurry applied to reed canary grass (*Phalaris arundinacea*) affected forage production. Also, recent poultry litter management practices have resulted in changes to the composition of the litter (cake versus total cleanout). At the same time, the use of chemical additives has potentially changed the N mineralization potential of the litter. A study to examine these potential changes on N mineralization is presented in this special issue.

In addition, increasing global atmospheric carbon dioxide (CO_2) concentration has led to concerns regarding its potential effects on terrestrial ecosystems and the long-term storage of C and N in soil. Studies are needed to understand how changes to plant inputs due to elevated CO_2 may impact soil N mineralization. In this special issue, research is presented that examines the responses to elevated CO_2 in a grass ecosystem invaded with a leguminous shrub *Acacia farnesiana* (L.) Willd (Huisache) on soil N and C dynamics. Also, a paper is presented which examines the impact of elevated atmospheric CO_2 concentration on the effects of terrestrial C and N dynamics, including CO_2 release back to the atmosphere and soil C sequestration.

2. Conclusion

Nitrogen mineralization varies across climates, regions, landscapes, and soil management depending on many factors. The increased research knowledge obtained in these studies will provide valuable information for improving management practices which will, in turn, increase the available supply of N to the soil-plant system. This special issue includes 11 new studies on N mineralization in production agriculture, but much work yet remains undone. As world population continues to increase, ongoing research on N mineralization will be needed to improve soil productivity and N supply to crops where industrially produced commercial fertilizer is not the sole nutrient source.

H. Allen Torbert
Dexter B. Watts

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 348754, 7 pages
doi:10.1155/2012/348754

Research Article

Nitrogen Fertilizer and Growth Regulator Impacts on Tuber Deformity, Rot, and Yield for Russet Potatoes

Mark S. Reiter,[1] Steven L. Rideout,[2] and Joshua H. Freeman[3]

[1] *Department of Crop and Soil Environmental Sciences, Eastern Shore Agricultural Research and Extension Center, Virginia Tech, Painter, VA 23420, USA*
[2] *Department of Plant Pathology, Physiology and Weed Science, Eastern Shore Agricultural Research and Extension Center, Virginia Tech, Painter, VA 23420, USA*
[3] *Department of Horticulture, Eastern Shore Agricultural Research and Extension Center, Virginia Tech, Painter, VA 23420, USA*

Correspondence should be addressed to Mark S. Reiter, mreiter@vt.edu

Received 8 January 2012; Revised 10 April 2012; Accepted 3 May 2012

Academic Editor: H. Allen Torbert

Potatoes (*Solanum tuberosum*) are an important high-value commodity for producers in the Mid-Atlantic Region. Current production recommendations were based on white potatoes, and practices for Russet potatoes have not been researched in this region. The objective of this study was to test impacts of N rate (0, 67, 134, 201, and 268 kg N ha^{-1}), N application timing (100% applied with planter, 2-way split (30% with planter and 70% band applied approximately 30 days after planting at dragoff), and three-way split (30% with planter, 50% band applied prior to drag-off, and 20% band applied at first sight of bloom)), and additions of the growth regulator maleic hydrazide (MH-30). We tested "Goldrush" and "Norkotah" Russet potato varieties on marketability, total yield, tuber deformity, and tuber soft rot incidence for sandy loam soils in the Mid-Atlantic. Overall, year variations were significant with substantial rots (up to 86.5%) occurring in year 3. Maleic hydrazide and N application timing had little consistent effect on any tested parameter. Nitrogen rate and variety factors had the greatest impacts on deformity, tuber rots, and yields for Russet potatoes in the Mid-Atlantic Region with 134 kg N ha^{-1} producing the highest total yields in 2009 and 2010. If tuber rots can be controlled, both "Goldrush" and "Norkotah" are acceptable varieties under the Mid-Atlantic production practices.

1. Introduction

Potatoes are an important crop to Virginia and the rest of the Mid-Atlantic Region that includes Delaware and Maryland, USA. Annually, the Mid-Atlantic states produce 4049 hectares (ha) of potatoes with an average yield of 30,091 kg tubers ha^{-1} worth $9.97 million (5 year averages) [1]. Sandy loam soils in the Mid-Atlantic Region are favorable for potato production. However, a close proximity to sensitive water bodies, such as the Chesapeake Bay, means that fertilizer use efficiency and reduction of nutrient losses from production fields are more important than ever before.

Intensive fertilizer management is necessary in sandy loam soils in the Mid-Atlantic to ensure proper nutrient supplies to growing crops. Sandy loam soils generally have overall low organic matter, low cation exchange capacities, and low total nitrogen (N) in the upper horizon, which means that little N is mineralized from soil organic N sources and N must be applied with fertilizer to match crop uptake needs [2]. For instance, Stanford and Smith [2] found that a Norfolk fine sandy loam had little initial N mineralized after 4 weeks of incubation (7.3 mg kg^{-1}) and this amount gradually fell for the next 30 weeks. Similarly, Van Veen and coworkers [3] found that any organic N applied to sandy loam soils was quickly mineralized into inorganic forms; therefore, soil supplies of N from crop to crop would be minimal in sandy loam crop production systems. Generally, only 1 to 3% of total organic N concentrations in the soil become available to a crop within the growing season in temperate regions of the world [4].

Nitrogen management is one of the most important aspects for potato production. Nitrogen fertilizer recommendations vary widely around the world. In loamy sand soils, Jamaati-E-Somarin and coworkers [5] found that

160 kg N ha^{-1} was sufficient for producing highest yields. Worthington et al. [6] found that 196 to 224 kg N ha^{-1} was necessary for the highest yields in Florida fine sands, with sidedress N applications being important for supplying necessary N fertilizer after leaching rain events. Nitrogen fertilizer recommendations for white potatoes in Virginia vary based on yield potential. For instance, producers yielding approximately 22,000 kg tubers ha^{-1} need 140 to 168 kg N ha^{-1} while higher producing yield goals require more N. For high yielding systems; growers are recommended to multiply yield goals in kg ha^{-1} by 0.006 to find recommended kg N ha^{-1} necessary [7]. Currently, producers in Virginia do not have separate guidelines for Russet potato varieties even though Russet varieties are more prone to deformity and other quality issues than white potatoes. Tuber deformity and secondary growth are historically correlated with moisture; however, other environmental factors that initiate and decrease growth, such as N fertility, may also be a cause [8].

Nitrogen application timing is one of the most important management techniques that producers can use to increase their fertilizer N use efficiency. For sandy loam and loamy sand soils, Virginia Cooperative Extension recommends two or three N fertilizer application splits to reduce potential N losses due to over irrigation or excessive rainfall. Fertilizer should be split between at planting, at dragoff (approximately 30 days after planting when potato plants are beginning to emerge, are bedded during cultivation, and the bed height is reduced), and immediately prior to bloom [7, 9]. Split N applications are recommended to increase overall yield and fertilizer use efficiency [10, 11]. However, N applications too late in the growing season can significantly delay maturity and decrease tuber quality [12].

Nutrient availability is not only important for overall yield in potato production, but is also important for disease management. Mackenzie [13] studied the relationship between N rate and potato yield in association with potato early blight in silt clay loam soils of Pennsylvania. Mackenzie [13] demonstrated that increasing N rates from 133 to 160 kg N ha^{-1} decreased overall rates of potato early blight infection. Research with potassium fertilizer also demonstrated that fertilizer rate and source impacted disease incidence. Panique and coworkers [14] found that potassium sulfate increased overall yields, but potassium chloride fertilizers decreased *Rhizoctonia solani* incidence. Therefore, fertilizer applications can significantly impact both foliar and tuber disease; however, the role of N fertilizer management in the Mid-Atlantic on tuber rots such as *Erwinia carotovora* ss. *carotovora and Pythium* sp. is not known.

Growth regulators have been researched for decades to help producers manage tuber sugar content, maturity, and sprouting after harvest and during storage [15, 16]. However, results are mixed depending on the factors studied. For instance, Yada et al. [17] found that foliar application of maleic hydrazide (MH-30) at a rate of 3.39 kg ha^{-1} had no significant effect on potato yield or sugar content, but did reduce sprout growth after harvest. Other research by Rex [18] found that foliar applications of chlormequat chloride, ethephon, and a combination of the two products reduced tuber size, increased tuber deformity, and reduced overall yield. Work by Caldiz and coworkers [19] found that MH-30 was safe to use on potato foliage did not cause any phytotoxicity symptoms, and did increase yield in several varieties. However, no yield impact was found in any variety, but in all cases tuber sprouting was reduced. An analysis by Davis and Groskopp [20] found that overall tuber yield was reduced from MH-30 treatments; however, this reduction was mainly from lower numbers of undersized tubers. Overall, growth regulators have demonstrated positive effects on yield and sprouting, but impacts on tuber rot is not known.

Previous studies did not focus on Russet potatoes and most producers in the Mid-Atlantic currently use recommendations for white potatoes. The objectives of this study included finding: (1) impacts of N rate and N application timing and (2) impact of maleic hydrazide growth regulator on tuber yield, deformity, and rot on sandy loam soils in the Mid-Atlantic for Russet potatoes.

2. Materials and Methods

The trial was conducted on a Bojac sandy loam soil (Coarse-loamy, mixed, semiactive, and thermic Typic Hapludult) (65% sand, 25% silt, and 10% clay; 0.75% organic matter) at Virginia Tech's Eastern Shore Agricultural Research and Extension Center near Painter, VA, USA (37.5845808N, −75.8210421W) [21]. Prior to treatment establishment, soil was sampled per replication to a 15 cm depth, dried, ground, and analyzed using the Dumas method for total N and total carbon (C) analysis using a Vario EL cube (elementar Americas, Mt. Laurel, NJ, USA) [22]. Treatments included a 2 × 4 × 3 factorial combination of treatments of variety × N rate × N application timing. Ammonium nitrate (340 g N kg^{-1}) was applied with total N rates of 67, 134, 201, and 268 kg N ha^{-1} at various application timing intervals. Nitrogen treatments were applied either in a single treatment (100% applied with planter), 2-way split (30% with planter and 70% band applied prior to dragoff (approximately 30 days after planting when potato plants are beginning to emerge and are bedded during cultivation, and the bed height is reduced)), or with the high yielding white potato timing methodology of a three-way split (30% with planter, 50% band applied prior to dragoff, and 20% band applied at first sight of bloom). A 0-N control was also included. At-planting fertilizer treatments were spread evenly across the treatment area and incorporated using a field cultivator; dragoff treatments were surface applied and incorporated with bed shaping, while early bloom treatments were surface band applied. "Goldrush" and "Norkotah" Russet cultivars were seeded into conventionally-tilled land in early April following incorporation of fertility treatments. The growth regulator, maleic hydrazide, was applied at the manufacturer's recommended rate of 3.36 kg active ingredient (ai) ha^{-1} (MH-30; 0.18 kg ai L^{-1}). The MH-30 was broadcast foliar-applied using a CO_2-pressurized sprayer calibrated to deliver 280 L ha^{-1} at three weeks past full bloom in late June. The MH-30 was applied to both cultivars and plots that had

combinations of all three N application timing treatments, and total N rates of 0, 134, and 268 kg N ha^{-1}. Comparable plots with identical variety, N rate, and N application timing treatments were maintained with and without MH-30 treatments, so direct comparisons of MH-30 impacts could be observed. Plots were two 7.62 m rows spaced 0.9 m apart and separated by a guard row, which did not receive any N fertilizer or growth regulator treatments. Treatments were replicated four times in a randomized complete block design. During the growing season, recommended practices for disease, weed, and insect control were followed as outlined by Wilson and coworkers [9]. The second row of each plot was mechanically harvested upon maturity in late July. Harvested tubers were sorted in the following categories: deformed (misshapen tubers), rotten, marketable (total of all tubers not rotten = misshapen + size B + size A + chef), and total (marketable + rotten tubers). Means were separated using Fisher's protected least significant difference (LSD) test at $\alpha = 0.10$ that was established *a priori*. The 0-N control plots were not included in the factorial combination PROC ANOVA analysis as only 1 control plot per replication was included per variety; therefore, the design was not balanced for all combinations of treatments if 0-N treatments were included.

3. Results and Discussion

Total N attributed to potato plants from sandy loam soil organic matter mineralization is expected to be low in the Mid-Atlantic due to relatively low total C concentrations (Table 1). Ambient soil total N concentrations ranged from 0.42 to 0.49 g N kg^{-1} (Table 1), which would equate to 864 to 1,008 kg total soil ha^{-1} at an average bulk density of 1.35 g kg^{-1} (2,057,400 kg soil ha^{-1} at 15.24 cm depth) for a Bojac sandy loam on the Eastern Shore of Virginia [23]. Due to these low soil total N concentrations, we would expect only 26 to 30 kg N ha^{-1} to be available to potato tubers in temperate conditions from ambient soil sources via mineralization [4]. Therefore, substantial inorganic N fertilizer sources are necessary for optimal production and are the overall focus of a potato grower's fertilizer program.

In each year, *Erwinia carotovora* ss. *carotovora* and *Pythium* sp. occurred naturally in these trials causing tuber rots in the field. Data were analyzed as a year × treatment effect to test differences of treatments across years. In all cases, year × treatment was significant ($P = 0.0063$, <0.0001, <0.0001, and <0.0001 for percentage of deformed tubers, total yield, marketable yield, and percentage of rots, resp.). Large weather variations are likely responsible for varying yearly effects of N fertilizer, variety, and growth regulator treatments. Total yields were the highest in 2010, followed by 2009, and the lowest in 2011, which was inversely proportion to rots (2011 > 2009 > 2010). Therefore, each year will be discussed separately.

3.1. 2009 Growing Season. Maleic hydrazide had no significant effect on deformed tubers (55.8% of nonrotten tubers), rotten tubers (34.3% of total yield), marketable yield (13517 kg ha^{-1}), or total yield (20040 kg ha^{-1}) ($P = 0.6380$, 0.8236, 0.3860, and 0.2909, resp.) when compared to plots with identical N application timing and total N rate treatments that did not receive MH-30. These yield results are similar to work by Yada et al. [17] and Caldiz and coworkers [19] where no yield advantage was seen on several Russet potato varieties when MH-30 was used.

Table 1: Soil total nitrogen, carbon, and C : N ratio for a sandy loam soil in the Mid-Atlantic by year.

Year	Total nitrogen	Total carbon	C : N ratio
	g kg^{-1}		g C g N^{-1}
2009	0.49 a†	5.26 a	10.72 b
2010	0.46 ab	4.88 a	10.52 b
2011	0.42 b	4.87 a	11.47 a
$LSD_{0.10}$	0.04	0.54	0.40
Pr > F	0.0716	0.3500	0.0081

†Means within each dependent variable with the same letter are not significantly different ($P \geq 0.10$; Fisher's Protected LSD) and can be compared within column.

Generally, only the N rate and cultivar main effects were significant in 2009. When averaged across N application timing and cultivar, the percentage of deformed tubers and total yield increased as N rate increased (Table 2). The 201 kg ha^{-1} N rate had more deformed tubers than the lower N rate of 67 kg N ha^{-1} (58.3 versus 45.8% of nonrotten tubers, resp.; $LSD_{0.10}$ = 9.5%). For total yield, at least 134 kg N ha^{-1} was necessary to achieve commercially acceptable yields (19267 kg ha^{-1}), which is similar to N rates currently recommended in Virginia for white potato production [7]. However, the Virginia agronomic efficiency is significantly lower than efficiencies for Russet potatoes grown in Oregon and Washington states. Lauer [24] found that Russet Burbank returned 223 kg of tubers per kg N fertilizer applied, while yields in our study returned 144 kg tubers per kg N fertilizer. For the N rate main effect, marketable yield was not significant and averaged 13061 kg tubers ha^{-1} ($P = 0.4200$) (Table 2). For the cultivar main effect, "Goldrush" had significantly higher yields than "Norkotah" albeit higher percentage of deformed tubers, averaged across N rate and N application timing (Table 3). An N rate × N application timing × variety cultivar interaction was significant for rotten tubers ($P = 0.0399$, Table 4). Wide variation was seen in this experiment regarding tuber rots, which resulted in a relatively large $LSD_{0.10}$ (15.9% rotten tubers as a percentage of total yield). Generally, treatments that one would expect to have higher N use efficiency (more N splits) and higher N rates had more tuber rots. For example, "Norkotah" 268 kg N ha^{-1} with 3-splits (41.9% rots) compared to "Norkotah" 67 kg N ha^{-1} with all N applied at planting (24.5% rots) or "Norkotah" 0-N fertilizer treatments (21.8% tuber rots) (Table 4).

3.2. 2010 Growing Season. Maleic hydrazide had no impact on deformity (16.8% deformed tubers as a percentage of nonrotten tubers) or rot (23.4% of total yield) in 2010. An N rate × N application timing × variety × MH-30 interaction in 2010 for total yield was significant ($P = 0.0474$) and is illustrated in Table 5. Generally, "Norkotah" yielded the

Table 2: Nitrogen rate main effect in 2009 for deformed tubers, rotten tubers as a percentage of total yield, marketable tuber yield, and total tuber yield for Russet potatoes grown on sandy loam soils in the Mid-Atlantic, averaged across Russet cultivars and N application timing.

Nitrogen rate	Deformed tubers	Rotten tubers	Marketable yield	Total yield
kg ha^{-1}	Percentage of tubers	Percentage of total yield	kg ha^{-1}	
0[†]	30.0	22.2	12728	15978
67	45.8 b[‡]	33.2 a	12248 a	17360 b
134	54.2 ab	34.0 a	14884 a	21515 a
201	58.3 a	35.5 a	12862 a	19267 ab
268	61.5 a	35.7 a	12248 a	21507 a
$LSD_{0.10}$	9.5	6.5	3053	3208
Pr > F	0.0445	0.8967	0.4200	0.0995

[†] No-fertilizer control plots were not included in Analysis of Variance and are included for informational purposes only.
[‡] Means within each dependent variable with the same letter are not significantly different ($P \geq 0.10$, Fisher's Protected LSD) and can be compared within column.

Table 3: Russet potato main effect in 2009 for deformed tubers, rotten tubers as a percentage of total yield, marketable tuber yield, and total tuber yield for Russet potatoes grown on sandy loam soils in the Mid-Atlantic, averaged across N rate and N application timing.

Variety	Deformed tubers	Rotten tubers	Marketable yield	Total yield
	Percentage of tubers	Percentage of total yield	kg ha^{-1}	
Goldrush	66.7 a[†]	34.5 a	16894 a	24549 a
Norkotah	43.2 b	34.6 a	10343 b	15276 b
$LSD_{0.10}$	6.7	4.6	1928	2025
Pr > F	< 0.0001	0.9726	<0.0001	<0.0001

[†] Means within each dependent variable with the same letter are not significantly different ($P \geq 0.10$, Fisher's Protected LSD) and can be compared within column.

Table 4: Nitrogen rate × N application timing × variety interaction in 2009 for rotten tubers as a percentage of total yield for Russet potatoes grown on sandy loam soils in the Mid-Atlantic.

Nitrogen rate	Goldrush			Norkotah		
	All at planting	2-splits	3-splits	All at planting	2-splits	3-splits
kg ha^{-1}	% of total yield					
0[†]		11.8			21.8	
67	47.2 ab[‡]	29.3 cdef	34.6 bcdef	24.5 ef	29.6 cdef	33.7 bcdef
134	31.2 cdef	31.9 bcdef	35.4 abcdef	35.3 abcdef	26.7 def	43.4 abc
201	23.2 f	50.8 a	33.0 bcdef	39.8 abcde	29.7 cdef	36.6 abcdef
268	36.4 abcdef	26.3 def	35.2 abcdef	31.6 bcdef	42.9 abc	41.9 abcd
$LSD_{0.10}$	15.9					
Pr > F	0.0399					

[†] No-fertilizer control plots were not included in Analysis of Variance and are included for informational purposes only.
[‡] Means with the same letter are not significantly different ($P \geq 0.10$, Fisher's Protected LSD) and any mean can be compared within the table.

Table 5: Nitrogen rate × N application timing × variety × MH-30 interaction in 2010 for marketable yield for Russet potatoes grown on sandy loam soils in the Mid-Atlantic.

Nitrogen rate and MH-30	Goldrush			Norkotah		
	All at planting	2-splits	3-splits	All at planting	2-splits	3-splits
kg ha^{-1}	kg ha^{-1}					
134 no MH-30	9148 j[†]	13325 hij	19556 efgh	28348 abcd	32261 abc	29262 abcd
134 with MH-30	17187 fghi	17035 ghi	12237 hij	28337 abcd	32789 ab	31356 abc
268 no MH-30	24800 cdef	22615 defg	22513 defg	28601 abcd	27707 abcd	28277 abcd
268 with MH-30	17696 fghi	10815 ij	26833 cde	27239 abcde	34883 a	32250 abc
$LSD_{0.10}$	7724					
Pr > F	0.0474					

[†] Means with the same letter are not significantly different ($P \geq 0.10$, Fisher's Protected LSD) and any mean can be compared within the table.

Table 6: Nitrogen rate × N application timing × MH-30 interaction in 2010 for total yield for Russet potatoes grown on sandy loam soils in the Mid-Atlantic.

Nitrogen rate	No MH-30			MH-30		
	All at planting	2-splits	3-splits	All at planting	2-splits	3-splits
kg ha^{-1}	kg ha^{-1}					
134	15185 c[†]	19144 bc	19378 bc	19200 bc	20211 abc	17756 c
268	24216 ab	20719 abc	20231 abc	18241 c	18204 c	25256 a
$LSD_{0.10}$	5766					
Pr > F	0.0588					

[†] Means with the same letter are not significantly different ($P \geq 0.10$, Fisher's Protected LSD) and any mean can be compared within the table.

Table 7: Nitrogen rate × variety interaction in 2010 for rotten tubers as a percentage of total yield, marketable tuber yield, and total tuber yield for Russet potatoes grown on sandy loam soils in the Mid-Atlantic, averaged across N application timing.

Nitrogen rate	Rotten tubers		Marketable yield		Total yield	
	Goldrush	Norkotah	Goldrush	Norkotah	Goldrush	Norkotah
kg ha^{-1}	Percentage of total yield		kg ha^{-1}		kg ha^{-1}	
0[†]	18.8	14.0	8507	7735	9839	8792
67	43.0 a[‡]	12.2 bc	9239 c	21266 b	13593 d	23777 bc
134	48.2 a	8.6 c	8396 c	27409 a	14009 d	29957 a
201	21.7 b	11.6 bc	21100 b	26972 a	26121 abc	30360 a
268	20.4 b	13.2 bc	19000 b	2444 c	23309 c	28195 ab
$LSD_{0.10}$	10.6		4744		4812	
Pr > F	0.0009		0.0033		0.0184	

[†] No-fertilizer control plots were not included in Analysis of Variance and are included for informational purposes only.
[‡] Means within each dependent variable with the same letter are not significantly different ($P \geq 0.10$, Fisher's Protected LSD).

Table 8: Growth regulator (MH-30) × variety interaction in 2011 for rotten tubers as a percentage of total yield and marketable tuber yield for Russet potatoes grown on sandy loam soils in the Mid-Atlantic, averaged across Nrates and N application timing.

Russet cultivar	Rotten tubers		Marketable yield	
	With MH-30	No MH-30	With MH-30	No MH-30
	Percentage of total yield		kg ha^{-1}	
Goldrush	52.3 b[†]	39.2 c	7157 b	9423 a
Norkotah	86.4 a	86.5 a	2815 c	2573 c
$LSD_{0.10}$	6.5		1705	
Pr > F	0.0177		0.0873	

[†] Means within each dependent variable with the same letter are not significantly different ($P \geq 0.10$, Fisher's Protected LSD).

Table 9: Nitrogen application timing × variety interaction in 2011 for rotten tubers as a percentage of total yield, marketable tuber yield, and total tuber yield for Russet potatoes grown on sandy loam soils in the Mid-Atlantic, averaged across N rates.

Nitrogen application timing	Rotten tubers		Marketable yield		Total yield	
	Goldrush	Norkotah	Goldrush	Norkotah	Goldrush	Norkotah
	Percentage of total yield		kg ha^{-1}			
No nitrogen[†]	35.5	82.9	7871	1773	11603	9400
At planting	34.8 c[‡]	85.2 a	7874 b	2811 c	11947 c	15698 ab
2-split	42.6 c	86.4 a	10396 a	2696 c	17354 a	16200 ab
3-split	48.0 bc	79.7 ab	8303 b	3970 c	15729 ab	14284 bc
$LSD_{0.10}$	32.1		1835		2710	
Pr > F	0.0131		0.0823		0.0458	

[†] No-fertilizer control plots were not included in Analysis of Variance and are included for informational purposes only.
[‡] Means within each dependent variable with the same letter are not significantly different ($P \geq 0.10$, Fisher's Protected LSD).

higher than "Goldrush" with only isolated effects of N application timing and MH-30 application. An N rate × N application timing × MH-30 interaction, averaged across variety, was significant for marketable yields in 2010 ($P = 0.0588$) (Table 6). Similar to total yield, significant effects were isolated but at high N rates with MH-30 the 3-split application timing yielded highest.

An N rate × variety interaction was significant in 2010 for rotten tubers, marketable yield, and total yield (Table 7). Overall, "Goldrush" tubers had more rot incidence than "Norkotah" tubers at 67 and 134 kg N ha^{-1}. Tuber rot generally mirrored marketable yield with 134 and 201 kg N ha^{-1} providing the highest marketable yields for "Norkotah," while "Goldrush" tuber yield was lower at similar N rates (Table 7). Compared to white potato recommendations [4], a yield of 29957 kg tuber ha^{-1} would suggest a need of 180 kg N ha^{-1} based on the yield × 0.006 factor for "Norkotah," while only 134 kg N ha^{-1} was necessary in both 2009 and 2010.

3.3. 2011 Growing Season. The 2011 growing season resulted in low yields compared to 2009 and 2010. Generally, yields were only 25 to 50% of yield expectations for potatoes in Virginia. A wet Spring coupled with excessive heat and drought likely contributed to low yields. In 2011, there was an MH-30 × variety interaction for rotten tubers and marketable yield (Table 8). For rotten tubers, "Norkotah" had no impact if MH-30 was included; however, "Goldrush" with MH-30 had more rotten tubers than treatments with no MH-30 (52.3 versus 39.2, resp., $LSD_{0.10} = 6.5$) (Table 8). Marketable yield followed an inversely proportional trend to rotten tubers with "Goldrush" with no MH-30 treatments having the highest marketable yields (9423 kg tubers ha^{-1}) (Table 8). A main effect was significant for deformed tubers with MH-30 treatments having 38.2% of nonrotten tubers being deformed with 27.6% being irregular if no MH-30 was used ($LSD_{0.10} = 6.3\%$), averaged across variety, N rate, and N application timing.

Nitrogen rate generally had no significant impacts on deformity, rot, or yield in 2011. However, N application timing × variety interactions, averaged over N rate, indicated that "Norkotah" tubers rotted twice as much as "Goldrush"; which resulted in significantly higher marketable yields for "Goldrush" (Table 9). A two-way N split (at planting and dragoff) was sufficient for providing the highest marketable and total tuber yields for "Goldrush" (10396 and 17354 kg ha^{-1}, resp.). A 3-split application timing decreased yield for "Goldrush," possibly due to reduced tuber formation and delayed maturity due to excessive N late in season as demonstrated by Ojala and coworkers [12]. Interestingly, total yield indicated that "Norkotah" generally yielded similar to "Goldrush" for total yield, so any management to reduce tuber rots would be beneficial.

4. Conclusions

In conclusion, N rate and variety factors had the greatest impacts on deformity, tuber rots, and yields for Russet potatoes in the Mid-Atlantic Region. Generally, findings indicate that 134 kg N ha^{-1} were adequate for producing the highest yields. If tuber rots can be controlled, both "Goldrush" and "Norkotah" are acceptable varieties under the Mid-Atlantic production practices. Neither maleic hydrazide nor application timing had a consistent impact on tuber rot, deformity, or yield.

Abbreviations

ai: Active ingredient
C: Carbon
ha: Hectare
kg: Kilogram
LSD: Least significant difference
MH-30: Maleic hydrazide
m: Meter
N: Nitrogen.

Acknowledgments

The authors gratefully acknowledge staff stationed at the Virginia Tech Eastern Shore Agricultural Research and Extension Center for assistance with plot establishment, maintenance, and data collection. They also thank the Virginia Irish Potato Board for funding this paper.

References

[1] United States Department of Agriculture—National Agricultural Statistics Service, *Quick Stats. U.S. & All States Data—Crops*, USDA-NASS, Washington, DC, USA, 2012, http://www.nass.usda.gov/Quickstats/.

[2] G. Stanford and S. J. Smith, "Nitrogen mineralization potentials of soils," *Soil Science Society of America Journal*, vol. 36, pp. 465–472, 1972.

[3] J. A. Van Veen, J. N. Ladd, J. K. Martin, and M. Amato, "Turnover of carbon, nitrogen and phosphorus through the microbial biomass in soils incubated with 14-C-, 15N- and 32P-labelled bacterial cells," *Soil Biology and Biochemistry*, vol. 19, no. 5, pp. 559–565, 1987.

[4] J. F. Power and J. W. Doran, "Nitrogen use in organic farming," in *Nitrogen in Crop Production*, R. D. Hauck, Ed., chapter 40, pp. 585–598, American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wis, USA, 1984.

[5] S. Jamaati-E-Somarin, A. Tobeh, K. Hashemimajd, M. Hassanzadeh, M. Saeidi, and R. Zabihi-E-Mahmoodabad, "Effects of nitrogen fertilizer and plant density on N-P-K uptake by potato tuber," *Indian Journal of Horticulture*, vol. 67, pp. 329–333, 2010.

[6] C. M. Worthington, K. M. Portier, J. M. White et al., "Potato (*Solanum tuberosum* L.) yield and internal heat necrosis incidence under controlled-release and soluble nitrogen sources and leaching irrigation events," *American Journal of Potato Research*, vol. 84, no. 5, pp. 403–417, 2007.

[7] M. S. Reiter, S. B. Phillips, J. G. Warren, and R. O. Maguire, *Nitrogen Management for White Potato Production*, Virginia Cooperative Extension and Virginia Agricultural Experiment Station, Blacksburg, Va, USA, 2009.

[8] J. E. Kraus, "Influence of certain factors on second growth on Russet Burbank potatoes," *American Potato Journal*, vol. 22, no. 5, pp. 134–142, 1945.
[9] H. P. Wilson, T. P. Kuhar, S. L. Rideout et al., *Commercial Vegetable Production Recommendations*, Virginia Cooperative Extension and Virginia Agricultural Experiment Station, Blacksburg, Va, USA, 2011.
[10] S. Roberts, W. H. Weaver, and J. P. Phelps, "Effect of rate and time of fertilization on nitrogen and yield of Russet Burbank potatoes under center pivot irrigation," *American Potato Journal*, vol. 59, no. 2, pp. 77–86, 1982.
[11] D. T. Westermann and G. E. Kleinkopf, "Nitrogen requirements of potatoes," *Agronomy Journal*, vol. 77, pp. 616–621, 1985.
[12] J. C. Ojala, J. C. Stark, and G. E. Kleinkopf, "Influence of irrigation and nitrogen management on potato yield and quality," *American Potato Journal*, vol. 67, no. 1, pp. 29–43, 1990.
[13] D. R. Mackenzie, "Association of potato early blight, nitrogen fertilizer rate, and potato yield," *Plant Disease*, vol. 65, pp. 575–577, 1981.
[14] E. Panique, K. A. Kelling, E. E. Schulte, D. E. Hero, W. R. Stevenson, and R. V. James, "Potassium rate and source effects on potato yield, quality, and disease interaction," *American Potato Journal*, vol. 74, no. 6, pp. 379–398, 1997.
[15] P. C. Marth and E. S. Schultz, "A new sprout inhibitor for potato tubers," *American Potato Journal*, vol. 29, no. 11, pp. 268–272, 1952.
[16] S. N. Rao and S. H. Wittwer, "Further investigations on the use of maleic hydrazide as a sprout inhibitor for potatoes," *American Potato Journal*, vol. 32, no. 2, pp. 51–59, 1955.
[17] R. Yada, R. Coffin, M. Keenan, M. Fitts, C. Dufault, and G. Tai, "The effect of maleic hydrazide (potassium salt) on potato yield, sugar content and chip color of kennebec and norchip cultivars," *American Journal of Potato Research*, vol. 68, pp. 705–709, 1991.
[18] B. L. Rex, "Effect of two plant growth regulators on the yield and quality of russet burbank potatoes," *Potato Research*, vol. 35, no. 3, pp. 227–233, 1992.
[19] D. O. Caldiz, L. V. Fernández, and M. H. Inchausti, "Maleic hydrazide effects on tuber yield, sprouting characteristics, and french fry processing quality in various potato (*Solanum tuberosum* L.) Cultivars grown under argentinian conditions," *American Journal of Potato Research*, vol. 78, no. 2, pp. 119–128, 2001.
[20] J. R. Davis and M. D. Groskopp, "Yield and quality of Russet Burbank potato as influenced by interactions of Rhizoctonia, maleic hydrazide, and PCNB," *American Potato Journal*, vol. 58, no. 5, pp. 227–237, 1981.
[21] United States Department of Agriculture—Natural Resources Conservation Servic, *Bojac Series*, USDA-NRCS, Washington, DC, USA, 2012, https://soilseries.sc.egov.usda.gov/OSD_Docs/B/BOJAC.html.
[22] J. M. Bremner, "Nitrogen—total," in *Methods of Soil Analysis: Part 3—Chemical mMethods*, J. M. Bigham, Ed., hapter 37, pp. 1085–1122, Soil Science Society of America, Madison, Wis, USA, 1996.
[23] United States Department of Agriculture—Natural Resources Conservation Service, *Map—Bulk Density, Bojac Series*, United States Department of Agriculture—Natural Resources Conservation Service, Washington, DC, USA, 2012, http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx.
[24] D. A. Lauer, "Russet burbank yield response to sprinkler-applied nitrogen-fertilizer," *American Potato Journal*, vol. 63, pp. 61–69, 1986.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 204629, 8 pages
doi:10.1155/2012/204629

Research Article

Impact of Poultry Litter Cake, Cleanout, and Bedding following Chemical Amendments on Soil C and N Mineralization

Dexter B. Watts,[1] Katy E. Smith,[2] and H. A. Torbert[1]

[1] *National Soil Dynamics Laboratory, USDA-ARS, 411 S. Donahue Drive, Auburn, AL 36832, USA*
[2] *Depatement of Math, Science, and Technology, University of Minnesota-Crookston, 2900 University Avenue, Crookston, MN 56716, USA*

Correspondence should be addressed to Dexter B. Watts, dexter.watts@ars.usda.gov

Received 7 January 2012; Accepted 2 April 2012

Academic Editor: Mark Reiter

Poultry litter is a great alternative N source for crop production. However, recent poultry litter management changes, and increased chemical amendment use may impact its N availability. Thus, research was initiated to evaluate the effect that broiler cake and total cleanout litter amended with chemical additives have on C and N mineralization. A 35-day incubation study was carried out on a Hartsells fine sandy loam (fine-loamy, siliceous, subactive, thermic Typic Hapludults) soil common to the USA Appalachian Plateau region. Three poultry litter components (broiler cake, total cleanout, and bedding material) from a broiler house were evaluated and compared to a soil control. Chemical amendments lime ($CaCO_3$), gypsum ($CaSO_4$), aluminum sulfate ($AlSO_4$), and ferrous sulfate ($FeSO_4$) were added to the poultry litter components to determine their impact on C and N mineralization. Litter component additions increased soil C mineralization in the order of broiler cake > total cleanout > bedding > soil control. Although a greater concentration of organic C was observed in the bedding, broiler cake mineralized the most C, which can be attributed to differences in the C : N ratio between treatments. Chemical amendment in addition to the manured soil also impacted C mineralization, with $AlSO_4$ generally decreasing mineralization. Nitrogen mineralization was also significantly affected by poultry litter component applications. Broiler cake addition increased N availability followed by total cleanout compared to soil control, while the bedding resulted in net N immobilization. Chemical amendments impacted N mineralization primarily in the broiler cake amended soil where all chemical amendments decreased mineralization compared to the no chemical amendment treatment. This short-term study (35-day incubation) indicates that N availability to crops may be different depending on the poultry litter component used for fertilization and chemical amendment use which could decrease N mineralization.

1. Introduction

Poultry litter is increasingly being demanded as an alternative nutrient source to commercial fertilizer in the southeastern US region. Poultry litter is regarded as one of the most valuable nutrient sources compared to other manures due to its relatively high N and phosphorus (P) content. However, poultry litter's N and P may be susceptible to environmental loss. This has resulted in the increased use of chemical amendments to specifically reduce surface water P loss and NH_3 volatilization. Concurrently, poultry litter management practices have also recently changed, with most poultry producers cleaning the litter out of their houses less often to reduce labor costs. The impact that increased chemical amendment use and the changes in poultry litter management practices will have on N availability to plants is largely unknown.

Previous studies have shown that the abatement of P transport from land applied manure to water bodies can be achieved with the use of chemical amendments containing aluminum (Al), iron (Fe), or calcium (Ca) [1–7]. These compounds (Al, Fe, or, Ca) work by binding to P in solution thereby forming insoluble compounds. For instance, Ca rich compounds (gypsum and lime) used as soil amendments tend to form insoluble P compounds, largely as a result of forming a Ca phosphate complex [8]. Aluminum and ferrous compounds (aluminum sulfate and ferrous sulfate) usually result in decreased pH. Consequently as pH

decreases, Al and Fe salts result in the precipitation of aluminum hydroxy phosphate and ferrous hydroxy phosphate [9].

Similarly, Al, Fe, and Ca compounds are believed to reduce ammonia volatilization from poultry litter. Ammonia volatilization occurs through enzymatic conversion and decomposition of organic nitrogenous compounds contained in poultry litter. Reece et al. [10] reported that NH_3 volatilization can be reduced when the litter pH falls below 7, while it is greatly increased when pH is above 8. Thus, chemical additives that manipulate poultry litter's pH are believed to be the most effective. Kithome et al. [11] evaluated effects of the chemical amendments gypsum ($CaSO_4$)and aluminum sulfate (Al_2SO_4) as reducing agents, influencing ammonia volatilization. A mixture of 20% Al_2SO_4 effectively reduced ammonia emissions by 74% compared to control, while gypsum was somewhat ineffective. Moore Jr. et al. [12] also reported significant reductions in ammonia volatilization from poultry manure amended with Al_2SO_4 and $FeSO_4$. Although these chemical additives show promise for reducing environmental degradation from litter, the question of what effect these compounds have on manure decomposition rate has not been explored. This leads to the following question: what effect do these amendments have on agricultural production? Understanding how soil amendments containing Al, Fe, and Ca affect the N mineralization capacity of manure will aid in developing better management practices for manure application.

Periodic removal of litter from poultry houses is important to promote bird health and limit manure buildup [13]. Traditionally, poultry producers cleaned their houses to the ground (total cleanout) each year. While this management is still practiced by some, most producers no longer follow this procedure. Decaking (removal of the cake or top layer of harden manure) is becoming the most popular practice utilized by producers to save money and labor. This procedure involves removing the top portion of the litter, leaving behind the old bedding material. As a result, producers do not replace bedding materials as often. Thus, total cleanout of the litter is typically reduced to once every three to five years. Given that the cake contains a more concentrated manure component and less bedding than litter removed during total cleanout, the difference between the two poultry litter sources may influence N mineralization rates following land application for fertilization.

Continual changes in management practices can impact the sustainability of agricultural production. In order to keep producers abreast of the benefits and drawbacks from these changing practices, research must be done to evaluate their impact on agricultural productivity. A better understanding of how some recent manure management practices affect the decomposition and the availability of N is required. Therefore, the objective of this study was to determine impacts of chemical amendments added to poultry litter components, on C and N mineralization in a laboratory incubation study.

2. Material and Methods

2.1. Site Description for Incubated Soil. Soil for this study was collected from a bermudagrass (Cynodon dactylon) pasture located at the Sand Mountain Agriculture Research and Extension Center in the Appalachian Plateau region of northeast Alabama, USA. Soil was from plots that had not received manure within the last 10 years. The soil was a Hartsells fine sandy loam (fine-loamy, siliceous, subactive, thermic Typic Hapludults). The regional climate is subtropical with no dry season; mean annual rainfall is 1325 mm, and mean annual temperature is 16°C [14]. Bulk soil samples were collected from the 0–20 cm depth and sieved through a 2 mm mesh screen to remove rocks and roots. Samples were stored in a cold room at 4°C until laboratory incubations were performed.

2.2. Experimental Treatments. A 35-day incubation study was conducted to measure C and N mineralization of soil amended with poultry litter containing different chemical amendments that had been previously reported to reduce P loss. Poultry litter components consisted of broiler cake, total cleanout, and bedding. Theses component were collected from a local broiler production facility in the Sand Mountain Region of north Alabama, USA. Broiler cake was collected following the decaking process during broiler house cleaning. Total cleanout litter was collected following the cleaning of a broiler house to the ground. Bedding material consisted of pine wood shavings. In order to apply small-uniform quantities of poultry litter for incubation, samples were air dried at 40°C and ground to pass through a 2 mm sieve. Characteristics of the poultry litter components are presented in Table 1. Laboratory grade chemicals consisting of gypsum ($CaSO_4$), lime ($CaCO_3$), ferrous sulfate ($FeSO_4$), and aluminum sulfate ($AlSO_4$) were used as the chemical amendments for this study.

2.3. Laboratory Analysis. Chemical analysis of soil and poultry litter component sources was performed by the Auburn University Soil Testing Laboratory as described by Hue and Evans [15]. Specifically, soil pH was determined on 1 : 1 soil/water suspensions with a glass electrode pH meter. Total C and N for the soil and poultry litter components were determined by dry combustion using a LECO TruSpec CN analyzer (LECO Corp., St. Joseph, MI). Concentrations of P, K, Ca, Mg, and so forth were determined using a Mehlich 1 (double acid) extracting solution for soil [16] and with the dry ash procedure for poultry litter components [17]; both were measured by inductively coupled Argon plasma emission spectrometry [18] using an ICAP 9000 (Thermo Jarrell Ash, Franklin, MA). Soil textural analysis (percentage sand, silt, and clay) for the incubated soil was determined using the hydrometer method [19].

2.4. Incubation Study. Methods described by Torbert et al. [20] were utilized for quadruple determinations of potential C and N mineralization. Twenty-five grams of soil (oven-dried weight basis), passed through a 2 mm sieve, were

Table 1: Nutrient properties of soil and poultry litter components bedding, total cleanout, and broiler cake used for incubations, dry weight basis.

Variables	pH	CEC	C	N	P P_2O_5	K K_2O	Ca	Mg	Al	B	Cu	Fe	Mn	Zn
					g kg^{-1}						mg kg^{-1}			
Poultry litter components														
Broiler cake	7.6	—	375	42.1	41.3	37.4	25.0	60.0	997	39.0	461	903	751	572
Total cleanout	8.5	—	262	31.3	48.4	32.3	37.5	64.0	2813	39.0	332	2115	509	439
Bedding	4.9	—	456	0.50	1.0	1.4	1.3	0.20	203	<0.1	13.36	4116	62	15
Soil														
Sandy loam	6.1	4.9	7.81	0.83	0.003	0.08	0.49	0.05	103	<0.1	1	9	11	1

placed in 118 mL (4-oz) plastic containers. The poultry litter component source (broiler cake, total cleanout, or bedding material) was added to soils at a rate of 350 kg total N ha^{-1} (based on application to a 15 cm soil furrow slice) and mixed homogeneously to ensure uniformity within and between samples. Following poultry litter component addition, chemical amendments ($CaSO_4$, $CaCO_3$, $FeSO_4$, and $AlSO_4$) were added to the soil at a rate that provided either Al, Fe, or Ca at an amount equivalent to the molar P content of the poultry litter component source (i.e., 651 kg ha^{-1} gypsum, 480 kg ha^{-1} lime, 930 kg ha^{-1} ferrous sulfate, and 700 kg ha^{-1} aluminum sulfate) with the greatest concentration. To facilitate uniform addition, chemical amendments were dissolved with deionized water and added to samples using a micropipette. Deionized water was added to bring the soil matric potential to approximately –20 kPa at a bulk density of 1.2 Mg m^{-3}. After sample preparation, the containers were placed in 1.06 L wide-mouth incubation jars, and 10 mL of water was added to the bottom of each jar (not sample) for humidity control. Jars were incubated in the dark at 25°C and removed after 15 and 35 days for analysis of C and N mineralization.

Soil C mineralization was determined using a 10 mL CO_2 trap (vial containing10 mL 1 N NaOH). The CO_2 trap was placed in each jar and hermetically sealed. After removal, 1 mL of a saturated $BaCl_2$ solution (~1N) was added to each sample to stop CO_2 adsorption, and the NaOH was then backtitrated with 1 N HCl using phenolphthalein as an indicator to determine the amount of CO_2 released from soil samples. Soil C mineralization was determined as the difference between CO_2-C captured in sample traps and from blanks (sealed jar without soil). The concentrations of CO_2 determined on day 15 and 35 days after incubation were added together to determine the total amount of C mineralized for the 35 d incubation period, as described by Anderson (1982).

Soil N mineralization was determined by evaluating inorganic N concentrations during incubation. Concentrations of ammonium (NH4) and nitrite (NO2)+ nitrate (NO_3) were determined by extraction using 2 M KCl as described by Keeney and Nelson [21] and measured colorimetrically using automated laboratory equipment (Bran-Luebbe, Norderstedt, Germany). Soil N mineralization was determined as the difference between final and initial inorganic N contents of the incubated sample.

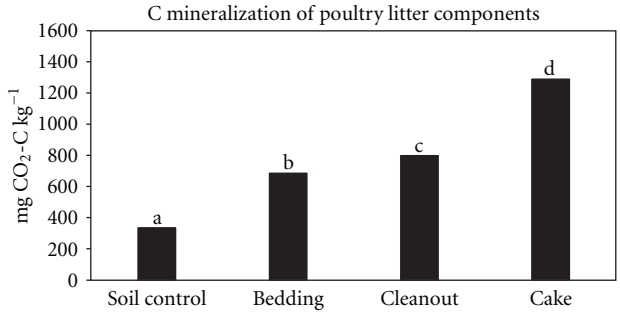


Figure 1: Effect of poultry litter components bedding, total cleanout, and broiler cake compared to a soil control on C mineralization following a 35-day incubation.

2.5. Statistics. The incubation study was analyzed as a completely randomized design with four replications. There were 3 poultry litter sources X 5 chemical amendments X 4 replications compared to a control (4 replicates) for a total of 64 experimental units. Statistical analyses were performed using the GLM procedure of SAS [22], and means were separated using least significant difference (LSD). A significance level of $\alpha < 0.05$ was established *a priori*.

3. Results and Discussion

Poultry litter is generally applied as a readily available N source to pastures in the southeastern USA. Thus, a common upland, well-drained, low microbial activity soil managed under bermudagrass pasture from the southeastern USA region was used to evaluate C and N mineralization for this study. The soil used for this incubation historically received minimal fertilization, no grazing, or harvesting for hay. Background nutrient concentrations for the soil

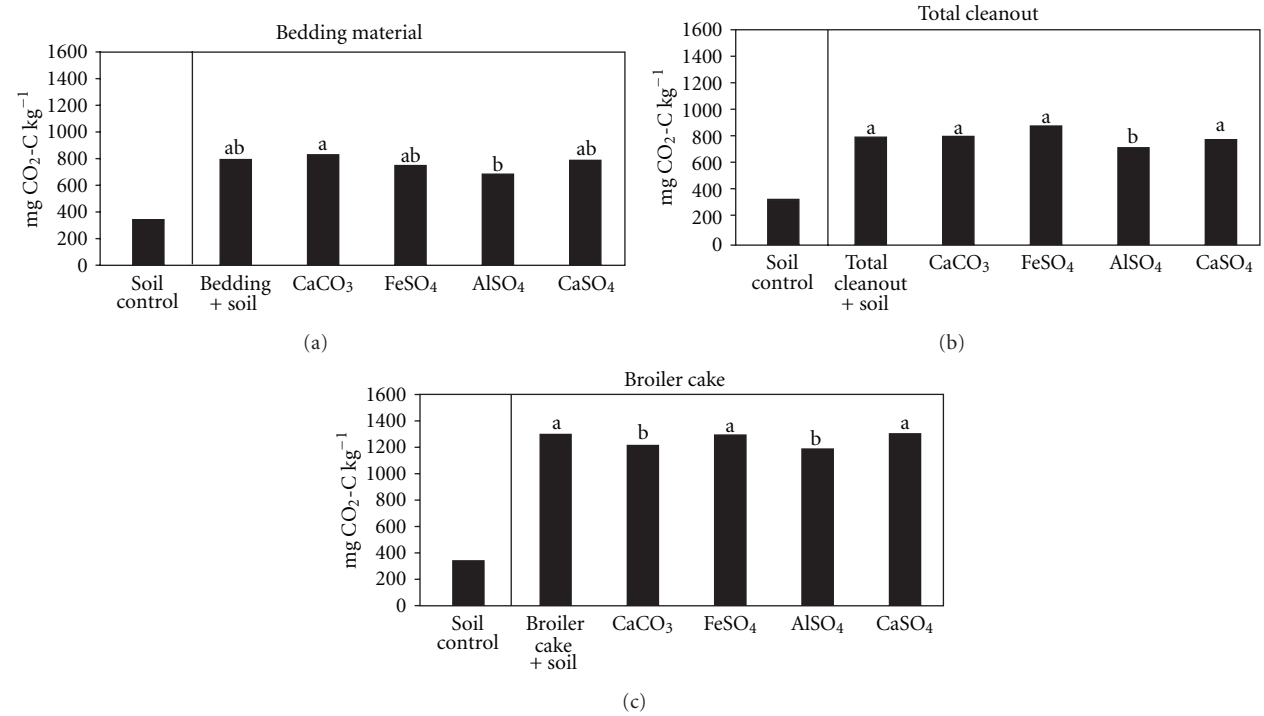


Figure 2: Effect of chemical amendments $CaCO_3$, $FeSO_4$, $AlSO_4$, and $CaSO_4$ on C mineralization of soil amended with poultry litter components bedding, total cleanout, and broiler cake following a 35-day incubation. Soil control is presented for comparison purposes of background levels.

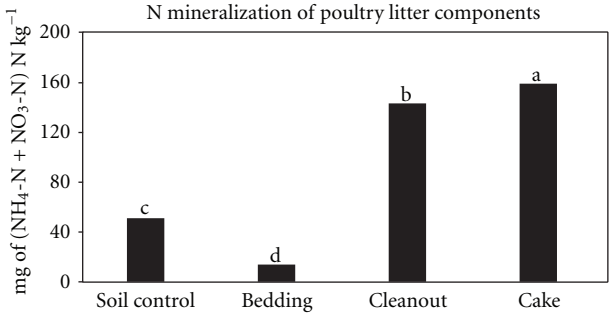


Figure 3: Effect of poultry litter components bedding, total cleanout, and broiler cake compared to a soil control on N mineralization following a 35-day incubation.

and poultry litter component sources used in this study are presented in Table 1. Nutrient concentrations varied among the poultry litter component sources. The bedding material had the greatest C concentrations compared to broiler cake and total cleanout. On the other hand, the bedding material N concentration was the lowest among the poultry litter component sources. Between the poultry litter component sources containing manure (broiler cake and total cleanout), broiler cake had greater C and N concentrations compared to the total cleanout, but C : N ratios were similar between the two sources. Total C, N, and C : N ratios were within the range reported by others in previous research [13, 23]. The focus of this incubation study was to evaluate C and N cycling in soil following poultry litter addition amended with chemical amendments. Information from this study will aid in a better understanding of the N-supplying potential of broiler cake and total cleanout amended with chemical additives containing Al, Fe, and Ca. For discussion purposes of this manuscript, C and N mineralization comparisons were made between the poultry litter components alone (without chemical amendments) and between chemical amendments for each poultry litter component source (broiler cake, total cleanout, and bedding) separately.

3.1. Soil Carbon Mineralization. Carbon mineralization is the conversion of organic C material into inorganic forms through soil microbial oxidation. This process occurs during decomposition. An evaluation of C mineralization can be used as an index to understand the decomposition rate of organic substances. Carbon evolution values observed on 15 d and 35 d were summed together and expressed as total C mineralized. Significant differences were observed in C mineralization rates resulting from the different poultry litter components (Figure 1). Cumulative soil C mineralization for the 35-day incubation period was 335 mg kg^{-1} for soil, 687 mg kg^{-1} for bedding, 801 mg kg^{-1} for total cleanout, and 1293 mg kg^{-1} for broiler cake. As expected, amending soil with the poultry litter components sources significantly increased C evolution, with broiler cake having the greatest C mineralization capacity compared to the other treatments. Carbon mineralization rates following the 35-day incubation

was in the order of broiler cake > total cleanout > bedding > soil control. Although bedding contained the most C, the percentage of total C mineralized was less than the other poultry litter component sources. This suggests that the bedding is a more stable material than the litter sources containing manure (broiler cake and total cleanout). Also, the difference in C mineralization between the bedding and the other poultry litter component sources was most likely due to the amount of available N. Although the litter sources containing manure had a similar C : N ratio, broiler cake had the highest C mineralization. Thus, since N was not a limiting factor, the poultry litter component source with the greatest C concentration mineralized the most when both broiler cake and total cleanout were applied at 350 kg N ha^{-1}. Also, it is important to note that the total cleanout contained a greater percentage of bedding material compared to the broiler cake. Thus, the total cleanout most likely contained more stable C compared to the cake. Since broiler cake consisted of a lower recalcitrant form of organic C, it contributed to a greater mineralization capacity. Although C mineralization data reported for this study represent the total C mineralized during the two sampling periods (15 and 35 days after incubation), it is important to note that a significantly greater C mineralization rate was observed during the second portion of the incubation for the bedding material (data not shown). The other poultry litter components generally resulted in a slightly lower C mineralization rate.

Addition of chemical amendments to the incubated soil also impacted carbon mineralization. Differences in C mineralization rates for each chemical compound added to the broiler cake, total cleanout, and bedding amended soil are illustrated in Figure 2. Chemical amendment additions impacted C mineralization differently depending on the poultry litter component (total cleanout, broiler cake, and bedding) evaluated. For instance, significant differences were observed among chemical amendments in the bedding treatment (i.e., $CaCO_3$ > $AlSO_4$) with $CaCO_3$ producing the greatest C mineralization; however when each chemical amendment was compared to the no chemical amendment bedding treatment (bedding + soil only), no significant differences were observed. Cumulative C mineralization in the total cleanout treatment was significantly increased with $FeSO_4$, producing significantly greater C mineralization rates compared to the other treatments. No significant differences were observed between the $CaSO_4$, $FeSO_4$, $AlSO_4$, and the no chemical amendment treatment (total cleanout + soil only). In the broiler cake treatment, no significant differences were observed between $CaSO_4$, $FeSO_4$, and the no chemical amendment broiler cake treatment (broiler + soil only), while $CaCO_3$ and $AlSO_4$ produced statistically lower C mineralization.

3.2. Soil Nitrogen Mineralization. Nitrogen in manure is primarily in an organic form. Before plant uptake can occur, it must be converted to inorganic forms of N. This is achieved via microbes as a by-product of organic matter decomposition in a process called N mineralization. Understanding of how recent changes in poultry litter management practices affect N mineralization can help improve N management for crop demands.

Similar to C mineralization, addition of the different poultry litter components impacted N mineralization. Mean N mineralization observed during the 35-day incubation is presented in Figure 3. Cumulative soil N mineralizations for the 35-day incubation period were 51 mg kg^{-1} for soil, 13 mg kg^{-1} for bedding material, 143 mg kg^{-1} for total cleanout, and 159 mg kg^{-1} for broiler cake. Addition of bedding resulted in a lower mineralization compared to the soil control (no chemical amendment or poultry litter addition), while broiler cake and total cleanout amended soils resulted in greater N availability. This was to be expected since wood shavings (bedding) are highly stable carbonaceous material with a C : N ratio of 143 : 1. As rule of thumb, a C : N ratio of 20 : 1 is the breakeven point between immobilization and N release. When organic materials with C : N ratios greater than 30 : 1 are added to soil, there is N immobilization during the initial decomposition process. Thus, the highly carbonaceous bedding material supplied the microbes with a new energy source, but insufficient N to build protein. Thus the soil microbes scavenged N from the soil, thereby reducing N availability, or, in other words, caused net N immobilization. Broiler cake produced the highest N mineralization capacity compared to the other poultry litter components evaluated during this incubation study. Although the broiler cake and total cleanout had similar C : N ratios (8.9 broiler cake and 8.4 total cleanout), broiler cake most likely contains a more energy-rich supply of C compared to the total cleanout. For instance, the carbon source (although not quantified) in the cake portion of the litter in broiler houses generally contains a higher concentration of manure while the total cleanout contains both manure and bedding.

Use of chemical amendments with the different poultry litter components resulted in N availability differences following the 35-day incubation (Figure 5). Generally, all of the chemical amendments negatively impacted N availability when compared with poultry litter component sources. There was a significant interaction between the poultry litter components and the chemical amendments. For the bedding material, adding chemical amendments produced a statistically similar N rate compared to the no chemical amendment bedding treatment (bedding + soil only). The $CaCO_3$ amendment produced the greatest N mineralization rate compared to the other chemical amendments applied to the bedding material. Nitrogen mineralization for $CaCO_3$, $FeSO_4$, and $CaSO_4$ was statistically similar to the no chemical amendment bedding treatment, while the $AlSO_4$ produced a statistically lower N mineralization rate. Addition of $AlSO_4$ to the bedding material, which was slightly acidic, could have caused Al toxicity to the soil microbes that were responsible for decomposition. Chander and Brookes [24] reported that Al concentrations at low pH can directly affect microbial biomass and indirectly decrease inputs of soil plant-derived substrates.

Results of N mineralization for chemical amendment additions to total cleanout are reported in Figure 4. Addition of chemical amendments $FeSO_4$, $AlSO_4$, and $CaSO_4$ to soil amended with total cleanout resulted in N mineralization

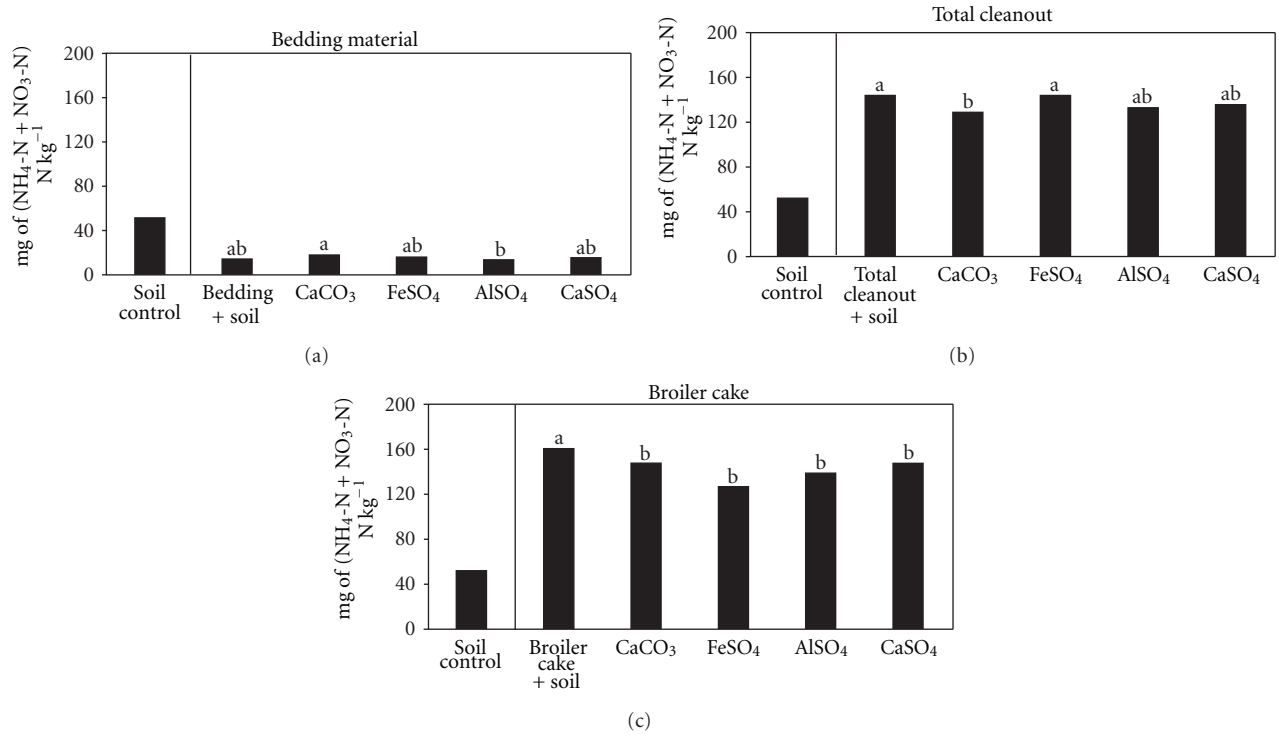


Figure 4: Effect of chemical amendments $CaCO_3$, $FeSO_4$, $AlSO_4$, and $CaSO_4$ on N mineralization of soil amended with poultry litter components bedding, total cleanout, and broiler cake following a 35-day incubation. Soil control is presented for comparison purposes of background levels.

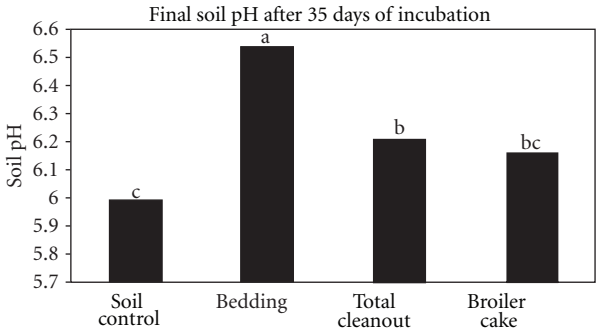


Figure 5: Effect of poultry litter components bedding, total cleanout, and broiler cake compared to a soil control on soil pH.

statistically similar to the no chemical amendment total cleanout treatment. However, addition of $CaCO_3$ to the total cleanout amended soil resulted in a statistically lower N mineralization rate compared to when no chemical amendments were applied. Originally, the pH of the total cleanout was 8.5; thus addition of the liming agent to an already basic material had a negative effect on the N mineralization.

When evaluating the effect of chemical additions to broiler cake amended soil, a slightly different effect was observed in the N mineralization. All of the chemical amendments evaluated resulted in statistically lower plant available N during the 35-day incubation compared to the no chemical amendment broiler cake treatment. Although all the chemical amendments decreased N mineralization, the rate of decrease among treatments with chemical amendments was statistically similar. These results suggest that chemical amendments added to land-applied poultry litter to decrease P loss with surface water runoff or in the poultry production facilities to reduce NH_3 volatilization during growout could affect N availability to crops.

3.3. Soil pH Change. Addition of the poultry litter component sources significantly impacted pH of the incubated soil. Soil pH during the 35-day incubation containing the poultry litter component sources and soil control are shown in Figure 5. Initial soil pH prior to the incubation was 6.1. The soil control pH was essentially unchanged following the 35-day incubation (6.0). Poultry litter additions significantly increased soil pH compared to the soil control. The greatest pH change was observed in soil amended with bedding material followed by total cleanout, broiler cake, and soil control. Generally, poultry litter is not a liming material, but because of the alkaline pH and large Ca content, the broiler cake and total cleanout amendments increased the incubated soil's pH. Increases in soil pH following poultry litter addition have been reported by others [25–27]. Moore and Edwards [27] reported an increase in pH from 5.1 to 5.8 after poultry litter was applied for 7 years. On the other hand, an unexpected increase was also observed with the bedding material, but this phenomenon cannot be explained from previous reports.

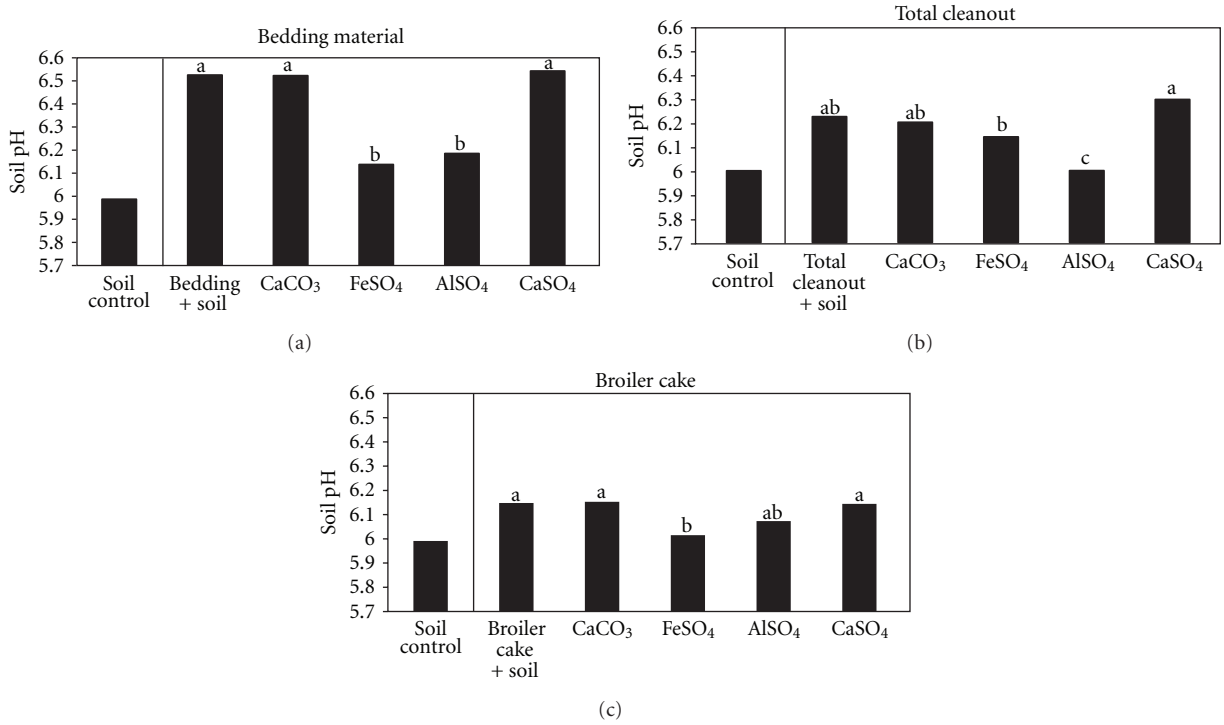


Figure 6: Effect of chemical amendments $CaCO_3$, $FeSO_4$, $AlSO_4$, and $CaSO_4$ on the pH of soil amended with poultry litter components bedding, total cleanout, and broiler cake following a 35-day incubation. Soil control is presented for comparison purposes of background levels.

Chemical amendment addition to soil with the poultry litter components also impacted pH levels (Figure 6). The general pH trend resulting from chemical amendment additions to the incubated soil containing the different poultry litter components was statistically similar between the no chemical amendment, $CaSO_4$, and $CaCO_3$ treatments; lower levels were observed for $AlSO_4$ and $FeSO_4$. Evaluation of the chemical amendment additions to the incubated soil with bedding was similar to the general trend with no significant difference being observed between the no chemical amendment, $CaSO_4$, and $CaCO_3$ treatments, while $AlSO_4$ and $FeSO_4$ resulted in significantly lower soil pH. Significant differences were also observed for the total cleanout. No significant difference was observed between the no chemical amendment, CaSO4, $FeSO_4$, and $CaCO_3$ treatments. The addition of $AlSO_4$ to the total cleanout treatment resulted in decreases to soil pH. An evaluation of broiler cake showed that $AlSO_4$ and $FeSO_4$ resulted in the lowest pH values similar to the bedding and total cleanout poultry litter component. However, $AlSO_4$ was the only chemical amendment that was statistically different from the no chemical amendment broiler cake treatment.

4. Conclusions

Nitrogen is the most limiting nutrient in a crop production system. When manure sources are used for N fertilization, N mineralization accounts for most of the crop N needs. An understanding of how manure management practices affect N availability is important to maintain crop yields. The information obtained from this incubation study may be useful when considering fertilization with poultry litter. For instance, should poultry litter cake or cleanout be used for N fertilization and which chemical amendment provides the best N availability? Carbon mineralization, which is a representation of microbial activity, was increased by the addition of the poultry litter components. Even though the bedding material had the greatest C source, broiler cake had the highest C mineralization rate. Carbon mineralization was in the order of broiler cake, total cleanout, and bedding. Evaluation of N mineralization showed that the bedding material resulted in severe N immobilization. On the other hand, broiler cake had the highest N mineralization rate followed by total cleanout. Addition of chemical amendments to the poultry litter components also impacted C mineralization. The greatest differences in C mineralization were observed with $AlSO_4$, generally decreasing mineralization. Chemical amendment additions also resulted in significant N mineralization differences. The greatest differences were observed in the broiler cake treatment. All chemical amendments applied to the broiler cake amended soil resulted in decreased N mineralization compared to the no chemical amendment treatment. For the total cleanout treatment, $CaCO_3$ was the only chemical amendment to decrease N mineralization compared to the no chemical amendment treatment. These results suggest that while the use of chemical amendments has been shown to reduce P loss and decrease NH_3 volatilization, a reduction in N mineralization may also occur. Also

changes in poultry litter management practices are likely to impact N availability in soil.

References

[1] P. A. Moore Jr. and D. M. Miller, "Decreasing phosphorus solubility in poultry litter with aluminum, calcium, and iron amendments," *Journal of Environmental Quality*, vol. 23, no. 2, pp. 325–330, 1994.

[2] D. L. Anderson, O. H. Tuovinen, A. Faber, and I. Ostrokowski, "Use of soil amendments to reduce soluble phosphorus in dairy soils," *Ecological Engineering*, vol. 5, no. 2-3, pp. 229–246, 1995.

[3] T. H. Dao, "Coamendments to modify phosphorus extractability and nitrogen/phosphorus ratio in feedlot manure and composted manure," *Journal of Environmental Quality*, vol. 28, no. 4, pp. 1114–1121, 1999.

[4] Z. Dou, G. Y. Zhang, W. L. Stout, J. D. Toth, and J. D. Ferguson, "Efficacy of alum and coal combustion by-products in stabilizing manure phosphorus," *Journal of Environmental Quality*, vol. 32, no. 4, pp. 1490–1497, 2003.

[5] M. Kalbasi and K. G. Karthikeyan, "Phosphorus dynamics in soils receiving chemically treated dairy manure," *Journal of Environmental Quality*, vol. 33, no. 6, pp. 2296–2305, 2004.

[6] H. A. Torbert, K. W. King, and R. D. Harmel, "Impact of soil amendments on reducing phosphorus losses from runoff in sod," *Journal of Environmental Quality*, vol. 34, no. 4, pp. 1415–1421, 2005.

[7] D. B. Watts and H. A. Torbert, "Impact of gypsum applied to grass buffer strips on reducing soluble p in surface water runoff," *Journal of Environmental Quality*, vol. 38, no. 4, pp. 1511–1517, 2009.

[8] D. Brauer, G. E. Aiken, D. H. Pote et al., "Amendment effects on soil test phosphorus," *Journal of Environmental Quality*, vol. 34, no. 5, pp. 1682–1686, 2005.

[9] Y. Ann, K. R. Reddy, and J. J. Delfino, "Influence of chemical amendments on phosphorus immobilization in soils from a constructed wetland," *Ecological Engineering*, vol. 14, no. 1-2, pp. 157–167, 1999.

[10] F. N. Reece, B. J. Bates, and B. D. Lott, "Ammonia control in broiler houses," *Poultry Science*, vol. 58, pp. 754–755, 1979.

[11] M. Kithome, J. W. Paul, and A. A. Bomke, "Reducing nitrogen losses during simulated composting of poultry manure using adsorbents or chemical amendments," *Journal of Environmental Quality*, vol. 28, no. 1, pp. 194–201, 1999.

[12] P. A. Moore Jr., T. C. Daniel, D. R. Edwards, and D. M. Miller, "Effect of chemical amendments on ammonia volatilization from poultry litter," *Journal of Environmental Quality*, vol. 24, no. 2, pp. 293–300, 1995.

[13] K. R. Sistani, G. E. Brink, S. L. McGowen, D. E. Rowe, and J. L. Oldham, "Characterization of broiler cake and broiler litter, the by-products of two management practices," *Bioresource Technology*, vol. 90, no. 1, pp. 27–32, 2003.

[14] R. H. Shaw, "Climate of the United States," in *Handbook of Soils and Climate in Agriculture*, V. J. Kilmer, Ed., CRC Press, Boca Raton, Fla, USA, 1982.

[15] N. V. Hue and C. E. Evans, *Procedures used for soil and plant analysis by the Auburn University Soil Testing Laboratory*, Auburn University, Auburn, Ala, USA, 1986.

[16] S. R. Olsen and L. E. Sommers, "Phosphorus," in *Methods of Soil Analysis Part 2*, A. L. Page, Ed., Agronomy Monograph 9, pp. 403–430, ASA and SSSA, Madison, Wis, USA, 1982.

[17] S. J. Donahue, *Reference soil test methods for the Southern Region of the United State*, Southern Cooperative Service, Bulletin 289, Georgia Agricultural Experiment Station, Athens, Ga, USA, 1983.

[18] P. N. Soltanpour, J. B. Jones, and S. M. Workman, "Optical emission spectrometry," in *Methods of Soil Analysis. Part 2*, A. L. Page et al., Ed., Agronomy Monograph 9, pp. 29–65, ASA and SSSA, Madison, Wis, USA, 2nd edition, 1982.

[19] G. J. Bouyoucos, "Hydrometer method for making particle analysis of soil," *Agronomy Journal*, vol. 54, pp. 464–465, 1962.

[20] H. A. Torbert, S. A. Prior, and D. W. Reeves, "Land management effects on nitrogen and carbon cycling in an ultisol," *Communications in Soil Science and Plant Analysis*, vol. 30, no. 9-10, pp. 1345–1359, 1999.

[21] D. R. Keeney and D. W. Nelson, "Nitrogen: inorganic forms," in *Methods of Soil Analysis. Part 1*, A. L. Page et al., Ed., Agronomy Monograph 9, pp. 643–698, ASA and SSSA, Madison, Wis, USA, 2nd edition, 1982.

[22] SAS Institute, *SAS, version 9.1*, SAS Institute, Cary, NC, USA, 2002.

[23] P. L. Ward, J. E. Wohlt, P. K. Zajac, and K. R. Cooper, "Chemical and physical properties of processed newspaper compared to wheat straw and wood shavings as animal bedding," *Journal of Dairy Science*, vol. 83, no. 2, pp. 359–367, 2000.

[24] K. Chander and P. C. Brookes, "Synthesis of microbial biomass from added glucose in metal-contaminated and non-contaminated soils following repeated fumigation," *Soil Biology and Biochemistry*, vol. 24, no. 6, pp. 613–614, 1992.

[25] E. E. Codling, R. L. Chaney, and C. L. Mulchi, "Use of aluminum- and iron-rich residues to immobilize phosphorus in poultry litter and litter-amended soils," *Journal of Environmental Quality*, vol. 29, no. 6, pp. 1924–1931, 2000.

[26] G. Gupta and S. Charles, "Trace elements in soils fertilized with poultry litter," *Poultry Science*, vol. 78, no. 12, pp. 1695–1698, 1999.

[27] P. A. Moore and D. R. Edwards, "Long-term effects of poultry litter, alum-treated litter, and ammonium nitrate on aluminum availability in soils," *Journal of Environmental Quality*, vol. 34, no. 6, pp. 2104–2111, 2005.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 437354, 11 pages
doi:10.1155/2012/437354

Research Article

The Effects of Some External Management Factors on the Nitrogen Composition of Cattle Manure on Smallholder Farms

H. A. Markewich,[1] A. N. Pell,[2] D. M. Mbugua,[3] D. J. R. Cherney,[1] H. M. van Es,[3] and J. Lehmann[3]

[1] *Department of Animal Science, Cornell University, 149 Morrison Hall, Ithaca, NY 14853, USA*
[2] *Department of Animal Science, Cornell University, 115 Day Hall, Ithaca, NY 14853, USA*
[3] *Department of Crop and Soil Sciences, Cornell University, 235 Bradfield Hall, Ithaca, NY 14853, USA*

Correspondence should be addressed to H. A. Markewich, hamarkewich@gmail.com

Received 16 November 2011; Accepted 12 March 2012

Academic Editor: Dexter B. Watts

Smallholder farmers in Kenya collect manure from confined cattle housing termed zero-grazing units. Zero-grazing designs may include urine collection, though the effectiveness of these designs in improving manure N content has not been established. The manure-urine mixtures produced in these units were simulated to determine urine effects on manure N composition. Manure and manure-urine mixtures were stored for 120 days during dry and rainy seasons in Kenya. Manure-urine mixtures leached 26% of their mineral N content during the dry season, but only 12% during the rainy season. After storage, manure-urine mixtures had less organic-N and fiber-N than manure alone during the dry season ($P < 0.01$), but not during the rainy season. Results suggest that the effect of cattle urine on manure N composition is greater during dry seasons than rainy. Manure should not be stored more than 30 days to minimize N loss to leaching. Farmers may take steps to reduce N loss by controlling leaching and protecting manure from rainfall.

1. Introduction

Smallholder farmers in Kenya who confine their livestock in zero-grazing units collect and store manure for use as a soil amendment during the growing seasons [1]. The design of zero-grazing units may allow for the collection and preservation of some of the cattle urine. A survey of 60 smallholders in the Kenyan highlands reported that more than half of the farms used zero-grazing units with sloped floors and manure storage piles just outside the animal confinement area at the base of the sloped floor [1]. Farmers perceived that manure decomposition speeds up when manure and urine are mixed. Liquids from the zero-grazing unit floor drained into the manure storage pile. Less than 10% of farms used designs that allowed direct urine collection such as channels to transfer liquid from the zero-grazing unit floor into a reservoir just outside of the confinement area [1]. The animal housing on the remaining farms had no design attributes to conserve urine. The liquid from the floor of the animal confinement area was either absorbed by bedding and feed refusals on the floor of the zero-grazing unit, or soaked into the soil [1, 2]. Whether the urine-conserving zero-grazing unit designs actually increased manure N content was not established. The designs may not prevent urinary N losses via volatilization [1, 2].

The N retention efficiency of zero-grazing systems is poor. More than 36% of N in a mixture of manure and urine may be lost between excretion and the end of storage. Urinary-N losses are greater when no refusals to absorb the urine are on the floor of the zero-grazing unit. Manure and urine N may be lost via leaching when the dirt floors of the zero-grazing units are cleaned out only a few times each year [3].

To determine whether the presence of urine increased manure N content, experiments were conducted with manure-urine mixtures similar to those produced on farms

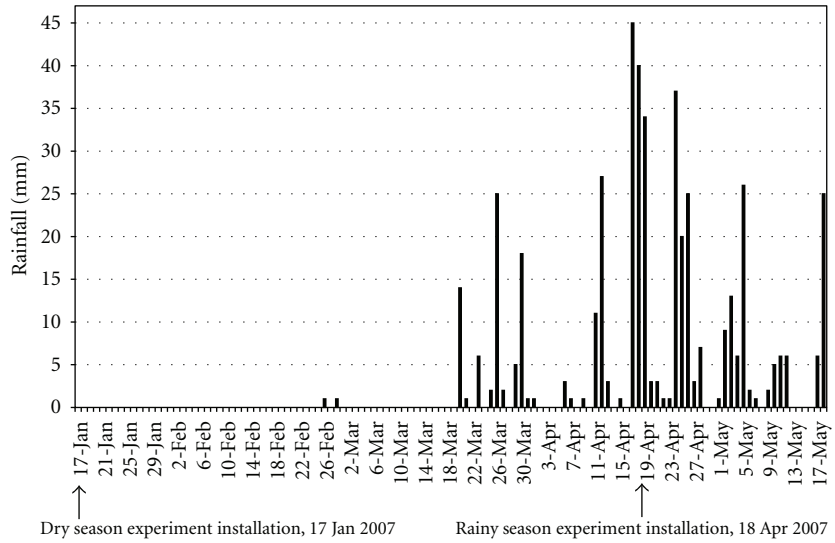


Figure 1: Rainfall at the experiment site, Kenya Agricultural Research Institute. Dry season experiment duration: 17 January to 01 June 2007. Rainy season experiment duration: 18 April to 18 May 2007.

with zero-grazing units designed to drain urine into the manure storage piles. In addition to total manure N content, it is important to consider the patterns of transformation of nitrogenous compounds in manure that occur during storage. One objective of this study was to examine the changes in specific manure N fractions resulting from inclusion of urine in manure storage systems. It was hypothesized that most N losses from stored manure occur within the first month of storage. In a study of stored beef cattle manure, N losses of more than 10% occurred after 42 days of storage [4]. A second hypothesis was that manure with urine contains more mineral N and less fiber-N and lignin-N than manure without urine. Urine is a major source of N in stored manure [5], so stored manure with urine may not be N-limited, allowing manure microbes to degrade the fibrous manure materials more rapidly. The third hypothesis was that manure mixed with urine leaches more mineral N than manure without urine. Large losses of urinary N via leaching and volatilization may occur from manure before its application to soil [6]. Finally, it was hypothesized that heavy rainfall increases N losses from leaching in newly stockpiled manure because the rain saturates the manure and the water-soluble N compounds in fresh manure leach from the manure and are not recovered. The effects of season on manure composition and leaching were investigated by starting one experiment during the dry season and one during the rainy season.

2. Materials and Methods

2.1. Experimental Design. Manure from two different farms in the Embu area was used to demonstrate the effects of farm and cattle diet management on manure quality. The larger farm maintained a milking herd of approximately 20 cows. The smaller farm milked 4 Holstein-Freisian cattles. The cattles on both farms were fed by the cut-and-carry method, but the cattle of the larger farm were fed energy concentrates and Napier grass (*Pennisetum purpureum*), a relatively high protein forage, more regularly. That the manure differed in terms of total N and organic matter (OM) content was established prior to the start of the dry season experiment to establish the effects of different management regimes on manure composition. Based on the initial manure composition and the management styles of the farms, the larger farm was designated the better-managed farm and the smaller farm the less well-managed farm. Based on this distinction in management quality, manure from the smaller farm will be referred to as "Low quality" and manure from the larger farm will be referred to as "Medium quality" from this point forward. The cattle urine used in the manure storage experiments was collected from the milking herds of both farms and mixed before being used in the experiment. The urine collection lasted approximately 10 days before the start of the dry season experiment and the start of the rainy season experiment. The urine was stored at 4°C before the beginning of each experiment. At the start of each experiment, samples of the urine were acidified to pH < 2.5 using concentrated H_2SO_4 to prevent NH_3 volatilization by microbes [7]. Before NH_4-N analysis, samples were stored at −20°C.

Two manure storage experiments were conducted, the first beginning in the dry season and carried out for 120 days, and the second beginning early in the long rainy season, ending after 30 days. The two experiments were designed to demonstrate the effects of rainfall on newly established manure storage systems.

The dry season manure storage experiment was conducted from January through June 2007 at the Embu office of the Kenya Agricultural Research Institute in Embu, Eastern Province, Kenya. Mid-January usually marks the beginning of the dry season in Embu, so the experiment was started on January 17, 2007. The installation of the dry season experiment marked the beginning of a 2-month period of very dry weather (Figure 1).

Five-liter plastic buckets were modified to collect leachate from the manure. The bottom of each bucket was removed and replaced with a circular piece of stainless steel 50-mesh screen with square apertures of 279 μ on a side. Each of the 120 buckets was assigned to one of four treatments and placed in one of four adjacent tables in a spatially balanced complete block design to ensure spatial homogeneity in the experiment [8]. A standard funnel-graduated cylinder rain gauge was positioned at the center of the tables and rainfall was measured daily. Three replicates of each treatment were incubated. On a fresh weight (FW) basis, 4.8 kg of manure was added to each bucket. A volume of 1.4 L of urine was added to half of the buckets, representing the daily urine volume-to-fecal mass excretion ratio of cattle in water-constrained environments [9]. The urine NH_4-N concentration was 0.03 M. Three buckets from each treatment were randomly selected for destructive sampling after 6, 12, 18, 24, 30, 50, 70, 90, and 120 days of incubation. After sampling, subsamples of the manure were immediately stored at -20°C.

The rainy season manure storage experiment, to assess the effects of rainfall on newly installed manure storage units, was installed on April 18, 2007. A preliminary period of ten days of moderate precipitation preceded the onset of the long rainy period in mid-April (Figure 1).

The goal of this experiment was to demonstrate the response of newly stockpiled manure to heavy rainfall. The design of the experiment was similar to that of the dry season experiment. Each of 60 buckets was assigned to one of the four treatments in a randomized complete block design. Three replicates of each treatment type were incubated. An initial mass of 4.1 kg (FW basis) of manure was added to each bucket. This initial manure mass differed from the mass used in the dry season experiment because the manure for this study had a greater water content. The manure for the rainy season study was collected early in the long rainy season. The rains dampened manure in the cattle pens before it was collected. An initial volume of 1.2 L of urine was added to half of the buckets, to represent the daily urine volume-to-fecal mass excretion ratio of cattle in water-constrained environments [9]. The NH_4-N concentration of the urine was 0.15 M. The higher NH_4-N concentration of the rainy season urine likely was because cattle were fed more high-protein forages such as Napier grass during the rainy season than during the dry season. Three buckets of each treatment were sampled destructively after 6, 12, 18, 24, and 30 days of incubation.

For both the rainy and dry season experiments, leachates were collected through a plastic funnel attached to the mesh bottom of each bucket into a 60 mL bottle containing 1 mL concentrated H_2SO_4. The acid immediately lowered the leachate pH to less than 2.5 upon entering the bottle in order to check the degradation of organic and inorganic-N by microbes [7]. The leachate collection bottles were replaced when full and upon the destructive sampling of each bucket. The mass and volume of the leachate collected were recorded. Leachates for each individual bucket were composited by date: after 6, 12, 18, 24, 30, 50, 70, 90, and 120 days of storage for the dry season experiment and after 6, 12, 18, 24, and 30 days of storage for the rainy season experiment.

2.2. Chemical Analyses. One gram of each frozen subsample was thawed and extracted in 50 mL water for 30 min using an orbital shaker set to 180 rpm. The NH_4-N contents of the extracts were measured using a colorimetric method [10] in the soil analysis laboratories of the Kenya Agricultural Research Institute in Embu, Kenya and of the World Agroforestry Centre Headquarters in Nairobi, Kenya.

Aliquots of the manure samples were dried at $\leq 60^\circ$C and ground to pass a 1 mm screen in preparation for analysis. The samples were analyzed at the Dairy One Laboratories, Ithaca New York, USA for total N by the Kjeldahl method [11] and for neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN) [12]. It was assumed that all mineral N volatilized from the manure during the drying process, so the measurement of total N by the Kjeldahl method was a direct measurement of organic N for the dried samples.

2.3. Statistical Analyses. The dry and rainy experiments were designed as multi-factor models with fixed effects where the fixed factors were manure quality, urine presence, and days in storage. All possible interactions between the fixed effects were tested. The absolute concentration data were analyzed using the PROC MIXED procedure of the SAS software [13]. Comparisons were made using the Student's t-test.

Using the PROC NLIN procedure of the SAS software [13], exponential decay models were developed to describe the NH_4-N, total organic N, NDIN, and ADIN the manure in storage over time. Decay models with one exponential term were fitted to the data using the equation $Y = \alpha e^{kx}$, where Y represents the NH_4-N, organic N, NDIN, or ADIN content of the manure, x represents the number of days the manure was incubated in storage, and k represents the rate of decay of the NH_4-N, organic N, NDIN, or ADIN in the manure. Regression of the data using double exponential decay equations failed to converge.

The PROC NLIN procedure of the SAS software was used to develop logarithmic models describing the cumulative NH_4-N losses from manure in storage via leaching [13]. Logarithmic models with one term were fitted to the data using the equation $Y = k\ln(x)$, where Y represents the leached NH_4-N, x represents the number of days the manure was incubated in storage, and k represents the rate of accumulation of the NH_4-N leached from the manure. Regression of the data using log-log equations failed to converge.

3. Results

Results of the mixed model analyses for the dry season experiment appear in Tables 1, 2, and 3. The mixed model analyses results for the rainy season experiment appear in Tables 4 and 5.

Cattle feces contain essentially no urea [14, 15]. Cattle urine contains both urea and NH_4. However, since the fecal pH was approximately pH 8, it is assumed that the urea of the urine immediately underwent hydrolysis to produce NH_4 when the urine and manure were mixed. In this study, NH_4-N represents mineral N.

Table 1: ANOVA results for dry matter (DM) mass remaining in manure in storage in the dry season experiment, as a percent of the initial manure DM mass and for N content (DM basis) of manure in storage in the dry season experiment, as a percent of the initial manure N content. Fixed effects are the manure quality (Medium versus Low quality), the presence or absence of urine in the manure, and the number of days the manure was incubated (0, 6, 12, 18, 24, 30, 50, 70, 90, or 120 days). Terms for the interaction between fixed effects are denoted by fixed effects terms separated by an asterisk (*). DF represents the degrees of freedom of the test.

Effect	DF	*P*-value			
		DM mass (% of initial DM mass)	N (% of DM)	ADIN (% of DM)	NDIN (% of DM)
Medium versus Low quality manure [A]	1	<0.0001	0.0218	<0.0001	<0.0001
Urine added versus no urine [B]	1	<0.0001	0.0024	0.0303	0.0052
Days in storage [C]	8	<0.0001	<0.0001	<0.0001	<0.0001
A*B	1	0.8085	0.0348	0.0875	0.0119
A*C	8	0.0058	0.0798	0.1258	0.1166
B*C	8	0.0749	0.0464	0.0092	0.0029
A*B*C	8	0.2125	0.8771	0.8403	0.3715

Table 2: ANOVA results for NH_4-N concentration (mg NH_4-N/kg manure, DM basis) of manure in storage in the dry season experiment. Fixed effects are the manure quality (Medium versus Low quality), the presence or absence of urine in the manure, and the number of days the manure was incubated (0, 6, 12, 18, 24, 30, 50, 70, 90, or 120 days). Terms for the interaction between fixed effects are denoted by fixed effects terms separated by an asterisk (*). DF represents the degrees of freedom of the test.

Effect	DF	*P*-value
		NH_4-N (mg/kg manure)
Medium versus Low quality manure [A]	1	0.0147
Urine added versus no urine [B]	1	0.0079
Days in storage [C]	9	<0.0001
A*B	1	0.7994
A*C	8	0.5932
B*C	9	0.0199
A*B*C	8	0.0453

Table 3: ANOVA results for NH_4-N content (mg NH_4-N per manure storage unit) of leachate collected from manure in storage in the dry season experiment. Fixed effects are the manure quality (Medium versus Low quality), the presence or absence of urine in the manure, and the number of days the manure was incubated (0, 6, 12, 18, 24, 30, 50, 70, 90, or 120 days). Terms for the interaction between fixed effects are denoted by fixed effects terms separated by an asterisk (*). DF represents the degrees of freedom of the test.

Effect	DF	*P*-value
		Leachate NH_4-N (mg/storage unit)
Medium versus Low quality manure [A]	1	0.4382
Urine added versus no urine [B]	1	0.0192
Days in storage [C]	8	0.0281
A*B	1	0.9802
A*C	8	0.9152
B*C	7	0.3538
A*B*C	7	0.9861

Figure 2 shows the NH_4-N dynamics in manure of the dry season experiments. After 30 days of storage, the NH_4-N concentration of manure alone ranged from 156 to 241 mg NH_4-N per kg of manure (DM basis) in individual treatments. The NH_4-N concentration of urine-amended manure ranged from 372 to 375 mg NH_4-N per kg of manure (DM basis) in individual treatments after 30 days of storage. The manure source had no effect on manure NH_4-N after 30 days or 120 days ($\alpha = 0.05$, Figure 2). In the dry season experiment, manure alone contained less NH_4-N per kg of manure (DM basis) than the treatments with urine after 30 days of storage ($P < 0.04$).

Exponential decay models to describe the NH_4-N dynamics of the Medium quality manure and Low quality manure in the rainy season experiment using two exponential terms did not converge. Exponential models with one exponential term to describe NH_4-N dynamics appear in Figure 3. During the early rainy season, forages are more nutritious and digestible which may explain the marked difference in the NH_4-N concentration of the urine used in the dry season and rainy season experiments. Neither the source of the manure nor the presence of urine in the manure affected the NH_4-N concentration of the manure in the rainy season experiment ($\alpha = 0.05$, Table 5). Average manure NH_4-N for all treatments after 30 days of storage during the rainy season was 572 mg per kg of manure (DM basis).

Figures 4(a)–4(c) show the organic N, NDIN, and ADIN dynamics in manure of the dry season experiment in terms of % remaining from the total amounts in the fresh manure at the start of the experiment. In terms of absolute content, there was no difference in the manure NDIN or manure ADIN after 30 days in storage ($\alpha = 0.05$), nor was there a difference between the total organic N content of the medium and low farm manure after 30 days of storage or at

Table 4: ANOVA results for N content (DM basis) of manure in storage in the rainy season experiment, as a percent of the initial manure N content, and for NH_4-N content (mg NH_4-N per manure storage unit) of leachate collected from manure in storage in the rainy season experiment. Fixed effects are the manure quality (Medium versus Low quality), the presence or absence of urine in the manure, and the number of days the manure was incubated (0, 6, 12, 18, 24, or 30 days). Terms for the interaction between fixed effects are denoted by fixed effects terms separated by an asterisk (*). DF represents the degrees of freedom of the test.

Effect	DF	*P*-value				
		DM mass (% of initial DM mass)	N (% of DM)	ADIN (% of DM)	NDIN (% of DM)	Leachate NH_4-N (mg/storage unit)
Medium versus Low quality [A]	1	0.1037	<0.0001	<0.0001	<0.0001	<0.0001
Urine added versus no urine [B]	1	0.9842	0.1039	0.1967	0.079	<0.0001
Days in storage [C]	4	0.0004	0.0077	0.0314	0.2868	<0.0001
A*B	1	0.4727	0.009	0.4382	0.2768	<0.0001
A*C	4	0.9315	0.932	0.8198	0.9332	<0.0001
B*C	4	0.5561	0.922	0.9606	0.9683	<0.0001
A*B*C	4	0.7194	0.1104	0.2577	0.435	<0.0001

Table 5: ANOVA results for NH_4-N concentration (mg NH_4-N/kg manure, DM basis) of manure in storage in the rainy season experiment. Fixed effects are the manure quality (Medium versus Low quality), the presence or absence of urine in the manure, and the number of days the manure was incubated (0, 6, 12, 18, 24, or 30 days). Terms for the interaction between fixed effects are denoted by fixed effects terms separated by an asterisk (*). DF represents the degrees of freedom of the test.

Effect	DF	*P*-value
		NH_4-N (mg/kg manure)
Medium versus Low quality manure [A]	1	0.8389
Urine added versus no urine [B]	1	0.2912
Days in storage [C]	5	0.0473
A*B	1	0.1899
A*C	5	0.7942
B*C	5	0.6891
A*B*C	5	0.904

the end of the experiment ($\alpha = 0.05$). The days in storage had an effect on the organic N, ADIN, and NDIN contents of the dry season manure over the entire experiment ($P < 0.0001$, Table 1), but no treatment effects on total organic N, NDIN, or ADIN were observed after 30 days in storage (Figures 4(a)–4(c)). Single-term exponential decay models for total N, NDIN, and ADIN dynamics in manure appear in Figures 4(a)–4(c). Exponential decay models using two exponential terms to describe total N, NDIN, and ADIN dynamics in the manure failed to converge.

In general, lignin-bound N is considered to be quite refractory and poorly available for microbial degradation [16, 17]. Non-fiber bound nitrogenous organic compounds are more readily available for degradation than hemicellulose-bound N, cellulose-bound N, and lignin-N (represented by NDIN), and cellulose-bound N and lignin-N (represented by ADIN). The portion of the labile organic N that is microbial N can be recycled when a microbe dies and lyses and its cell contents, including nitrogenous compounds, are metabolized by other living microbes. The magnitudes of the N fractions in manure of the rainy season experiment were not affected by urine amendments ($\alpha = 0.05$, Table 4). After 30 days of storage, the Medium quality manure alone contained 9.2% organic N, 4.9% NDIN, and 3.9% ADIN (DM basis). The Low quality manure contained 11.0% organic N, 5.1% NDIN, and 4.1% ADIN (DM basis, Figures 5(a)–5(c)). The source of the manure did affect N composition ($P < 0.0001$ for organic N, $P < 0.0001$ for ADIN, $P < 0.0001$ for NDIN) with higher levels of ADIN in the Low quality manure ($P < 0.04$) after 30 days of storage. The time in storage had an effect on the organic N ($P < 0.008$) and ADIN ($P < 0.04$) throughout the experiment, but at the end of the rainy season experiment, the total organic N and ADIN content of the two manures did not differ ($\alpha = 0.05$, Table 4). The length of storage had no effect on NDIN ($\alpha = 0.05$, Table 4). Exponential decay models with one exponential term were developed for total N, NDIN, and ADIN dynamics in manure (Figures 5(a)–5(c)). Decay models with two exponential terms did not converge.

Single-term logarithmic rate equations describing cumulative leaching of NH_4-N from manure in the dry season experiment appear in Figure 6. Logarithmic models with two terms to describe cumulative NH_4-N leaching failed to converge. From each of the 4 treatments, more than 50% of the final amount of NH_4-N loss from the manure via leaching occurred during the first 30 days of storage.

Inclusion of urine significantly increased the amount of NH_4-N leached from manure in the rainy season experiment ($P < 0.0001$, Table 4). Total NH_4-N losses from urine-amended manure in the rainy season experiment were larger than from urine-amended manure in the dry season experiment. Neither the manure source nor the urine treatment affected the amount of NH_4-N leached during the 30 day storage period ($\alpha = 0.05$; Figure 7). The final cumulative leaching loss from urine-amended manure storage units was larger than that from units containing only manure. After 30 days of storage during the rainy season, the urine-amended manure storage units lost an average of 337 mg NH_4-N and

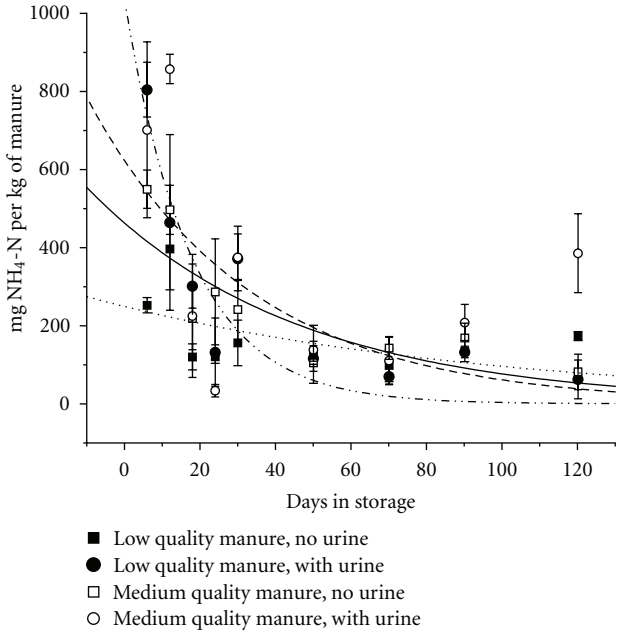


Figure 2: NH_4-N dynamics in manure of the dry season storage experiment. Error bars represent standard error of the mean values. Continuous lines represent the best-fit regression equations for NH_4-N dynamics in manure during the dry season experiment. Y represents the amount of NH_4-N (mg/kg manure DM) in the manure. The solid line (—) represents the Medium quality manure, no urine treatment. The dashed line (- - - -) represents the urine-amended Medium quality manure treatment. The dotted line (· · · ·) represents the Low quality manure, no urine treatment. The dot-dash line (-···-···) represents the urine-amended Low quality manure treatment. Medium quality manure, no urine: $Y = 462.3e^{-0.0180x}$, $R^2 = 0.807$. Medium quality manure, with urine: $Y = 621.8e^{-0.0232x}$, $R^2 = 0.619$. Low quality manure, no urine: $Y = 249.1e^{-0.00955x}$, $R^2 = 0.715$. Low quality manure, with urine: $Y = 1033.7e^{-0.0570x}$, $R^2 = 0.812$. The rate equations for NH_4-N dynamics between the manure sources did not differ at significance level $\alpha = 0.05$.

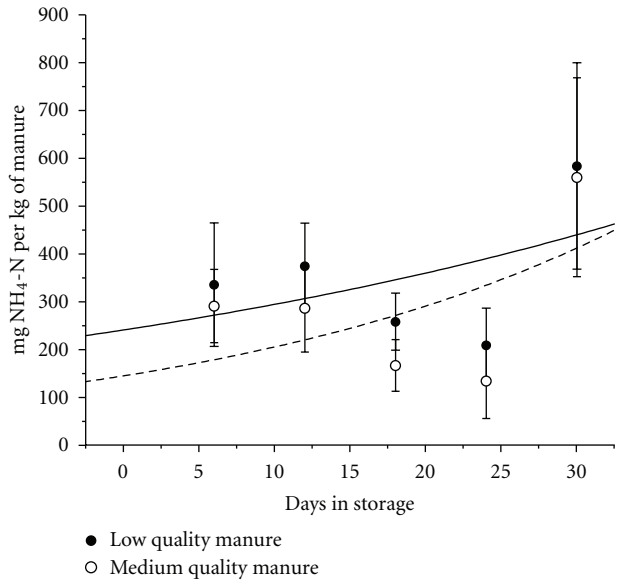


Figure 3: NH_4-N dynamics in manure of the rainy season storage experiment. Error bars represent standard error of the mean values. Continuous lines represent the best-fit regression equations for NH_4-N dynamics in manure during the dry season experiment. The solid line (—) represents the best-fit regression for the Medium quality manure treatments. The dashed line (- - - -) represents the best-fit regression for the Low quality manure treatments. Y represents the amount of NH_4-N (mg/kg manure DM) in the manure. Medium quality manure: $Y = 144.7e^{0.0349x}$, $R^2 = 0.508$. Low quality manure: $Y = 240.7e^{0.0201x}$, $R^2 = 0.571$. The rate equations for NH_4-N dynamics between the manure sources did not differ at significance level $\alpha = 0.05$.

the storage units containing manure alone lost an average of 97 mg NH_4-N. These cumulative leaching losses represent up to 12% of the initial manure NH_4-N from urine-amended manure and up to 57% of the initial manure NH_4-N from manure alone.

After 120 days of storage in the dry season experiment, urine-amended manure lost up to 59% of its initial DM mass while manure alone lost up to 45% of the initial DM. These represent mass losses of up to 0.6 kg DM from urine-amended manure and 0.5 kg DM from manure alone after 120 days of storage. At the end of the rainy season experiment, there were no treatment effects on mass loss ($\alpha = 0.05$).

Figure 2 shows the NH_4-N dynamics in manure of the dry season experiment. Exponential regression equations with one term to describe the NH_4-N dynamics were developed using manure data for the dry season experiment (Figure 2). Attempted exponential decay models to describe NH_4-N dynamics with two exponential terms did not converge. In the dry season experiment, the manure NH_4-N dynamics fit an exponential decay pattern (Figure 2). Most of the NH_4-N loss from the manure occurred during the first 50 days of incubation (Figure 2), before heavy rainfall began at 63 days of incubation (Figure 1). At the end of 120 days of storage in the dry season experiment, manure NH_4-N fell to less than 200 mg per kg of manure, with the exception of the urine-amended Medium quality manure. In this treatment, the manure NH_4-N concentration increased after 50 days in storage. At 120 days of storage, the NH_4-N concentration of the Medium quality manure amended with urine was nearly 400 mg per kg of manure (Figure 2). This effect was likely due not to an increase in the pool of NH_4-N in the manure, but the result of an increase in the manure NH_4-N concentration as the manure decomposed. As manure decomposes, its C-containing compounds are converted to CO_2 and CH_4. The loss of C mass reduces the manure C : N, and the content of manure N compounds increase [18].

In the dry season experiment, manure with urine treatments did not contain significantly more NH_4-N at the end of the 120 day experiment than the treatments with manure alone ($\alpha = 0.05$). The manure NH_4-N concentration averaged 176 mg NH_4-N per kg of manure (DM basis) with a range of 63 to 385 mg NH_4-N per kg of manure (DM basis). The manure source had no effect on manure NH_4-N after 120 days ($\alpha = 0.05$, Figure 2).

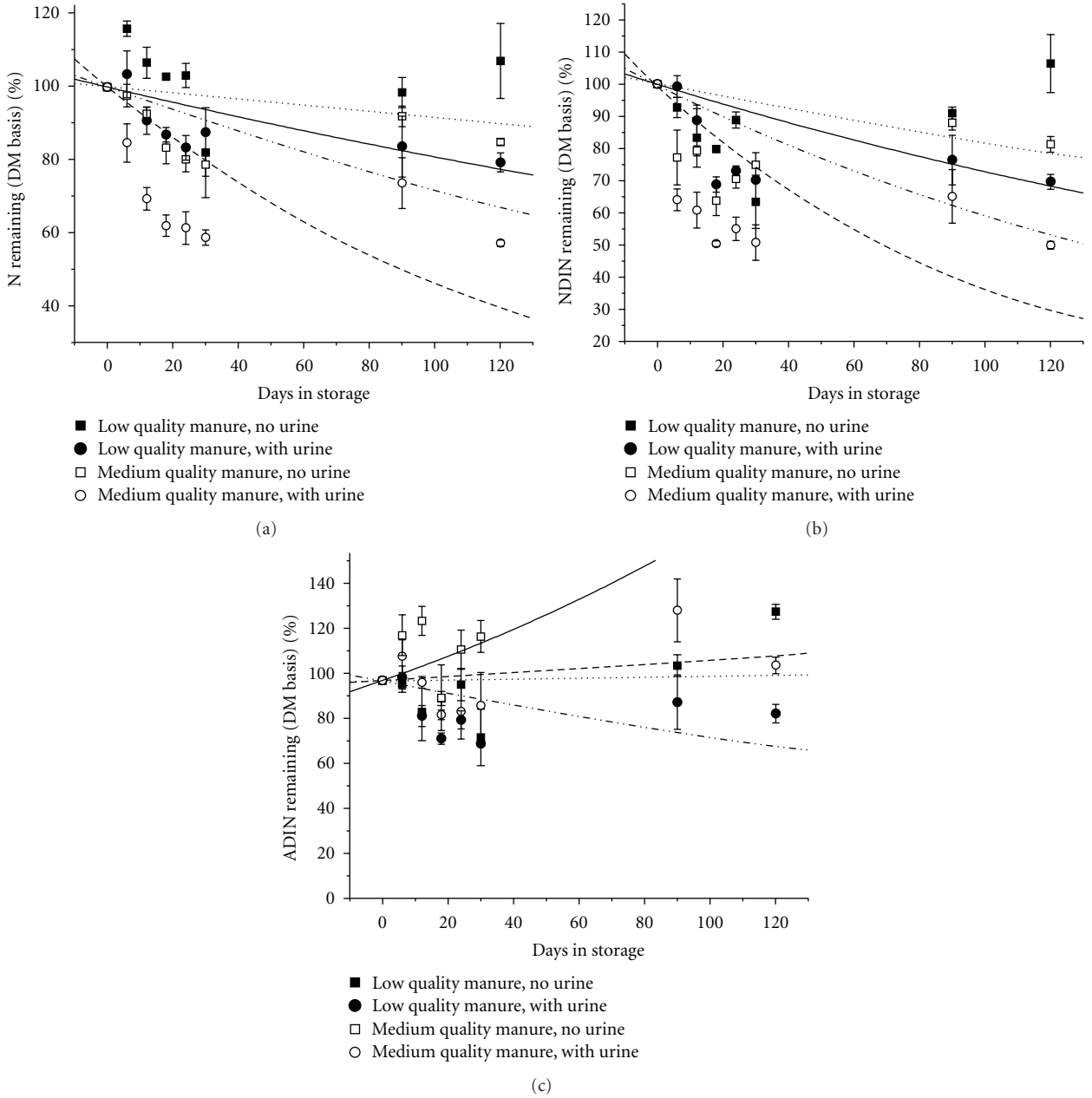


Figure 4: Total organic N, neutral detergent insoluble N (NDIN), and acid detergent insoluble N (ADIN) dynamics in manure in the dry season experiment (DM basis). Error bars represent standard error of means. Continuous lines represent best-fit regression equations for organic N dynamics in manure during the dry season experiment. Y represents the amount of organic N, NDIN, or ADIN remaining in the manure (% of initial content, DM basis). The solid line (—) represents the Medium quality manure, no urine treatment. The dashed line (- - - -) represents the urine-amended Medium quality manure treatment. The dotted line (· · · ·) represents the Low quality manure, no urine treatment. The dot-dash line (-·-·-·) represents the urine-amended Low quality manure treatment. (a) Total organic N dynamics (% N, DM basis). Medium quality manure, no urine: $Y = 100e^{-0.00213x}$, $R^2 = 0.978$. Medium quality manure, with urine: $Y = 100e^{-0.00768x}$, $R^2 = 0.916$. Low quality manure, no urine: $Y = 100e^{-0.00087x}$, $R^2 = 0.971$. Low quality manure, with urine: $Y = 100e^{-0.00327x}$, $R^2 = 0.982$. The rate equations for NDIN dynamics between the four treatments did not differ at significance level $\alpha = 0.05$. (b) NDIN dynamics (%NDIN, DM basis). Medium quality manure, no urine: $Y = 100e^{-0.00318x}$, $R^2 = 0.940$. Medium quality manure, with urine: $Y = 100e^{-0.00994x}$, $R^2 = 0.823$. Low quality manure, no urine: $Y = 100e^{-0.00202x}$, $R^2 = 0.945$. Low quality manure, with urine: $Y = 100e^{-0.00529x}$, $R^2 = 0.961$. The rate equations for organic N dynamics between the four treatments did not differ at significance level $\alpha = 0.05$. (c) ADIN dynamics (%ADIN, DM basis). Medium quality manure, no urine: $Y = 100e^{0.00528x}$, $R^2 = 0.976$. Medium quality manure, with urine: $Y = 100e^{0.000918x}$, $R^2 = 0.970$. Low quality manure, no urine: $Y = 100e^{0.000219x}$, $R^2 = 0.951$. Low quality manure, with urine: $Y = 100e^{-0.00288x}$, $R^2 = 0.964$. Rate constants for ADIN dynamics in manure with no urine added differed between the manure sources ($P < 0.0001$). No difference was observed in the urine treatments ($\alpha = 0.05$).

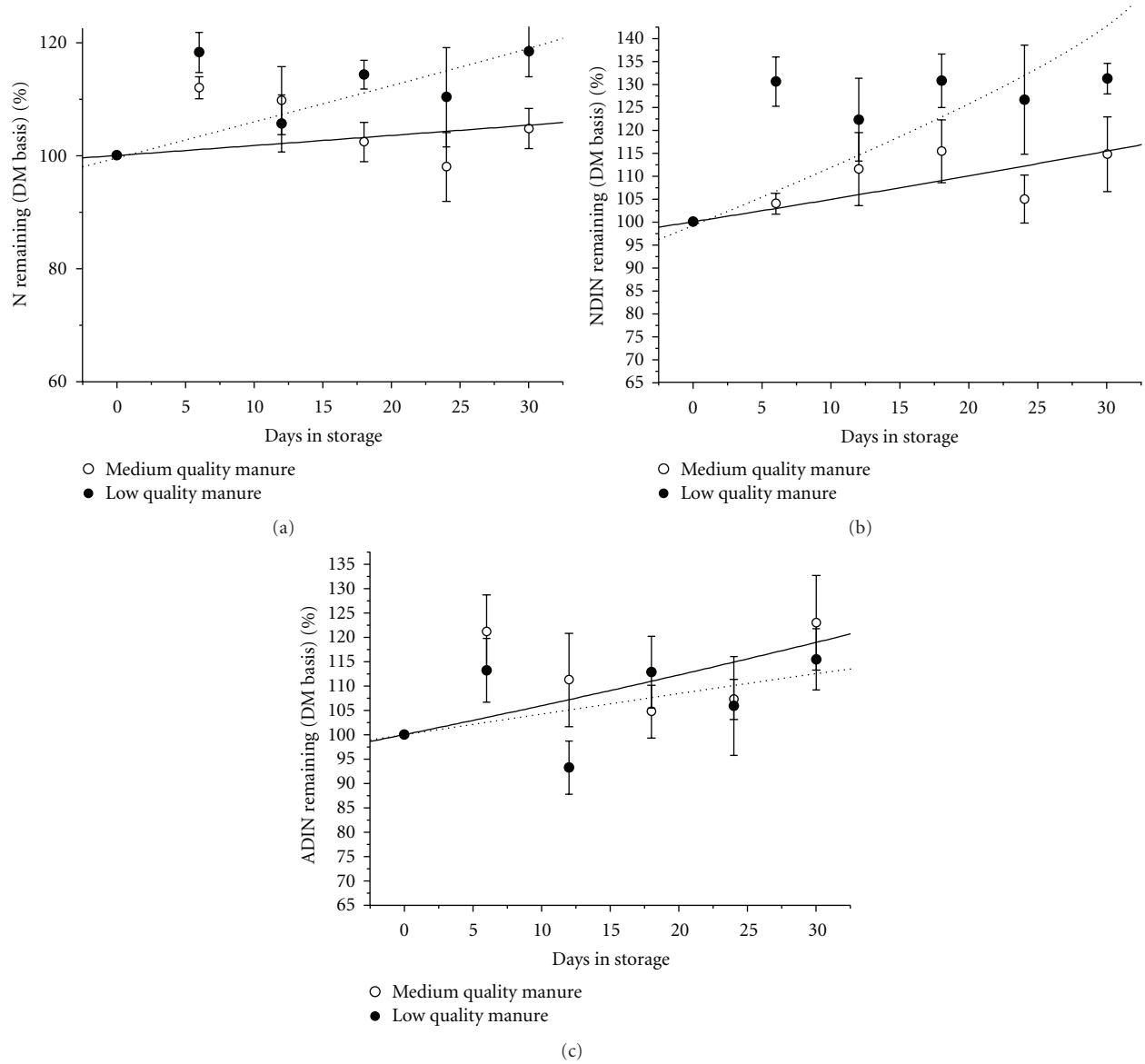


Figure 5: Total organic N, neutral detergent insoluble N (NDIN), and acid detergent insoluble N (ADIN) dynamics in manure in the rainy season experiment (DM basis). Error bars represent standard error of means. Continuous lines represent regression equations for organic N dynamics in manure during the rainy season experiment. Solid lines (—) represent the best fit regression lines for Medium quality manure treatments. Dashed lines (- - - -) represent the best fit regression lines for Low quality manure treatments. Y represents the amount of organic N, NDIN, or ADIN remaining in the manure (% of initial content, DM basis). (a) Total organic N dynamics (%N, DM basis). Medium quality manure: $Y = 100e^{0.00174x}$, $R^2 = 0.989$. Low quality manure: $Y = 100e^{0.00576x}$, $R^2 = 0.986$. The rate equations for organic N dynamics between the two manure sources did not differ at significance level $\alpha = 0.05$. (b) Neutral detergent insoluble N dynamics (%NDIN, DM basis). Medium quality manure: $Y = 100e^{0.00478x}$, $R^2 = 0.984$. Low quality manure: $Y = 100e^{0.0112x}$, $R^2 = 0.973$. The rate equations for NDIN dynamics between the two treatments did not differ at significance level $\alpha = 0.05$. (c) Acid detergent insoluble N dynamics (%ADIN, DM basis). Medium quality manure: $Y = 100e^{0.00578x}$, $R^2 = 0.974$. Low quality manure: $Y = 100e^{0.00394x}$, $R^2 = 0.973$. The rate equations for ADIN dynamics between the two treatments did not differ at significance level $\alpha = 0.05$.

The presence of urine lowered the fiber-bound N content of the manure in storage. After 120 days of storage during the dry season experiment, the Medium quality manure alone contained 4.9% NDIN, and 3.9% ADIN (DM basis). Urine-amended Medium quality manure contained 3.9% NDIN, and 3.2% ADIN (DM basis). At the end of the dry season experiment, the Low quality manure alone contained 6.2% NDIN, and 5.2% ADIN (DM basis). Low quality manure containing urine contained 5.0% NDIN, and 4.1% ADIN. At the end of the dry season experiment, the Low quality manure contained more NDIN and ADIN than the medium manure ($P < 0.02$ and $P < 0.03$, resp.). The average organic N content of manure after 120 days of storage was 8.6% (DM basis). Manure from both sources without urine contained

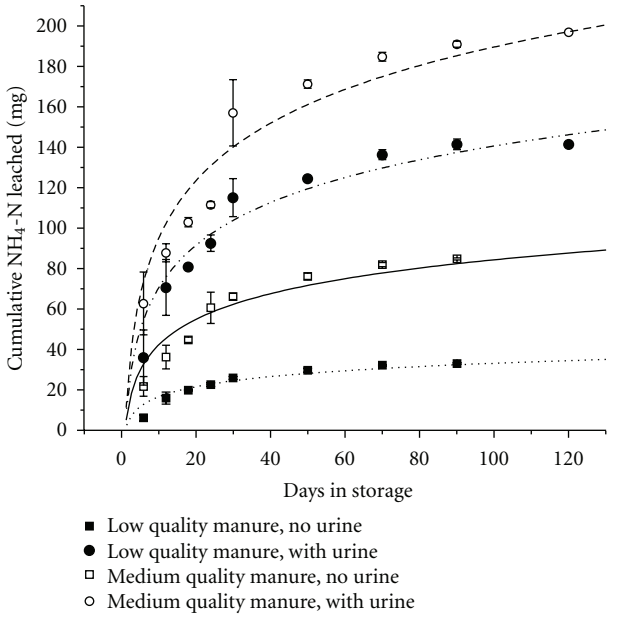


Figure 6: Cumulative NH_4-N (mg NH_4-N per manure storage unit) leached from manure of the dry season storage experiment. Error bars represent standard error of the mean values. Continuous lines represent best-fit regression equations for cumulative NH_4-N (mg) leached from manure during the dry season experiment. The solid line (—) represents the Medium quality manure, no urine treatment. The dashed line (- - - -) represents the urine-amended Medium quality manure treatment. The dotted line (· · · ·) represents the Low quality manure, no urine treatment. The dot-dash line (-·-·-·) represents the urine-amended Low quality manure treatment. Y represents the cumulative NH_4-N (mg) leached from the manure. Medium quality manure, no urine: $Y = 18.3144\ln(x)$, $R^2 = 0.989$. Medium quality manure, with urine: $Y = 41.1785\ln(x)$, $R^2 = 0.993$. Low quality manure, no urine: $Y = 7.1964\ln(x)$, $R^2 = 0.988$. Low quality manure, with urine: $Y = 30.5345\ln(x)$, $R^2 = 0.994$. The rate equations for leachate NH_4-N between the four treatments did not differ at significance level $\alpha = 0.05$.

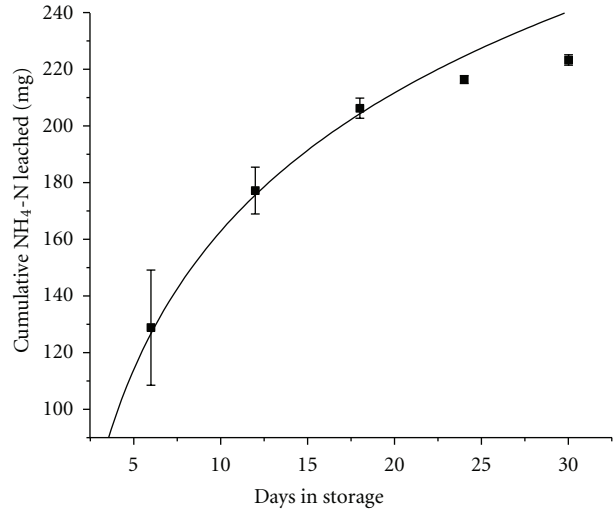


Figure 7: Cumulative NH_4-N (mg NH_4-N per manure storage unit) leached from manure of the rainy season storage experiment. Error bars represent standard error of the mean values. The continuous line represents best-fit regression equations for cumulative NH_4-N (mg) leached from manure during the dry season experiment. X represents the number of days in storage. The solid line represents all treatments. Y represents the cumulative NH_4-N (mg) leached from the manure. All treatments: $Y = 70.7127\ln(x)$, $R^2 = 0.998$.

more NDIN at the end of 120 days than did the urine treatments ($P < 0.05$). While the time in storage did have an effect on the organic N, ADIN, and NDIN contents of the dry season manure over the entire experiment ($P < 0.0001$, Table 1), total organic N and ADIN did not differ between the treatments after 120 days in storage ($\alpha = 0.05$). Exponential decay models with one exponential term were developed for total N, NDIN, and ADIN dynamics in manure (Figures 4(a)–4(c)). Exponential regression with two terms to describe total N, NDIN, and ADIN dynamics in manure failed to converge.

Logarithmic regression using equations with two terms to describe NH_4-N leaching losses did not converge. Cumulative leaching of NH_4-N from manure in the dry season experiment is described by the single-term logarithmic rate equations in Figure 6. The presence of urine had a significant positive effect on the amount of NH_4-N leached from manure in the dry season experiment ($P < 0.02$, Table 3). In total, the urine-amended manure units lost an average of 199 mg NH_4-N while storage units containing only manure lost an average of 104 mg NH_4-N over 120 days of storage. These cumulative leaching losses represents up to 26% of the initial manure NH_4-N from urine-amended manure and up to 61% of the initial manure NH_4-N from manure alone. The source of the manure, low farm or medium farm, had no effect on NH_4-N leaching rates ($\alpha = 0.05$, Table 3).

4. Discussion

The 14% greater loss of mass from urine-amended manure observed in this study, a loss of 0.6 kg manure DM in urine-amended manure and 0.5 kg manure DM from manure alone, was a larger difference than expected. The observation supports the perception of smallholder farmers that urine speeds up the decomposition of manure in storage [1]. In previous studies of composted manure, the manure lost mass over time due to the mineralization of nutrients and other degradation processes that cause structural disintegration [19]. In this study, mass loss was observed. The manure continued to lose solid mass after 30 days of storage as a result of microbial activity. Farmers may find the faster degradation of urine-amended manure unacceptable because it results in a smaller volume of manure available to apply to the soil at planting. However, urine may increase the rate of decomposition of the feed refusals in manure, thereby lowering the C : N ratio of the manure and lowering the risk of N immobilization when manure is applied to soil.

The results partially support the hypothesis that most N losses from stored manure occur within the first month of storage. In the dry season and rainy season experiments,

NH_4-N losses were large during the first 30 days of storage. In both experiments, the pools of total organic N, the more degradable hemicellulose-bound N, and the refractory fiber-bound and lignin bound N had increased in content by the end of the storage period (Figures 4(a)–4(c) and 5(a)–5(c)). The increase in the recalcitrant N fractions is probably due to manure mass loss: as the manure loses mass over time, the concentration of the recalcitrant N fractions inflates [18].

Although some low molecular weight organic-N compounds may have escaped the system via the leachate, the bulk of the loss of the available N is due to the mineralization activities by microbes. The labile organic N remaining in the manure after 30 days is likely of microbial origin. The ADIN that remains, 5.1% of manure DM in the rainy experiment, likely is either part of the lignin fraction or indigestible components of microbial cell walls. The ADIN is very slowly mineralized by microbes [16, 17].

The hypothesis that manure containing urine would have more NH_4-N and less fiber-N and lignin-N than manure without urine was only partly upheld by the results of this study. In the dry season, the results were as expected. The presence of urine significantly decreased the organic N content in the manure. This effect may be due to the urine providing the microbes with a larger pool of NH_4-N for metabolism and growth than the NH_4-N pool in the manure solution alone. The less N-constrained environment of urine-amended manure supports larger bacterial populations than that of manure alone, so the microbial degradation of refractory manure compounds is more rapid when urine is present in the manure mixture. The results of another study of stockpiled manure on small Kenyan farms indicated that urine, when added to manure, had no effect on the nutrient composition of the manure [2]. This result may have occurred because the manure piles sampled in the study differed in age or because the urinary N was lost to volatilization before being mixed with manure. The manure came from actual manure storage units on many small farms in central Kenya, and manure age was not controlled [2].

Urine may also boost the mineralization of organic N, thereby lowering the total organic N content of the manure, by increasing the water content of the manure. This effect was observed in this study, with urine amendments producing manure with less fiber- and lignin-bound N than manure with no amendments (Figures 4(b) and 4(c)). The wetter environment and increased saturation of the manure may allow microbes easier access to sites where organic N is present. Also, solubilization of OM may be facilitated in the more saturated system. Dissolved organic matter (OM) is more easily degraded by microorganisms than solid OM [20]. The failure of urine to significantly affect the organic N composition of the manure in the rainy season experiment may be due to the fact that the heavy rainfall resulted in urine-amended and nonamended manure with similar water contents, so that any difference between the treatments was obscured (Figures 4(a)–4(c) and 5(a)–5(c)).

The hypothesis that the presence of urine in stored manure would leach a greater volume of leachate with a higher content of NH_4-N than manure without urine was supported in the rainy season experiment. Urine did increase the NH_4-N content of the leachate in both the dry season and rainy season experiments. These results suggest measures to prevent leaching losses, such as sheltering the manure from rain and creating a leachate catchment system in which the leachate is collected and returned to the manure pile, should be taken for urine-rich manure in order to conserve manure NH_4-N.

The hypothesis that rainfall increases N losses from manure via leaching was not supported by the data for the urine-amended manure. Total NH_4-N losses from urine-amended manure in the rainy season experiment were 100 mg more (1.5 times greater) than the amount lost from urine-amended manure in the dry season experiment. However, as a percentage of the initial manure NH_4-N concentration, the urine-amended manure in the dry season lost more NH_4-N than in the rainy season: a loss of 26% of the initial NH_4-N in the dry season experiment versus a 12% loss of the initial NH_4-N in the rainy season experiment. Heavy rainfall increased the volume of the leachate, and the NH_4-N content decreased accordingly for the no urine treatments. In spite of this result, the loss of 337 mg NH_4-N from urine-amended manure observed in the rainy experiment is an N loss that should be prevented in order to conserve manure N.

5. Conclusions

The results suggest the need to protect manure from rain to prevent leaching N losses. Collecting the leachate as it escapes the manure in storage and pouring it back onto the manure would be another way to conserve manure N. If manure is piled on a downhill slope immediately adjacent to fields that require fertilizing, the N-rich leachate may drain into the soil of those fields. If plastic sheeting, an affordable and readily available commodity in Kenya, is first placed on the ground and manure piled on top of it, more of the leachate would drain onto the field. Plastic sheeting could also be used to collect leachate to return to the manure. Contamination of drinking water by uncontrolled leachate runoff and seepage into soil can also be avoided by the use of plastic sheeting. A limitation of using plastic sheeting to collect leachate is the potential for large N losses from the leachate via NH_3 volatilization when the leachate is exposed to air. Covering stored manure using plastic sheeting or another material impervious to rainfall may conserve manure N by preventing leaching losses.

Manure that remains in storage for many months continues to lose mass because of microbial activity and compacts due to gravity. The result is manure with far less N than its initial content and less bulk manure to spread on the fields at planting time. To use the manure N before it is lost via leaching, it is recommended that manure not be stored more than 30 days. If the manure cannot be applied to major crops such as maize during the growing seasons, it might be applied to the kitchen gardens. These gardens often contain vegetables that provide important vitamins and minerals to the diets of farm residents. Selling manure to neighboring farms would generate income but could exacerbate the negative nutrient balances observed on most of the farms in the Kenyan highlands.

Abbreviations

OM: Organic matter
NDIN: Neutral detergent insoluble N
ADIN: Acid detergent insoluble N.

Acknowledgments

The authors would like to thank The World Agroforestry Centre (ICRAF) and staff, the Kenya Agricultural Research Institute and staff, the Bradfield Award at Cornell University, the Cornell Center for the Environment Graduate Research Award, Dr. James B. Robertson, and Dr. Stephen Njoka.

References

[1] J. K. Lekasi, J. C. Tanner, S. K. Kimani, and P. J. C. Harris, "High potential production system portfolio of the natural resources systems programme renewable natural resources knowledge strategy department for international development. ryton-on-dunsmore," in *Manure Management in the Kenya Highlands: Practices and Potential*, The Henry Doubleday Research Association (HDRA), Coventry, UK, 2nd edition, 2001.

[2] J. K. Lekasi, J. C. Tanner, S. K. Kimani, and P. J. C. Harris, "Cattle manure quality in maragua district, central kenya: effect of management practices and development of simple methods of assessment," *Agriculture, Ecosystems and Environment*, vol. 94, no. 3, pp. 289–298, 2003.

[3] M. C. Rufino, E. C. Rowe, R. J. Delve, and K. E. Giller, "Nitrogen cycling efficiencies through resource-poor African crop-livestock systems," *Agriculture, Ecosystems and Environment*, vol. 112, no. 4, pp. 261–282, 2006.

[4] M. K. Luebbe, G. E. Erickson, T. J. Klopfenstein, and M. A. Greenquist, "Aerobic composting or anaerobic stockpiling of feedlot manure. Nebraska Beef Cattle Reports," 2008, http://digitalcommons.unl.edu/animalscinbcr/25/.

[5] S. Tamminga, "Nutrition management of dairy cows as a contribution to pollution control," *Journal of Dairy Science*, vol. 75, pp. 345–357, 1992.

[6] H. K. Murwira, M. J. Swift, and P. G. H. Frost, "Animal-soil interactions: manure as a key resource in sustainable agriculture. livestock and sustainable nutrient cycling in mixed farming systems of sub-saharan africa. Vol. II: technical papers," in *Proceedings of an International Conference*, J. M. Powell, S. Fernandez-Rivera, T. O. Williams, and C. Renard, Eds., pp. 131–148, International Livestock Centre for Africa, Addis Ababa, Ethiopia, November 1993.

[7] J. C. Marini and M. E. Van Amburgh, "Nitrogen metabolism and recycling in Holstein heifers," *Journal of Animal Science*, vol. 81, no. 2, pp. 545–552, 2003.

[8] H. M. van Es, C. P. Gomes, M. Sellmann, and C. L. van Es, "Spatially-balanced complete block designs for field experiments," *Geoderma*, vol. 140, no. 4, pp. 346–352, 2007.

[9] J. E. Vercoe, "Breed and nutritional effects on the composition of faeces, urine, and plasma from Hereford and Brahman x Hereford steers fed on high and low quality diets," *Australian Journal of Agricultural Research*, vol. 18, pp. 1003–1013, 1967.

[10] A. L. Chaney and E. P. Marbach, "Modified reagents for determination of urea and ammonia," *Clinical Chemistry*, vol. 8, no. 2, pp. 130–132, 1962.

[11] AOAC, "Protein (crude) in animal feed, forage (plant tissue), grain, and oilseeds," Official Methods of Analysis of the Association of Official Analytical Chemists 954.01, AOAC, Gaithersburg, Md, USA, 2000.

[12] G. Licitra, T. M. Hernandez, and P. J. Van Soest, "Standardization of procedures for nitrogen fractionation of ruminant feeds," *Animal Feed Science and Technology*, vol. 57, no. 4, pp. 347–358, 1996.

[13] SAS Institute Inc (v 9.1), SAS software, Version 9.1.3 of the SAS System for Windows. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc. Cary, NC, USA, SAS Institute Inc.

[14] R. E. Muck and B. K. Richards, "Losses of manurial nitrogen in free-stall barns," *Agricultural Wastes*, vol. 7, no. 2, pp. 65–79, 1983.

[15] D. W. Bussink and O. Oenema, "Ammonia volatilization from dairy farming systems in temperate areas: a review," *Nutrient Cycling in Agroecosystems*, vol. 51, no. 1, pp. 19–33, 1998.

[16] T. K. Kirk, W. J. Connors, and R. D. Bleam, "Preparation and microbial decomposition of synthetic [^{14}C]lignins," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 72, no. 7, pp. 2515–2519, 1975.

[17] D. L. Crawford and R. L. Crawford, "Microbial degradation of lignocellulose: the lignin component," *Applied and Environmental Microbiology*, vol. 31, no. 5, pp. 714–717, 1976.

[18] O. Martins and T. Dewes, "Loss of nitrogenous compounds during composting of animal wastes," *Bioresource Technology*, vol. 42, no. 2, pp. 103–111, 1992.

[19] D. B. Dresbøll, J. Magid, and K. Thorup-Kristensen, "Long-term stability and mineralization rate of compost is influenced by timing of nutrient application during composting of plant residues," *Compost Science and Utilization*, vol. 14, no. 3, pp. 215–221, 2006.

[20] D. Said-Pullicino and G. Gigliotti, "Oxidative biodegradation of dissolved organic matter during composting," *Chemosphere*, vol. 68, no. 6, pp. 1030–1040, 2007.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 420624, 12 pages
doi:10.1155/2012/420624

Research Article

Cover Crop Biomass Harvest Influences Cotton Nitrogen Utilization and Productivity

F. Ducamp,[1] F. J. Arriaga,[2] K. S. Balkcom,[2] S. A. Prior,[2] E. van Santen,[1] and C. C. Mitchell[1]

[1] *Department of Agronomy and Soils, Auburn University, 202 Funchess Hall, Auburn, AL 36849, USA*
[2] *USDA-ARS National Soil Dynamics Laboratory, 411 S. Donahue Drive, Auburn, AL 36832, USA*

Correspondence should be addressed to F. J. Arriaga, francisco.arriaga@ars.usda.gov

Received 11 January 2012; Accepted 16 February 2012

Academic Editor: H. Allen Torbert

There is a potential in the southeastern US to harvest winter cover crops from cotton (*Gossypium hirsutum* L.) fields for biofuels or animal feed use, but this could impact yields and nitrogen (N) fertilizer response. An experiment was established to examine rye (*Secale cereale* L.) residue management (RM) and N rates on cotton productivity. Three RM treatments (no winter cover crop (NC), residue removed (REM) and residue retained (RET)) and four N rates for cotton were studied. Cotton population, leaf and plant N concentration, cotton biomass and N uptake at first square, and cotton biomass production between first square and cutout were higher for RET, followed by REM and NC. However, leaf N concentration at early bloom and N concentration in the cotton biomass between first square and cutout were higher for NC, followed by REM and RET. Seed cotton yield response to N interacted with year and RM, but yields were greater with RET followed by REM both years. These results indicate that a rye cover crop can be beneficial for cotton, especially during hot and dry years. Long-term studies would be required to completely understand the effect of rye residue harvest on cotton production under conservation tillage.

1. Introduction

Nitrogen is the most difficult nutrient to manage when growing cotton. About 5,445,749 ha of the cotton were planted in the USA in 2003 [1]. Applying optimum N rates is necessary to maximize economic yields and minimize the negative impacts that N overapplication can have on the crop and environment [2]. Higher N rates than required can result in excessive vegetative growth which increases the proportion of immature bolls, reduces lint quality and cotton yields, and increases disease and insect damage [3–6]. However, N deficiencies can reduce vegetative and reproductive growth and decrease yields [3]. Many parameters combine to determine the optimum N rates for cotton, such as soil type, location, N application method, tillage system, water availability, use of winter cover crops, and potential yield [7].

Conservation systems for cotton production in the southeastern US have increased in adoption to approximately 50% of the 2.9 million ha planted in this area [8]. The use of winter cover crops has been well documented as an effective method for improving soil chemical, biological, and physical properties [9, 10]. Among winter crop species, winter cereals like rye can have many benefits because they produce high amounts of biomass, are easy to establish and kill, and provide good ground cover during the winter [8, 11]. However, the high biomass grass cover crops can produce combined with their high C/N ratios and can lead to N immobilization, which can increase the N fertilizer demand for maximizing cotton yields [10, 12, 13]. Additionally, the probability of N immobilization increases when the N fertilizer is broadcast over a soil covered with grass residue [7].

Higher N fertilizer requirements for cotton following small grain cover crops were reported by Howard et al. [7], Varco et al. [14], and Mitchell [15]. Varco et al. [14] reported that 120 kg N ha^{-1} was required to achieve maximum cotton lint yields after a rye cover crop compared to 96 kg N ha^{-1} needed after winter fallow, but lint yields were greater after rye than winter fallow. Howard et al. [7] stated that for achieving similar yields, 101 and 67 kg N ha^{-1} were required for maximizing lint yields when cotton followed corn stover and native winter weed vegetation, respectively. However, it is expected that the long-term use of high biomass cover

crops in conservation tillage systems will increase the soil organic carbon levels with a simultaneous increase of organic fractions of N in the soil, and once a new equilibrium is reached, N rates for crops could be reduced due to an increase of N provided through mineralization [16].

Recently, it has been proposed that winter cover crop biomass could be used as an alternative source of energy or for animal feed. Alternative uses for cover crop biomass would help farmers to increase revenue while diversifying market opportunities [17]. Cover crop biomass removal could cause significant changes in soil C and N dynamics and also impact crop yields and their response to N fertilization. Crop biomass removal can cause reductions in soil organic C levels with a subsequent deterioration of soil physical, chemical, and biological properties [18–23]. As a result of these changes in soil properties, reductions in crops yields are expected to occur [24, 25]. The impact of residue removal on soil properties and crop productivity has been well documented, but no research has been conducted emphasizing the potential impact of winter cover crop biomass removal on cotton yields and its response to N fertilization under conservation tillage.

We speculate that when rye residue is removed, N rates required for maximizing cotton production could be reduced because of the lower effect of N immobilization under conditions of low levels of residue with a high C/N ratio. Even though differences in soil properties in response to new management practices require some time to occur, we consider that short-term rye residue removal may produce enough changes in the soil environment to cause reductions in cotton yields. The objectives of this research were (i) to determine the effect of rye residue management on cotton growth parameters and yield, (ii) to quantify the impact of rye residue management and cotton response to N fertilization, and (iii) to determine if optimum N rates for cotton can be reduced under rye residue removal conditions.

2. Materials and Methods

A 2-year field experiment under supplemental irrigation was established in November 2005 at the Alabama Agricultural Experiment Station's E.V. Smith Research Center, Field Crops Unit (32° 25′ 19″N, 85° 53′ 7″W), near Shorter in central Alabama, USA. The soil was a Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludult). This region is characterized by a humid subtropical climate, with an average annual precipitation of about 1100 mm [8]. The experimental area was previously managed with conventional tillage. Three rye RM schemes and four N rates were evaluated for cotton production. Rye RMs were no cover (NC), residue removed (REM), and residue retained (RET). Each RM was evaluated with cotton N fertilization rates of 0, 50, 100, and 140 kg ha^{-1} applied at the first pinhead square stage. The RMs were the main plots (18 m long by 8 m wide) and N rates for cotton were the subplots (9 m long by 4 m wide).

2.1. Soil Management. Before planting rye the first year, the entire area was deep-tilled with a noninversion, bent-leg subsoiler to a depth of 46 cm to remove any soil compaction present, and leveled with a field cultivator. In early May each year the experimental area was tilled in-row (1 m between rows) with a narrow-shanked subsoiler to a depth of 40 cm. The in-row tillage was conducted using a tractor equipped with an automatic steering system with centimeter level precision. The NC treatment was kept free of weeds during winter by applying herbicide when required.

2.2. Crop Management. Rye (cultivar "Elbon") was drilled at 100 kg ha^{-1}, in early November each year, using a no-till drill. Plots planted with rye received 40 and 30 kg N ha^{-1} as ammonium nitrate applied manually three weeks after planting and in late February, respectively. In the RET treatment, rye was rolled down at the early milk development stage [26] in late April each year and then sprayed with glyphosate (N-phosphonomethyl glycine) at a rate of 0.9 kg a.i. ha^{-1}. At the same time rye was terminated in the RET treatment, the aboveground rye biomass in the REM treatment was harvested with a small forage harvester to a height of 10 cm over the soil surface and removed from the plots.

The entire experimental area received an application of 21, 10, 42, and 6 kg ha^{-1} of nitrogen, phosphorus (P) as P_2O_5, potassium (K) as K_2O, and sulfur (S) as SO_4, respectively, each year by early May, based on the Alabama Cooperative Extension System soil test recommendations [27]. Cotton, cultivar DP 454 BG/RR (Delta Pine and Land Co., Scott, MS), was planted on May 19 and 18 in 2006 and 2007, respectively, using a four-row vacuum planter at a rate of 17 seeds m^{-1}. Row spacing was one meter. Herbicides, insecticides, defoliant, and boll opener applied to cotton were based on the Alabama Cooperative Extension System recommendations. The entire research area received supplemental irrigation of 70 and 160 mm during the 2006 and 2007 cotton seasons, respectively, using a linear-movement sprinkler irrigation system. Nitrogen treatments for cotton were applied manually as ammonium nitrate at the first pinhead square stage (37 days after planting (DAP)). Cotton was chemically defoliated and a boll opener was applied when 60–70% of the bolls in RET were opened. Before cotton harvest, one meter of each end of the plots was cut off with a rotary mower. After harvesting, cotton stalks were shredded with a rotary mower.

2.3. Data Collection. Cotton population, leaf blade samples, and seed cotton yield were determined from the two middle rows of each subplot and cotton biomass from the two exterior rows of each subplot. Cotton population was determined by counting the number of plants from a 3 m length in each of the two middle rows of the subplots at 37 DAP. Ten upper-most fully developed blades of leafs were collected from recently matured leaves in the upper canopy of each subplot, at 37 and 65 DAP in 2006 and at 37 and 69 DAP in 2007. Total aboveground cotton biomass was determined at 37 and 92 DAP in 2006 and 2007 by randomly cutting eight plants per subplot. Leaf blade and whole plant samples were oven dried at 55°C until constant weight, finely ground to pass a 1 mm sieve, and analyzed for total N by dry combustion using a LECO TruSpec analyzer (LECO

Table 1: Analysis of variance for cotton population, leaf N concentration, plant N concentration, cotton biomass, and N uptake at first square as affected by year and rye residue management. P values within a row in bold are significant at $\alpha \leq 0.05$.

Source of variation	Cotton population	Leaf N concentration	Plant N concentration	Cotton biomass	N uptake
			$P > F$		
Year	**≤0.01**	**≤0.01**	**≤0.01**	0.64	0.15
RM†	**≤0.01**	**≤0.01**	**≤0.01**	**≤0.01**	**≤0.01**
Year∗RM	0.58	**0.02**	**≤0.01**	0.33	0.58

† Rye residue management.

Corp., St. Joseph, MI). Total cotton biomass was estimated using the dry weight per plant and cotton population. Plant N uptake at each sampling time was calculated based on the total biomass and plant N concentration. The cotton biomass and N uptake between first square and cutout were calculated by subtracting the amount at first square from the amount at cutout for each parameter. Seed cotton yield was determined at 139 and 125 DAP in 2006 and 2007, respectively, by harvesting a 14.6 m^2 (2 m wide by 7.3 m long) area from each subplot using a spindle picker.

2.4. Weather. Daily average temperature data for both years were taken from an automated weather station located at the Experimental Station, beginning when cotton was planted and ending at the cutout stage of cotton development. Daily heats units (HUs) between planting and cutout were calculated as the difference between the average daily temperature and a base temperature of 15.6°C [28]. Rainfall and irrigation during each season were measured directly in the experimental area with a rain gauge connected to a data-logger.

2.5. Experimental Design and Statistical Analyses. The experiment was arranged in a randomized complete block design (RCBD) with a split-plot restriction on the randomization and four replications. Rye RM was the main factor and N rates for cotton the subfactor. As N treatments were applied at the first pinhead stage of cotton development, data collected before this N application were analyzed using the MIXED procedure of SAS [29] only considering the RM effect (RCBD). The LSMEANS PDIFF option was used to establish mean differences between RM treatments. Data collected after applying N treatments to cotton were analyzed through covariance analysis using the MIXED procedure of SAS [29] considering N as covariate. Replication and its interactions were considered as random effects. Treatments and year were considered fixed effects. When a significant interaction including year occurred, data were presented separately for each year. When Year × RM × N or RM × N interactions were not significant, the LSMEANS PDIFF option was used to establish means differences between RM treatments. The covariance analysis was used to evaluate linear and quadratic effects of N rates on cotton parameters measured and to fit the best linear or quadratic regression model. Linear or quadratic effects were considered significant when $P \leq 0.15$ [30]. Treatment effects and differences of least squares means were considered significant when $P \leq 0.05$.

3. Results and Discussion

3.1. Climate Data. Rainfall and irrigation during both years were different in amount and distribution. In 2006, rainfall and irrigation between one week before planting cotton and cutout were 247 and 70 mm, respectively. For the same period during 2007, they were 207 and 176 mm, respectively. Rainfall in 2006 and 2007 was 23 and 36% lower than the 10-year average. In 2006, rainfall was below the 10-year average until midseason, after which it was similar or greater. However, in 2007 rainfall was below the 10-year average early and late in the cotton season and it was not uniformly distributed, with 75% of rainfall occurring during the first 10 days of July, resulting in a higher amount of irrigation applied during 2007. The main difference in HU between years occurred at the end of the cotton season. For the last 20-day period before cutout, HUs in 2007 were 20% higher than that in 2006, indicating that higher temperatures occurred during this period in 2007 with respect to 2006.

3.2. Cotton Population. Rye residue management had a significant effect on cotton population 37 DAP, across years (Table 1). Rye residue retained had a significantly higher population than NC ($P \leq 0.01$), but population for REM was not significantly different with respect to the other two treatments. Population for RET was 4 and 7% greater than REM and NC, respectively. Tillage operations were identical among RM, so differences in cotton populations can be attributed to differences in soil water content among treatments during the establishment period of the crop. Higher soil water content was measured in RET compared to REM and NC until 20–25 days after cotton planting in both years (data not shown) which probably contributed to better plant establishment.

Cotton population was also significantly different ($P \leq 0.01$) between years when averaged across RM. Higher cotton populations were observed in 2006 compared to 2007. In both years, the quality of the seed bed at planting and the soil water content between planting and the following two weeks were similar, indicating that other factors could be responsible for this difference between years. Accumulated HUs during the first 13 days after planting were 24% lower in 2007 compared to 2006, indicating that this period of 2007 was colder than 2006. These low temperatures could explain the population reduction in 2007, which probably contributed to slower plant growth, extending the period of time that young plants are susceptible to water deficit and

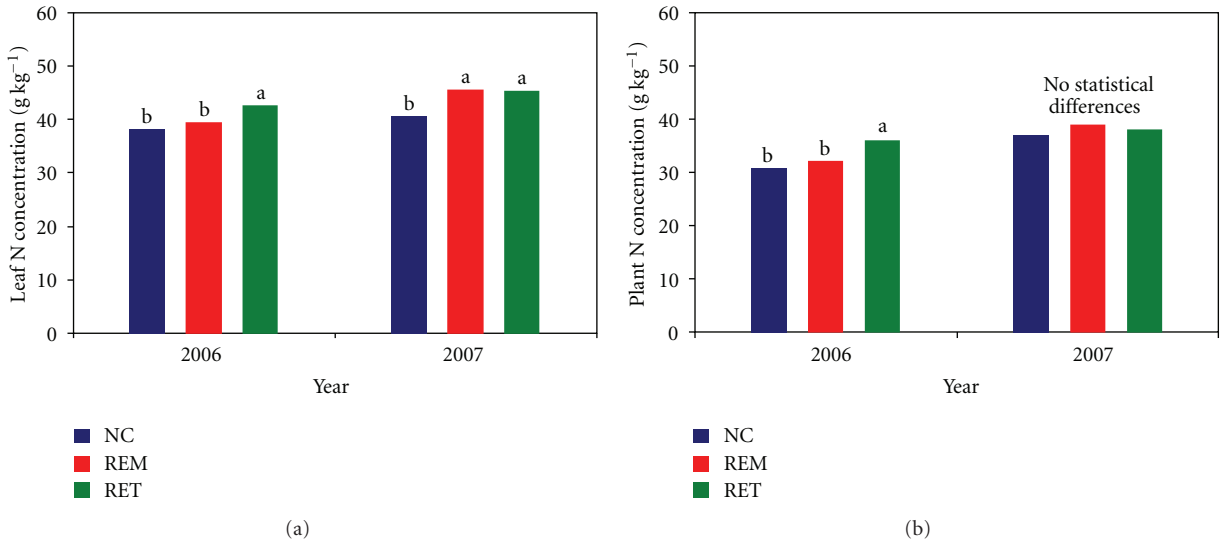


Figure 1: Effect of rye residue management and year on (a) leaf N concentration and (b) plant N concentration, at first square. Columns sharing the same letter for each parameter inside of year are not significantly different ($P \leq 0.05$). NC: no winter cover crop; REM: rye residue removed; RET: rye residue retained.

pest damage. Cotton populations in 2006 and 2007 were about 150,000 and 140,000 plants ha^{-1}, respectively, which were in a range considered high for cotton production, even though seed cotton yields can be stable for a wide range of plant densities [31]. However, this yield stability may be threatened if dry periods occur later in the growing season.

3.3. Leaf and Plant N Concentration at First Square. There was a significant Year × RM interaction for leaf and plant N concentration at first square (Table 1). In 2006, RET had a significantly higher leaf N concentration than NC and REM ($P \leq 0.01$ and $P = 0.03$, resp.), and NC was not significantly different from REM (Figure 1(a)). In 2007, RET and REM had significantly higher leaf N concentration than NC ($P \leq 0.01$ and $P \leq 0.01$, resp.), but differences between these two treatments were not significant. Leaf N concentration values ranged between 38 and 43 g kg^{-1} in 2006 and between 40 and 46 g kg^{-1} in 2007. These values were lower than the 54 g kg^{-1} critical level reported by Bell et al. [32] at first pinhead square, for cotton in the southern USA. The N applied before planting and the N possibly provided through mineralization were not enough to increase leaf N concentration to this critical value. Nonetheless, Bell et al. [32] also stated that high cotton yields can still be achieved by crops having low leaf N at first pinhead square, if N deficiencies are corrected at this stage of development and leaf N concentrations at early bloom are in the sufficiency range. Further, leaf N concentration levels in our study were in the sufficiency range reported by Mills and Jones [33].

In 2006, RET had a significantly higher plant N concentration than REM and NC ($P \leq 0.01$ and $P \leq 0.01$, resp.), but REM was not significantly different from NC (Figure 1(b)). However, in 2007 differences among RM treatments were not significant and plant N concentration values were very similar for each treatment. Plant N concentration for RM in 2006 followed a similar pattern to leaf N concentration, but their values were lower. This is expected because plant samples that include older tissues other than upper leaves are characterized by lower N concentrations. Further, there was a higher accumulation of HU during June 2007 compared to June 2006, which could help explain the greater leaf and plant N concentrations at first square. Additionally, the amount of soil mineral N at first square was 19% higher in 2007 compared to 2006 averaged across RM (data not shown), indicating that N availability during June was probably greater in 2007. However, soil mineral N amounts for all RM treatments in both years appeared to be sufficient, indicating that N availability was not a limiting factor for cotton growth at first square.

3.4. Cotton Biomass and N Uptake at First Square. Rye RM had a significant influence on cotton biomass and N uptake at first pinhead square, averaged across years (Table 1). The RET treatment had significantly higher cotton biomass than NC and REM ($P \leq 0.01$ and $P \leq 0.01$, resp.; Figure 2). Rye residue removed had a cotton biomass 35% higher than NC, but this difference was not significant. Similar results were obtained for N uptake, with RET having values 96 and 166% higher than REM and NC ($P \leq 0.01$ and $P \leq 0.01$, resp.). No significant differences occurred between REM and NC, but N uptake was 39% higher for REM (Figure 2). Differences in N uptake among RM treatments can be partially explained by differences in cotton biomass and plant N concentration. When averaged across years, plant N concentration was 34, 35.7, and 37.1 g N kg^{-1} for NC, REM, and RET, respectively. Although these two growth parameters influenced N uptake in the same manner, cotton biomass could have had the highest impact on N uptake, because its variability between RM treatments was higher in proportion to plant N concentration.

Table 2: Analysis of variance for the effect of year, rye residue management, and N fertilization on leaf N concentration at early bloom, cotton biomass, cotton biomass N concentration and N uptake between first square and cutout, and seed cotton yield. *P* values within a row in bold are significant at $\alpha \leq 0.05$.

Effect	Leaf N concentration†	Cotton biomass‡	Cotton biomass N concentration‡	N uptake‡	Seed cotton yield
			P > *F*		
Year	**≤0.01**	**0.04**	**≤0.01**	**≤0.01**	**≤0.01**
RM$^{\delta}$	**0.03**	**≤0.01**	**≤0.01**	0.63	**≤0.01**
N	**≤0.01**	**≤0.01**	**≤0.01**	**≤0.01**	**≤0.01**
Year∗RM	0.23	0.95	0.30	0.79	**≤0.01**
Year∗N	0.57	**0.02**	0.15	**≤0.01**	**≤0.01**
RM∗N	0.76	0.07	0.82	**0.05**	**0.02**
Year∗RM∗N	0.16	0.99	0.45	0.83	**≤0.01**

†At early bloom.
‡Between first square and cutout.
$^{\delta}$RM: rye residue management.
N: nitrogen.

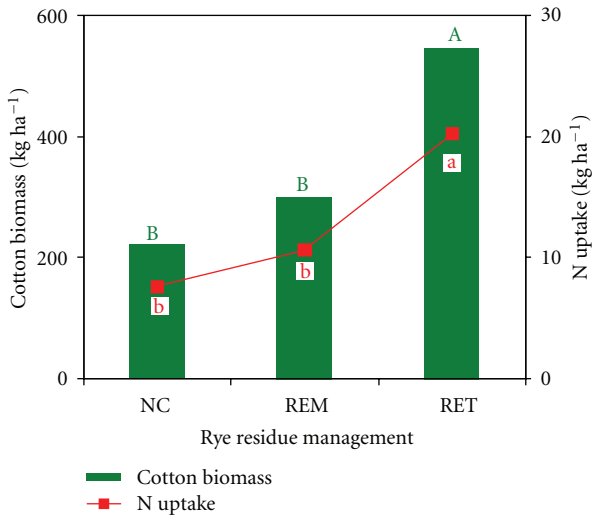


Figure 2: Effect of rye residue management on cotton biomass and N uptake at first square, averaged across years. Means followed by the same letter for each parameter are not significantly different ($P \leq 0.05$). NC: no winter cover crop; REM: rye residue removed; RET: rye residue retained.

These results show that in both years RET provided better conditions for cotton growth and N uptake early in the season. This could have been a consequence of greater N availability between planting and first square, as indicated by the residual levels of N at this time, and also because of the higher soil water content during this period of time. Although plant populations were greater with RET than REM and NC, plant biomass was also greater with RET at first square (130 and 77% greater relative to NC and REM, resp.). Further, the lack of a Year × RM interaction and a Year effect on cotton biomass and N uptake suggests that, at least until this stage of development, growth of the cotton crop was very similar in both seasons.

3.5. Leaf N Concentration at Early Bloom. Leaf N concentration at early bloom was significantly affected by RM, N rate, and year, but interactions were not significant (Table 2). No winter cover crop and REM had significantly higher leaf N concentration compared to RET ($P \leq 0.01$ and $P \leq 0.01$, resp.). It is possible that the rye residue immobilized some of the soil mineral N between first square and early bloom, decreasing the availability of N for cotton and reducing the N concentration in cotton tissues. Another possible explanation can be that higher cotton biomass production in RET could have caused a N dilution effect within cotton tissues. Cotton biomass was not measured at this growth stage, but plant heights at this sampling time averaged across years and N rates showed that plants in RET were taller than in REM and NC (data not shown), indicating a possible higher cotton biomass and potential N dilution effect. Similar results were reported by Fridgen and Varco [34] and Balkcom et al. [35], who found a dilution of leaf N when cotton biomass production was high.

Averaged across years and RM, leaf N concentration responded in a quadratic manner to fertilizer N rate, with a maximum leaf N concentration of 40 g kg^{-1} observed at the highest N rate applied (Figure 3(a)). This value was very close to the sufficiency range of 43 g kg^{-1} reported by Bell et al. [32]. However, our results do not agree with Fridgen and Varco [34], who reported a higher leaf N concentration using similar N rates as we did. Further, a maximum leaf N concentration was not achieved even with the highest N rate applied. This indicates that to reach the maximum leaf N concentration in the conditions of this experiment would have required a greater fertilizer N application than 140 kg ha^{-1}.

Year also significantly affected leaf N concentration at early bloom. The leaf N concentration was significantly lower ($P \leq 0.01$) in 2007, with a decrease of 22% with respect to 2006 (Figure 3(b)). Rainfall distribution and HU during 2007 could explain this trend between years. A more detailed analysis of the rainfall effect on cotton growth parameters will be provided when data of cotton biomass N concentration are presented hereinafter.

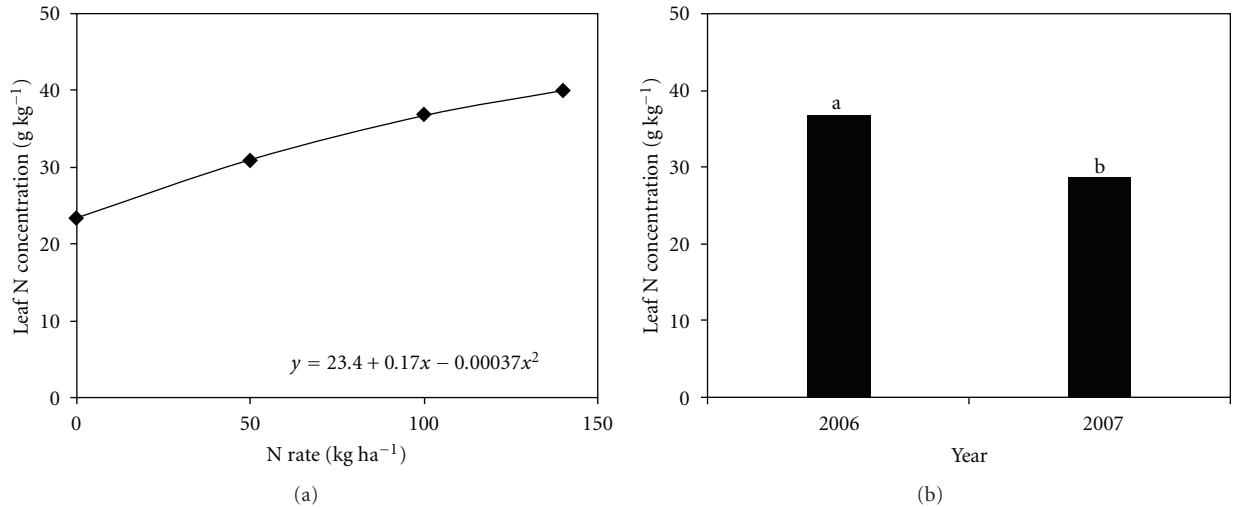


Figure 3: Leaf N concentration at early bloom as affected by (a) N rate (averaged across years and rye residue management) and (b) year (averaged across rye residue management and N rates). Columns followed by different letters are significantly different ($P \leq 0.05$).

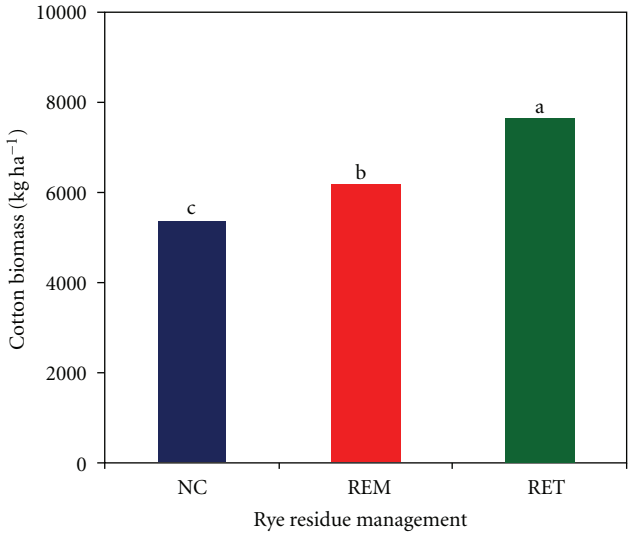


Figure 4: Effect of rye residue management on cotton biomass production between first square and cutout, averaged across years and N rates. Columns sharing the same letter are not significantly different ($P \leq 0.05$). NC: no winter cover crop; REM: rye residue removed; RET: rye residue retained.

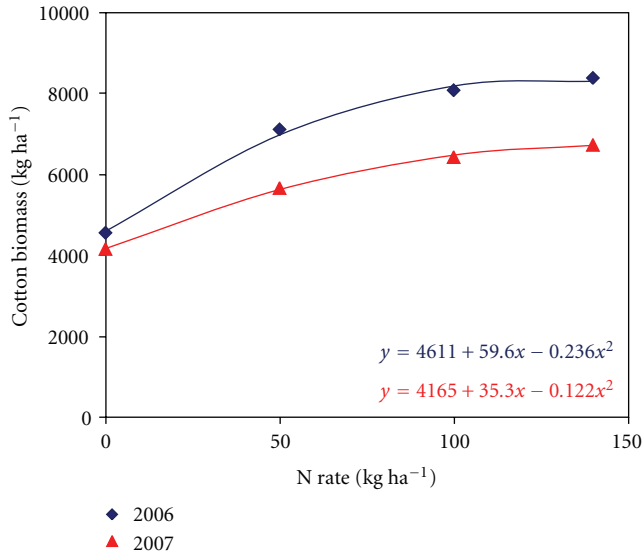


Figure 5: Cotton biomass production between first square and cutout as affected by year and N rate, averaged across rye residue management.

3.6. Cotton Biomass Production between First Square and Cutout. Rye RM had a significant effect on cotton biomass production between first square and cutout, averaged across years, and N rates (Table 2). Rye residue retained had significantly higher cotton biomass production than REM and NC (both of which were $P \leq 0.01$), and REM was significantly higher than NC ($P \leq 0.01$) (Figure 4). Cotton biomass production for RET was 24 and 43% higher than REM and NC, respectively, while REM was 16% higher than to NC. These results demonstrate that RET provided better conditions for cotton growth. Govaerts et al. [19] reported that keeping residue on the soil surface improves infiltration, increasing water available for plants.

The cotton biomass response to N produced a significant interaction with year, when averaged across RM treatments (Table 2). In both seasons, cotton biomass response to N was quadratic (Figure 5). The small increase between the 100 and 140 kg ha^{-1} N rates indicates that the N rate required to maximize cotton biomass would be similar to the highest rate used in this experiment. Cotton biomass in 2006 was similar to the one reported by Bassett et al. [36] for an N rate of 134 kg ha^{-1}, but it was extremely low compared to the findings of Boquet and Breitenbeck [2]. In spite of the similar trend between both seasons, cotton biomass was lower for all N rates in 2007 than that in 2006. The difference for the no N control was very low between years, with a decrease of

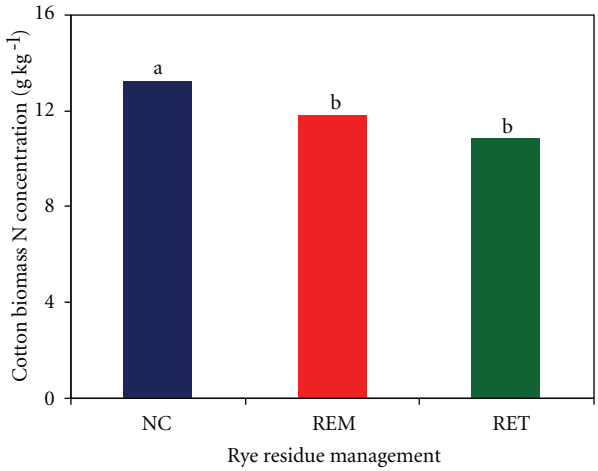


FIGURE 6: Effect of rye residue management on cotton biomass N concentration between first square and cutout, averaged across years and N rates. Columns sharing the same letter are not significantly different ($P \leq 0.05$). NC: no winter cover crop; REM: rye residue removed; RET: rye residue retained.

9% in 2007 compared to 2006. However, about 20% less biomass was produced in 2007 than that in 2006 when N was applied independent of the N rate used. This cotton biomass reduction could be explained by the lower rainfall and nonuniform distribution during 2007. Additionally, the last 20 days before cutout in 2007 were characterized by elevated temperatures as indicated by the higher accumulation of HU units relative to 2006. High temperatures and low rainfall in 2007 could have imposed a stress to the crop causing lower biomass production. This may have occurred even though irrigation was applied since low amounts of water were applied with each irrigation event (10 to 12 mm) and the high temperatures would have increased water loss due to evapotranspiration. These results agree with Balkcom et al. [35], who reported lower cotton biomass in hot and dry years regardless of irrigation.

3.7. Cotton Biomass N Concentration. The N concentration in cotton biomass accumulated between first square and cutout was significantly affected by RM, N, and year, but interactions were not significant (Table 2). No winter cover crop had 12 and 22% significantly higher cotton biomass N concentration compared to REM and RET, respectively ($P = 0.03$ and $P \leq 0.01$, resp.; Figure 6). Rye residue removed had a numerically higher cotton biomass N concentration compared to RET (9%), but this difference was not significant. As previously mentioned, N immobilization probably occurred in both growing seasons but at low levels. This would indicate that the reduction in cotton biomass N concentration in REM and RET relative to NC could be explained by the higher cotton biomass compared to NC (Figure 4) which may have contributed to a dilution of N in cotton tissues. Rye residue retained and REM accumulated 43 and 16% more biomass between first square and cutout than NC, respectively, but their increment in N uptake relative to NC was only 18 and 5%, respectively. This result also suggests an occurrence of N dilution in the accumulated biomass. Gerik et al. [3] and Bell et al. [32] reported that under conditions of high availability of N, cotton plants increase vegetative growth very quickly which leads to a N dilution in the biomass produced and a subsequent drop in tissue N concentration.

Cotton biomass N concentration response to N rates was linear (averaged across years and RM), indicating that the highest N rate applied did not maximize N concentration in the biomass (Figure 7(a)). This trend was similar to that observed for leaf N concentration, even though that response to N was quadratic.

Cotton biomass N concentration was significantly influenced by year (Table 2). In 2007, there was a significant decrease ($P \leq 0.01$) of about 28% in cotton biomass N concentration compared to 2006 (Figure 7(b)). A similar pattern was also observed at early bloom for leaf N concentration and cotton biomass production. There was a simultaneous decrease in cotton biomass and N concentration, but the reduction in cotton biomass N concentration was greater with respect to cotton biomass (28 versus 18%, resp.), providing strong evidence that N dilution in plant tissues occurred. These results indicate that the 2007 crop was affected by N dynamics in the soil-plant system. The rainfall regime during 2007 may have played an important role in these findings. The high rainfall that occurred during the first 10 days of July (about 150 mm) was twice than the 70 mm of available water that the soil in the experimental area can retain to a depth of 50 cm. The excess rainfall above the soil water holding capacity could have leached part of the N fertilizer out of the root zone. These high rainfall events at the beginning of July in 2007 occurred only one week after the N fertilizer was applied to cotton.

3.8. Nitrogen Uptake between First Square and Cutout. Table 2 shows that there was a significant RM × N interaction for N uptake between first square and cutout. Nitrogen uptake response to N rates was linear for RET and REM, whereas for NC this relationship was best described by a quadratic model (Figure 8(a)). The response of N uptake per kg of N added up to the highest N rate was 0.49, 0.61, and 0.68 for NC, REM, and RET, respectively. Cotton plants in RET absorbed more N independent of the N rates applied. Even though RET had higher values of N uptake than REM and NC at all N rates, these differences were magnified with increasing rates of N fertilizer (Figure 8(a)). The highest N uptake for each RM treatment occurred at the highest N rate applied, where at this rate, N uptake for RET was 32 and 15% higher than NC and REM, respectively, and it was 15% higher for REM compared to NC. The linear relationship between N uptake and N rate for RET and REM indicates that the highest N rate applied was not enough to maximize N uptake under the conditions of this experiment. Conversely, NC had a quadratic relationship with a very low N uptake increment between the 100 and 140 kg ha^{-1} N rates, indicating that the N rate required for maximizing N uptake was very similar to the highest N rate we applied. Our results

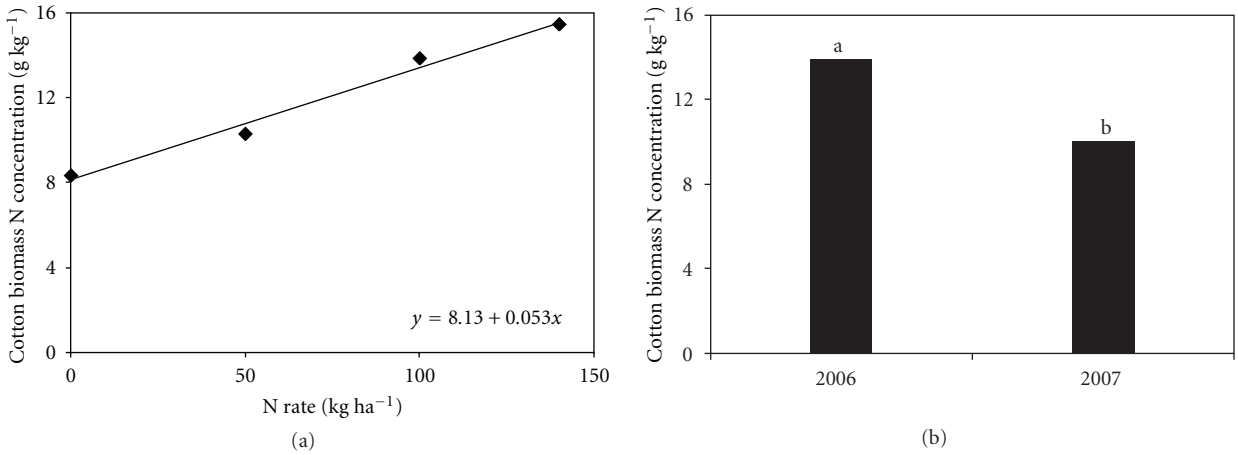


Figure 7: Cotton biomass N concentration between first square and cutout as affected by (a) N rate (averaged across years and rye residue management) and (b) year (averaged across rye residue management and N rates). Columns followed by the same letter are not significantly different ($P \leq 0.05$).

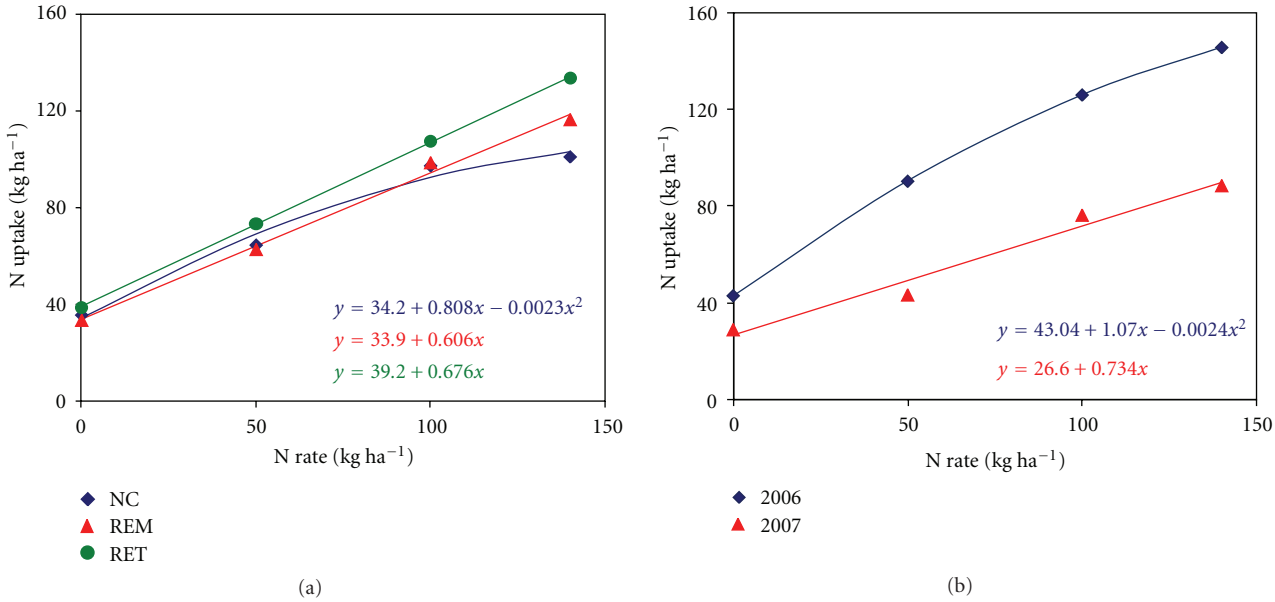


Figure 8: Cotton N uptake between first square and cutout as affected by (a) rye residue management and N rate (averaged across years) and (b) year and N rate (averaged across rye residue management).

for RET were similar to the findings of Basset et al. [36], who found a total N uptake of 142 kg ha^{-1} for irrigated cotton that received 134 kg N ha^{-1}. However, a study by Mullins and Burmester [37] revealed greater N taken up with an N rate of 112 kg ha^{-1}. The N uptake by cotton plants at the highest N rate (140 kg N ha^{-1}) represented 72, 83, and 95% of the N added, for NC, REM, and RET, respectively. This would indicate that RET provided better growing conditions for cotton that possibly improved the N use efficiency of the fertilizer applied.

The amount of N absorbed by a crop depends on its biomass production and its N tissue concentration. When averaged across years, the interaction RM × N was not significant for cotton biomass N concentration ($P = 0.82$) and cotton biomass production ($P = 0.07$) between first square and cutout (Table 2). Even though no significant interaction existed, cotton biomass N concentration values were slightly higher for NC, followed by REM and RET, but cotton biomass was higher for RET, followed by RM and NC, at each N rate (Table 3). This tendency supports the findings of higher biomass production with RET compared to REM and NC (particularly for the 100 and 140 kg ha^{-1} N rates) and its greater N uptake. These results agree with Gastal and Lemaire [38], who stated that N taken up by crops is mainly affected by the crop growth rate.

There was a significant Year × N interaction for cotton N uptake between first square and cutout (Table 2). Nitrogen uptake response was quadratic in 2006 and linear in 2007

Table 3: Effects of rye residue management and N fertilization on cotton biomass production and cotton biomass N concentration between first square and cutout, averaged across years.

N rates	Cotton biomass			Cotton biomass N concentration		
	NC†	REM	RET	NC	REM	RET
kg ha^{-1}		kg ha^{-1}			g ha^{-1}	
0	3,620	4,190	5,281	9.8	7.9	7.3
50	5,430	6,060	7,684	11.4	10.1	9.4
100	6,371	7,034	8,365	15.0	13.9	12.7
140	5,954	7,468	9,258	16.7	15.4	14.3

† NC: no winter cover crop.
REM: rye residue removed.
RET: rye residue retained.

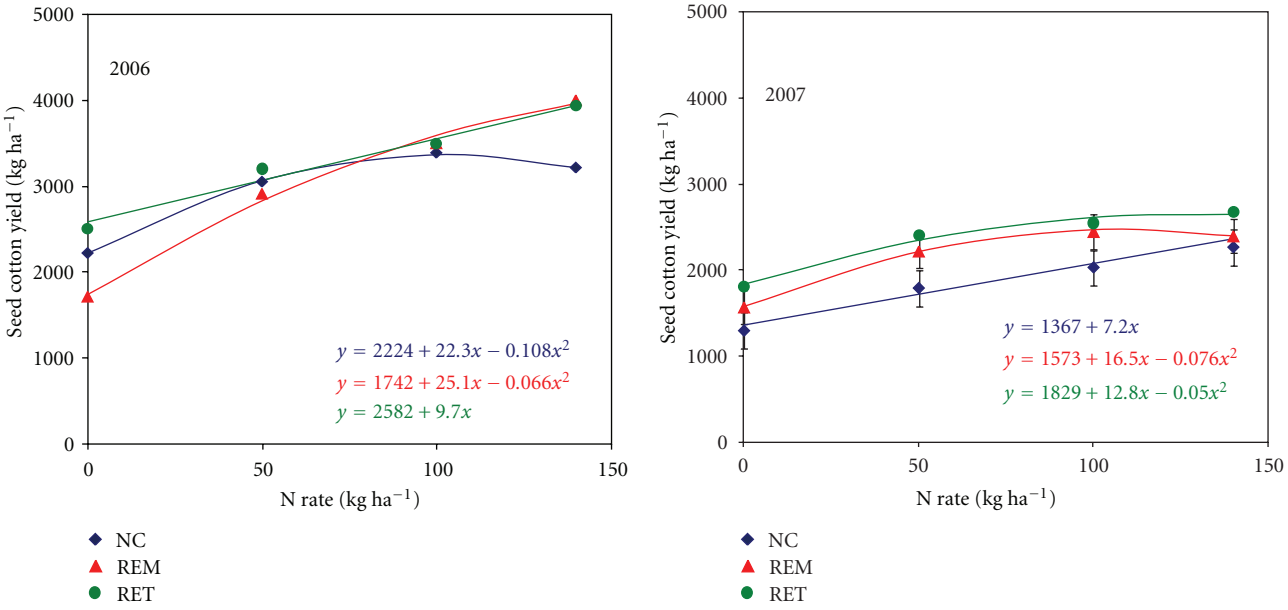


Figure 9: Seed cotton yield as affected by rye residue management and N rate, for the 2006 and 2007 seasons. NC: no winter cover crop; REM: rye residue removed; RET: rye residue retained.

(Figure 8(b)). In both years, the uptake response to N occurred up to the highest N rate applied, with 0.7 and 0.4 kg of N taken up per kg of N added in 2006 and 2007, respectively. Nitrogen uptake was lower for 2007 compared to 2006 for all N rates, but differences were greater when N was applied. Nitrogen uptake in 2007 was 33 and 39% lower for the no N control and for the 140 kg ha^{-1} N rate, respectively, relative to 2006 (Figure 8(b)). This observed reduction in N uptake can be attributed to the lower cotton biomass production in 2007 for all N rates and the reduction in cotton biomass N concentration measured for both years (Figures 5 and 7(b)).

3.9. Seed Cotton Yield. A significant Year × RM × N interaction occurred for seed cotton yield (Table 2). In 2006, observed seed cotton yields ranged from 1,740 (REM, no N control) to 3,970 kg ha^{-1} (REM, 140 kg of N ha^{-1}). The seed cotton yield response to N for RET was linear, while REM and NC were quadratic (Figure 9(a)). Highest observed and predicted yields corresponded to RET and REM with the application of 140 kg N ha^{-1}, with both treatments producing similar yields (Figure 9). Seed cotton yield response to N for RET and REM occurred up to the highest N rate applied without reaching a maximum. The fact that a plateau yield was not achieved in RET and REM indicates that the maximum yield potential with a cover crop was greater than that with no cover in 2006. Further, seed cotton yield in NC reached an estimated maximum at a N rate of 102 kg ha^{-1} and higher N rates caused a decrease in yields. The highest predicted seed cotton yield for RET and REM was about 17% higher than that for NC, showing that growing a cover was the best scenario for obtaining higher yields during 2006, whether or not the cover crop residue was removed or left on the soil surface. Our results for RET and REM are similar to the findings of Reiter et al. [12] who stated that conservation-tilled cotton on a Decatur silt loam responded up to 134 kg N ha^{-1}. Seed cotton yields observed in RET and

REM were also similar to results of Clawson et al. [39], who found cotton response to N up to 151 kg ha^{-1}. However, Wiatrak et al. [40] reported a linear increase in lint yields up to 200 kg N ha^{-1}, a rate considerably greater than that used in this experiment.

The seed cotton yield response to N during 2006 was 11.3, 15.9, and 9.7 kg of seed cotton per kg of N added for NC, REM, and RET at N rates of 102, 140, and 140 kg ha^{-1}, respectively. The highest yield increase with respect to the no N control corresponded to REM, followed by RET and NC (128, 53, and 45%, resp.), at the previously mentioned N rates. The lower response to N for RET can be somewhat explained by the greater seed cotton yield for the no N control relative to REM and NC. The REM treatment had the lowest seed cotton yield when no N was applied, with yield lower by 22 and 33% compared to NC and RET, respectively. Lower yields in REM compared to NC for the no N control were unexpected, since all cotton growth parameters for REM were at least similar or better than for NC when N was not applied. This yield decrease could be related to a factor or combination of factors directly affecting some of the yield components. However, the application of 50 and 100 kg of N ha^{-1} was enough to increase yields up to levels similar to NC and RET, and with 140 kg of N ha^{-1} the yield for REM was one of the highest. This observed trend for REM indicates that a severe N deficiency possibly occurred with this treatment when no N was added.

In 2007, seed cotton yields ranged from 1,295 kg ha^{-1} (NC, no N control) to 2,677 kg ha^{-1} (RET, 140 kg of N ha^{-1}). Rye residue retained and REM had a quadratic seed cotton yield response to N fertilization, while the yield increase for NC followed a linear trend (Figure 9). Rye residue retained had the highest predicted yield with 125 kg N ha^{-1}, followed by REM and NC at N rates of 106 and 140 kg N ha^{-1}, respectively (Figure 9). Boquet et al. [41] and Varco et al. [14] reported an optimum N rate of about 118 kg ha^{-1} for conservation tillage cotton, a value similar to the one we found for RET in 2007. Rye residue retained required 21 kg N ha^{-1} more than REM for maximizing yields but it had a higher yield. The highest estimated seed cotton yield for RET was 12 and 8% higher than that for NC and REM, respectively. Nitrogen rates above the optimum for REM tended to slightly decrease yields. A similar reduction in seed cotton yields occurred for NC in 2006 with N rates higher than 102 kg ha^{-1}. Cotton yield reductions with application of high N rates were reported by McConnell et al. [42] and Boquet et al. [43]. High N levels in soil can cause excessive vegetative growth, with a subsequent competition between vegetative and reproductive structures, which generally is detrimental to bolls and lint development, lint quality, and yield [4]. Regardless of its linear response to N, the decreasing yield with increasing N rate for NC indicates that the 140 kg N ha^{-1} we applied was near the optimum rate. In 2007, not only did NC have the lowest yield but also it required the highest N rate for achieving its highest seed cotton yield. Yields during 2007 were highly dependent on residue management. The best situation for achieving high seed cotton yields was to have a cover crop and keeping the residue on the soil surface.

The seed cotton yield response to N in 2007 was very similar among RM treatments, 7, 8, and 7 kg of seed cotton per kg of N for NC, REM, and RET (at N rates of 140, 106, and 140 kg ha^{-1}, resp.). No winter cover crop had the highest yield increase relative to the no N control, followed by REM and RET (75, 53, and 43%, resp.), for the previously indicated N rates. As in 2006, in 2007 RET had the lowest yield increment compared to the no N control, even though it had the highest estimated seed cotton yield. This pattern is explained by its greater seed cotton yield when no N was added.

In both years, RET had higher seed cotton yield than REM and NC in the no N control. This result was not expected because the presence of rye residue with a high C/N ratio on the soil surface has been commonly associated with the occurrence of N immobilization, which reduces levels of soil mineral N and decreases yields [10]. In situations with no N added this effect would have a greater negative impact on crop yields. However, results of cotton N uptake between first square and cutout in the no N control averaged across years (Figure 8(a)) followed a similar trend as seed cotton yield. The cotton N uptake in RET was 9 and 15% higher than that in NC and REM, respectively, for the no N control. These results indicate that under the conditions of our experiment N immobilization was not high enough to reduce seed cotton yields in RET.

4. Conclusions

Rye residue management treatments significantly influenced cotton growth parameters and seed cotton yield. In general, cotton population, leaf and plant N concentration, cotton biomass and N uptake at first square, and cotton biomass production between first square and cutout were higher for RET. However, leaf N concentration at early bloom and cotton biomass N concentration between first square and cutout were higher for NC. Leaf N concentration at early bloom, cotton biomass, and N concentration between first square and cutout increased with increasing N rates, when averaged across RM treatments. The highest N uptake was measured in RET, at the highest N rate. In 2006, the highest predicted seed cotton yield corresponded to RET and REM with the application of 140 kg N ha^{-1} (about 3,950 kg ha^{-1}). In 2007, RET had the highest predicted seed cotton yield with 125 kg N ha^{-1}(2,657 kg ha^{-1}) followed by REM with 106 kg N ha^{-1} (2,466 kg ha^{-1}). In both years, the lowest predicted yield was for NC. In 2006, the increase in cotton biomass for RET compared to REM did not necessarily result in an increase in seed cotton yields. However, a stronger association between cotton biomass production and seed cotton yields was observed in the hot and dry 2007 season. Even though RET had low leaf N concentration values at early bloom, it had high yields in both years, indicating that in our study leaf N concentration was not a good predictor of seed cotton yields. Results of this study show that short-term effects of rye residue removal can occur mainly in vegetative cotton parameters, but its effect on seed cotton yield and cotton response to N fertilization would depend more on the characteristics of the season. No rye residue removal

effect would be expected in years with average temperatures and rainfall. However, during hot and dry years, rye residue removal may lead to a decrease in cotton yields. We anticipate that cotton N requirements under rye residue removed conditions would not be lower compared to residue retained. The year dependence of rye residue removal impact on seed cotton yields and cotton response to N fertilization suggests that long-term studies are required to strengthen conclusions concerning this management practice.

Abbreviations

RM: Rye residue management
NC: No winter cover crop
REM: Rye residue removed
RET: Rye residue retained
N: Nitrogen
P: Phosphorus
K: Potassium
S: Sulfur
DAP: Days after planting
HU: Heat units.

Acknowledgments

The authors would like to acknowledge the staff of the Field Crops Unit at the E. V. Smith Research Center, especially the late Mr. Bobby Durbin, for their support with this experiment. This paper contributes to the USDA-Agricultural Research Service cross-location Renewable Energy Assessment Project (REAP).

References

[1] Economic Research Service (ERS), "Cotton and Wool Yearbook," 2003, http://usda.mannlib.cornell.edu/usda/ers/89004/2003/table04uplandplantedacreage.xls.

[2] D. J. Boquet and G. A. Breitenbeck, "Nitrogen rate effect on partitioning of nitrogen anti dry matter by cotton," *Crop Science*, vol. 40, no. 6, pp. 1685–1693, 2000.

[3] T. J. Gerik, B. S. Jackson, C. O. Stockle, and W. D. Rosenthal, "Plant nitrogen status and boll load of cotton," *Agronomy Journal*, vol. 86, no. 3, pp. 514–518, 1994.

[4] J. S. McConnell, R. E. Glover, E. D. Vories, W. H. Baker, B. S. Frizzell, and F. M. Bourland, "Nitrogen fertilization and plant development of cotton as determined by nodes above white flower," *Journal of Plant Nutrition*, vol. 18, no. 5, pp. 1027–1036, 1995.

[5] A. S. Hodgson and D. A. MacLeod, "Seasonal and soil fertility effects on the response of waterlogged cotton to foliar-applied nitrogen fertilizer," *Agronomy Journal*, vol. 80, pp. 259–265, 1988.

[6] C. H. Harris and C. W. Smith, "Cotton production affected by row profile and nitrogen rates," *Agronomy Journal*, vol. 72, pp. 919–922, 1980.

[7] D. D. Howard, C. O. Gwathmey, M. E. Essington, R. K. Roberts, and M. D. Mullen, "Nitrogen fertilization of no-till cotton on loess-derived soils," *Agronomy Journal*, vol. 93, no. 1, pp. 157–163, 2001.

[8] H. H. Schomberg, R. G. McDaniel, E. Mallard, D. M. Endale, D. S. Fisher, and M. L. Cabrera, "Conservation tillage and cover crop influences on cotton production on a Southeastern U.S. Coastal Plain Soil," *Agronomy Journal*, vol. 98, no. 5, pp. 1247–1256, 2006.

[9] G. W. Langdale, R. L. Wilson, and R. R. Bruce, "Cropping frequencies to sustain long-term conservation tillage systems," *Soil Science Society of America Journal*, vol. 54, no. 1, pp. 193–198, 1990.

[10] S. M. Dabney, J. A. Delgado, and D. W. Reeves, "Using winter cover crops to improve soil and water quality," *Communications in Soil Science and Plant Analysis*, vol. 32, no. 7-8, pp. 1221–1250, 2001.

[11] S. M. Brown, T. Whitwell, J. T. Touchton, and C. H. Burmester, "Conservation tillage systems for cotton production (USA)," *Soil Science Society of America Journal*, vol. 49, no. 5, pp. 1256–1260, 1985.

[12] M. S. Reiter, D. W. Reeves, and C. H. Burmester, "Nitrogen management for cotton in a high-residue cover crop conservation tillage system," in *Making Conservation Tillage Conventional: Building a Future on 25 Years of Research, Proceedings of the 25th Southern Conservation Tillage Conference for Sustainable Agriculture*, E. van Santen, Ed., pp. 136–141, Auburn University, Auburn, Ala, USA, 2002.

[13] M. G. Wagger, "Time of dessication effects on plant composition and subsequent nitrogen release form several winter annual cover crops," *Agronomy Journal*, vol. 81, pp. 236–241, 1989.

[14] J. J. Varco, S. R. Spurlock, and O. R. Sanabria-Garro, "Profitability and nitrogen rate optimization associated with winter cover management in no-tillage cotton," *Journal of Production Agriculture*, vol. 12, no. 1, pp. 91–95, 1999.

[15] C. C. Mitchell, "Fertility," in *Conservation Tillage Cotton Production Guide—ANR-952*, C. D. Monks and M. G. Paterson, Eds., pp. 7–9, Agronomy and Soils Department, Auburn University, Auburn, Ala, USA, 1996.

[16] D. Dinnes, D. Jaynes, T. Kaspar, T. Colvin, C. A. Cambardella, and D. L. Karlen, "Plant-soil-microbe N relationships in high residue management systems," 2007, http://www.sdnotill.com/Newsletters/Relationships.pdf.

[17] G. Siri-Prieto, D. W. Reeves, and R. L. Raper, "Tillage requirements for integrating winter-annual grazing in cotton production: plant water status and productivity," *Soil Science Society of America Journal*, vol. 71, no. 1, pp. 197–205, 2007.

[18] C. E. Clapp, R. R. Allmaras, M. F. Layese, D. R. Linden, and R. H. Dowdy, "Soil organic carbon and 13C abundance as related to tillage, crop residue, and nitrogen fertilization under continuous corn management in Minnesota," *Soil and Tillage Research*, vol. 55, no. 3-4, pp. 127–142, 2000.

[19] B. Govaerts, M. Fuentes, M. Mezzalama et al., "Infiltration, soil moisture, root rot and nematode populations after 12 years of different tillage, residue and crop rotation managements," *Soil and Tillage Research*, vol. 94, no. 1, pp. 209–219, 2007.

[20] S. S. Malhi, R. Lemke, Z. H. Wang, and B. S. Chhabra, "Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions," *Soil and Tillage Research*, vol. 90, no. 1-2, pp. 171–183, 2006.

[21] W. W. Wilhelm, J. M. F. Johnson, J. L. Hatfield, W. B. Voorhees, and D. R. Linden, "Crop and soil productivity response to corn residue removal: a literature review," *Agronomy Journal*, vol. 96, no. 1, pp. 1–17, 2004.

[22] H. Tsuji, H. Yamamoto, K. Matsuo, and K. Usuki, "The effects of long-term conservation tillage, crop residues and P fertilizer

on soil conditions and responses of summer and winter crops on an Andosol in Japan," *Soil and Tillage Research*, vol. 89, no. 2, pp. 167–176, 2006.

[23] J. R. Salinas-Garcia, A. D. Báez-González, M. Tiscareño-López, and E. Rosales-Robles, "Residue removal and tillage interaction effects on soil properties under rain-fed corn production in Central Mexico," *Soil and Tillage Research*, vol. 59, no. 1-2, pp. 67–79, 2001.

[24] D. K. Cassel and M. G. Wagger, "Residue management for irrigated maize grain and silage production," *Soil and Tillage Research*, vol. 39, no. 1-2, pp. 101–114, 1996.

[25] J. W. Doran, W. W. Wilhelm, and J. F. Power, "Crop residue removal and soil productivity with no-till corn, sorghum, and soybean," *Soil Science Society of America Journal*, vol. 48, no. 3, pp. 640–645, 1984.

[26] J. C. Zadoks, T. T. Chang, and C. F. Konzak, "A decimal code for the growth of cereals," *Weed Research*, vol. 14, pp. 415–421, 1974.

[27] J. F. Adams and C. C. Mitchell, "Soil test nutrient recommendations for Alabama crops," 2000, http://www.ag.auburn.edu/agrn/croprecs/CropRecs/cc10.html.

[28] S. Peng, D. R. Krieg, and S. K. Hicks, "Cotton lint yield response to accumulated heat units and soil water supply," *Field Crops Research*, vol. 19, no. 4, pp. 253–262, 1989.

[29] R. C. Littell, G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger, *SAS for Mixed Models*, SAS Institute, Cary, NC, USA, 2nd edition, 2006.

[30] C. C. Mitchell and S. Tu, "Long-term evaluation of poultry litter as a source of nitrogen for cotton and corn," *Agronomy Journal*, vol. 97, no. 2, pp. 399–407, 2005.

[31] C. W. Bednarz, D. C. Bridges, and S. M. Brown, "Analysis of cotton yield stability across population densities," *Agronomy Journal*, vol. 92, no. 1, pp. 128–135, 2000.

[32] P. F. Bell, D. J. Boquet, E. Millholln et al., "Relationship between leaf-blade nitrogen and relative seed cotton yields," *Crop Science*, vol. 43, pp. 1367–1374, 2003.

[33] H. A. Mills and J B. Jones, *Plant Analysis Handbook II: A Practical Sampling, Preparation, Analysis, and Interpretation Guide*, Micro-Macro, Athens, Ga, USA, 1996.

[34] J. L. Fridgen and J. J. Varco, "Dependency of cotton leaf nitrogen, chlorophyll, and reflectance on nitrogen and potassium availability," *Agronomy Journal*, vol. 96, no. 1, pp. 63–69, 2004.

[35] K. S. Balkcom, J. N. Shaw, D. W. Reeves, C. H. Burmester, and L. M. Curtis, "Irrigated cotton response to tillage systems in the Tennessee Valley," *Journal of Cotton Science*, vol. 11, no. 1, pp. 2–11, 2007.

[36] D. M. Bassett, W. D. Anderson, and C. H. E. Werkhoven, "Dry matter production and nutrient uptake in irrigated cotton (*Gossypium hirsutum*)," *Agronomy Journal*, vol. 62, pp. 299–303, 1970.

[37] G. L. Mullins and C. H. Burmester, "Dry matter, nitrogen, phosphorus, and potassium accumulation by four cotton varieties," *Agronomy Journal*, vol. 82, pp. 729–736, 1990.

[38] F. Gastal and G. Lemaire, "N uptake and distribution in crops: an agronomical and ecophysiological perspective," *Journal of Experimental Botany*, vol. 53, no. 370, pp. 789–799, 2002.

[39] E. L. Clawson, J. T. Cothren, and D. C. Blouin, "Nitrogen fertilization and yield of cotton in ultra-narrow and conventional row spacings," *Agronomy Journal*, vol. 98, no. 1, pp. 72–79, 2006.

[40] P. J. Wiatrak, D. L. Wright, J. J. Marois, W. Koziara, and J. A. Pudelko, "Tillage and nitrogen application impact on cotton following wheat," *Agronomy Journal*, vol. 97, no. 1, pp. 288–293, 2005.

[41] D. J. Boquet, R. L. Hutchinson, and G. A. Breitenbeck, "Long-term tillage, cover crop, and nitrogen rate effects on cotton: yield and fiber properties," *Agronomy Journal*, vol. 96, no. 5, pp. 1436–1442, 2004.

[42] J. S. McConnell, W. H. Baker, D. M. Miller, B. S. Frizzell, and J. J. Varvil, "Nitrogen fertilization of cotton cultivars of differing maturity," *Agronomy Journal*, vol. 85, no. 6, pp. 1151–1156, 1993.

[43] D. J. Boquet, E. B. Moser, and G. A. Breitenbeck, "Boll weight and within-plant yield distribution in field-grown cotton given different levels of nitrogen," *Agronomy Journal*, vol. 86, no. 1, pp. 20–26, 1994.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 508986, 12 pages
doi:10.1155/2012/508986

Research Article

Nitrogen Transformations in Broiler Litter-Amended Soils

Kokoasse Kpomblekou-A and Andrae Genus

Department of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL 36088, USA

Correspondence should be addressed to Kokoasse Kpomblekou-A, kka@mytu.tuskegee.edu

Received 22 December 2011; Accepted 19 March 2012

Academic Editor: Dexter B. Watts

Nitrogen mineralization rates in ten surface soils amended with (200 μg N g^{-1} soil) or without broiler litter were investigated. The soil-broiler litter mixture was incubated at 25 ± 1°C for 28 weeks. A nonlinear regression approach for N mineralization was used to estimate the readily mineralizable organic N pools (N_0) and the first-order rate constant (k). The cumulative N mineralized in the nonamended soils did not exceed 80 mg N kg^{-1} soil. However, in Decatur soil amended with broiler litter 2, it exceeded 320 mg N kg^{-1} soil. The greatest calculated N_0 of the native soils was observed in Sucarnoochee soil alone (123 mg NO_3^- kg^{-1} soil) which when amended with broiler litter 1 reached 596 mg N kg^{-1} soil. The added broiler litter mineralized initially at a fast rate (k_1) followed by a slow rate (k_2) of the most resistant fraction. Half-life of organic N remaining in the soils alone varied from 33 to 75 weeks and from 43 to 15 weeks in the amended soils. When N_0 was regressed against soil organic N ($r = 0.782^{**}$) and C ($r = 0.884^{***}$), positive linear relationships were obtained. The N_0 pools increased with sand but decreased with silt and clay contents.

1. Introduction

In general, nitrogen (N) is said to be the most difficult nutrient to manage in agriculture because of challenges in estimating the amount of N available for plant uptake and synchronizing N release from sources to meet a specific crop demand [1]. Even though the Earth's atmosphere contains 78% N in the form of dinitrogen (N_2) gas, most of this N is unavailable for plant uptake [2] with the exception of leguminous plants which can fix N. In the plant root zone, N is present in organic forms, including plant and microbial protein and amino acids, all together forming soil organic matter [3] from which the N is slowly converted into plant-available forms. During mineralization, organic N is converted into plant-useable inorganic forms (NH_4^+–N and, NO_3^-–N) that are released into soil and subjected to various fates. For farmers in general and organic farmers in particular, N mineralization is an important process to understand because several environmental conditions govern this process [3]. Presently, there is an array of commercial inorganic N fertilizers available; however, their costs are prohibitory and out of range for many limited resource farmers. Thus, a careful management of organic N sources is one of the most important priorities for farmers; this in turn will limit unfavorable N losses into the environment.

Because of the rapid growth of the organic farming segment of the United States agriculture, there is a high demand for alternative plant nutrient sources, especially, organic sources. It is estimated that organic produce sales reached approximately $23.0 billion in 2009 [4] from 2.6 billion in 1997 [5]. This rapid growth in organic food demand urges researchers to obtain a more thorough understanding of organic amendment in organic farming systems. The United States poultry industry produced 9.2 billion broiler chickens and 90.6 billion eggs in 2007 with nearly 82% of the broilers and 31% of the eggs produced in the southeastern states of Georgia, Arkansas, Alabama, Mississippi, and North Carolina. These five states account for approximately 60% of all broiler meat produced in the United States [6, 7]. Broiler production in the United States has been transformed dramatically from small backyard operations to more integrated farms. As time progressed, broiler production became an industry that established itself in the South due to favorable climate, low labor costs, and advantages in feed production [8]. In 2009, approximately

8.6 billion broiler chickens equaling 50 billion pounds valued at $22 billion were raised in the USA, and the state of Alabama accounted for approximately 12% of this total [9]. This robust broiler industry generates substantial quantities of poultry litter as waste at a rate of 1 to 1.4 tons per 1000 birds. It is estimated that broiler litter in Alabama contains 41.1 g kg^{-1} Kjeldahl N [10]. The broiler litter generated has historically been applied to pasture and agricultural land in close proximity to poultry production facilities [11]. Consequently, negative environmental impacts of these waste products are a major concern worldwide. One major problem that occurs is the presence and build-up of trace elements in soil over time [12]. Despite the fact that broiler litter contains trace elements that may affect N transformations in soils; it remains a cheap organic fertilizer used in organic farming systems.

Excessive applications of broiler litter to farmlands have resulted in NO_3^- contamination of both ground and surface water bodies. Accurate estimates of N availability from broiler litter is a prerequisite to determine application rates necessary for optimum plant growth and minimal NO_3^- leaching. A method to estimate the amount of N available in broiler litter to increase crop yields and reduce N losses to the environment was assessed [13]. In this method, broiler litter application rates were based on predicted available N (PAN). It was assumed that 80% of the inorganic N in the broiler litter would be recovered and 60% of the organic N would be mineralized within 140 days. In addition, other methods used to predict N availability from broiler litter include complex mechanistic and simple kinetic models [14]. Mechanistic models are more process based and require large amounts of input data. On the other hand, kinetic models rely on laboratory incubations to obtain certain parameters; however, these kinetic models do not account for N turnover processes [15]. The various types of kinetic models used to describe inorganic N production from soil alone or amended with organic material include sigmoid, hyperbolic, single exponential, and linear models [16, 17]. However, explanations behind the theoretical implications of the parameters in these various mathematical equations have been limited [18]. Therefore, the two first-order kinetic models that remain widely used include the single [19] and double [20] exponential models.

The Stanford and smith (1972) model is represented by

$$N_m = N_0\left(1 - e^{-kt}\right), \quad (1)$$

where, N_m is the cumulative mineral-N (mg N kg^{-1} soil) at time t. N_0 is defined as the potentially mineralizable N and k as the mineralization rate constant.

The double exponential model separates the mineralizable organic N into active and slow pools and is represented by

$$N_m = N_0\left(1 - e^{-k_0 t}\right) + N_1\left(1 - e^{-k_1 t}\right), \quad (2)$$

where N_m is the cumulative amount of N mineralized at time t, N_0, and N_1 are the sizes of the active and slow pools of mineralizable N, respectively; k_0 and k_1 are the corresponding mineralization rate constants for each pool.

In the study reported here, the single exponential model [19] was used. The model suggests that the potentially mineralizable N (N_0) of a soil and its rate constant (k) can be estimated by incubating the soil at optimum conditions and measuring the N mineralized (N_m) and time of incubation (t). The main assumption is that organic N mineralization at optimum temperature and moisture follows first-order kinetics. The objectives of the study were to (1) determine N mineralization rates in ten Alabama soils amended and nonamended with broiler litter, (2) compare potentially mineralizable organic N pools in the soils, (3) compare half-life of N remaining in the soils, and (4) establish relationships between the soils' potentially mineralizable organic N and soil properties.

2. Materials and Methods

Alabama surface (0–15 cm) soil samples were collected from Barbour County (Troup soil), Bullock County (Maytag and Sucarnoochee soils), Dekalb County (Colbert, Hartsells, and Linker soils), Coffee County (Dothan soil), Talladega County (Decatur soil), and Tallapoosa County (Appling and Cecil soils). The physical and chemical properties of the soils are presented in Table 1. Following sampling, the soils were air-dried and ground to pass through a 2 mm sieve. A subsample was finely ground to pass a 100-mesh (<149-μm) sieve for analysis of total C and N by Vario EL III Automated Analyzer (CHNS Analyzer, Hanau, Germany) using a combustion method. In the analysis reported in Table 1, soil pH was measured (soil : water ratio 1 : 2.5) using a glass electrode, organic C by the Mebius method [21], total N by Semimicro-Kjeldahl procedure [22], inorganic N by steam distillation [23], and partial-size distribution by the pipette method [24]. Selected properties of the broiler litter samples used in the study are shown in Table 2 where total C and N were determined by the elemental CHNS Analyzer (Vario EL III Elemental Analyzer, Hanau, Germany). The pH was determined (broiler litter : water ratio 1 : 5) using a glass electrode. The NH_4^+–N and NO_3^-–N were determined by steam distillation [23]. Properties of the soil and the broiler litter samples shown in Tables 1 and 2, respectively, were previously reported [25].

The experimental setup was a 10 × 3 factorial in a completely randomized design. The treatments included a control (soil alone with no broiler litter added), soil-amended with broiler litter 1, and soil-amended with broiler litter 2. The broiler litter was added at a rate to give a concentration of 200 μg N g^{-1} soil. A 20 g soil sample (<2-mm, OD) and an equal amount of acid-washed silica sand were weighted into a weighing dish and mixed thoroughly. Before the experiment, the silica sand was washed in 10% HCl solution and let to stand for 1 hour after which the silica sand was washed three times in deionized water. The washed silica sand was allowed to dry under the hood for several days. A sample of the silica sand was analyzed for inorganic N by steam distillation and showed absence of NH_4^+– and NO_3^-–N. The soil-silica sand mixture was then treated with broiler litter 1 or 2. A thin glass wool was

Table 1: Selected properties of the soils used†.

Soil series†	pH	Organic Carbon	Total nitrogen	Inorganic N		Texture		
				NH_4^+–$(NO_2^- + NO_3^-)$–N		Clay	Silt	Sand
		$g\,kg^{-1}$		$mg\,kg^{-1}$			$g\,kg^{-1}$	
Appling	5.95	26.4	3.90	14.3	9.57	75	125	800
Cecil	5.87	19.0	3.38	14.4	20.8	75	225	700
Colbert	6.05	6.88	2.53	13.1	23.0	—	125	875
Decatur	5.90	14.3	3.91	8.91	8.73	—	100	900
Dothan	6.48	9.37	2.57	12.0	21.2	75	650	275
Hartsells	6.06	9.58	2.67	3.91	5.39	125	300	575
Maytag	5.95	7.37	2.55	2.55	3.05	125	325	550
Linker	6.31	9.71	2.73	3.10	7.02	25	350	625
Sucarnoochee	5.91	11.4	2.82	6.87	3.39	75	475	450
Troup	6.64	20.1	3.30	2.16	5.07	125	75	800

† Appling: Fine, kaolinitic thermic kanapludult; Cecil: Fine, kaolinitic thermic, Typic kanapludults; Colbert; Fine, smetitic, thermic Vertic Hapludults; Decatur; Clayey, kaolinitic thermic Rhodic Paleudults; Dothan; Fine-loamy, siliceous, thermic Plinthic Paleudults; Hartsells; Fine-loamy, siliceous subactive thermic Typic Hapludults; Maytag; Fine montmorillonitic, thermic, oxyaquic Hapluderts; Linker; Fine-loamy, siliceous semiactive thermic Typic Hapludults; Sucarnoochee; montmorillonitic, thermic chromic Epiaqurand; Troup; Loamy, siliceous, Thermic Grossarenic Paleudults. From Sissoko and Kpomblekou-A 2010 [25].

Table 2: Selected properties of the broiler litter used†.

Broiler litter ID	pH	Organic C	Total N	Inorganic N		C/N	Bedding material	Litter age
				NH_4^+	$(NO_2^- + NO_3^-)$			
			$g\,kg^{-1}$					Month
1	8.4	229	27.5	1.61	1.03	8.32	Pine sawdust	9
2	8.6	351	46.1	4.39	1.95	7.61	Peanut hulls	9

† From Kpomblekou-A [10].

inserted at the bottom of a leaching tube to retain the mixture. To prevent any disturbance of the soil during the leaching procedure, a thin glass wool pad was also placed on the top of the mixture in the leaching tube. The leaching tube was placed on a flask and immediately leached with 100 mL of 5 mM $CaCl_2$ to remove any initial inorganic N (time zero). A suction of 60 cm Hg (6 kpa) was applied to remove the remaining solution. The volume of the leachate obtained was adjusted to 100 mL with deionized water. The leaching tube was covered with parafilm and a small hole was inserted for aeration and placed in an incubator (Low Temperature Incubator 815, Precision Scientific Winchester, VA) at $25 \pm 1^\circ$C for 22 weeks. The inorganic N mineralized in the leaching tube was leached every two weeks for 28 weeks with 100 mL $CaCl_2$(5 mM) and filtered through a membrane filter (0.5 μm, Osomics Inc., Minnetonka, MN). The filtrate was analyzed for NH_4^+–N and $(NO_2^- + NO_3^-)$–N by steam distillation [23]. Controls for each soil without broiler litter were included. Results presented are average of duplicate samples.

2.1. Model Description and Statistical Analysis. The nonlinear regression [26] approach for N mineralization in (1) was used to estimate the readily mineralizable organic pools (N_0) in the broiler litter and the first-order rate constant (k). The Statistical Analysis System (SAS) computer language was used to calculate N_0 and k [27]. From the slopes of the linear segments of curves obtained by plotting the natural log of organic N remaining against time [28], the decomposition rates (k_i) of the organic N pool was calculated. The half-life ($t_{1/2}$) of the most resistant N fraction in the organic materials was calculated by using the k_i value of the resistant fraction of the broiler litter samples ($t_{1/2} = 0.693/k_i$). The fitting of the mathematical models was done using SAS-ProcNLIN [29], an interactive method using MARQUADART [30] algorithm. Estimated potentially mineralizable organic N (N_0) values were used in a 10×3 factorial arrangement and analyzed by SAS-Proc GLM. Therefore, when soil differences or treatment differences were detected base on overall analysis of variance, least significant difference (LSD) was used to evaluate differences between treatments and between soils. However, in this study, no soil by treatment interactions was detected.

3. Results and Discussion

3.1. Nitrogen Mineralization. The total N content of the soils varied considerably (Table 1). These variations are attributed to climate, vegetation, and topography [31] that are major components of soil formation factors. In cultivated soils, total N content tends to decline over time if external organic N sources are not incorporated into the soil to compensate for losses due to increasing microbial N mineralization. In general, N mineralization increases as temperature rises to a

Table 3: Organic carbon, sulfur, and total nitrogen contents of soil-amended with broiler litter sample 1 or 2 before aerobic incubation studies†.

	Broiler litter 1			Broiler litter 2		
	Organic carbon	Total nitrogen	Organic sulfur	Organic Carbon	Total nitrogen	Organic sulfur
	$g\,kg^{-1}$					
Appling	33.4	4.62	0.60	52.7	7.64	0.96
Cecil	23.5	3.99	0.67	29.6	4.64	0.65
Colbert	11.1	2.94	0.32	13.2	3.05	0.48
Decatur	18.0	3.48	0.58	24.6	4.25	0.45
Dothan	11.2	2.91	0.53	19.6	3.76	0.49
Hartsells	15.2	3.25	0.50	15.9	3.54	0.57
Maytag	13.1	3.03	0.41	20.5	3.76	0.50
Linker	15.4	3.31	0.56	18.1	3.60	0.54
Sucarnoochee	15.2	3.14	0.53	20.5	3.74	0.29
Troup	22.0	3.40	0.52	23.7	3.93	0.40
Median	15.3	3.28	0.53	20.5	3.76	0.50
Mean	22.0	3.40	0.52	23.7	3.93	0.40

point where microbial growth is reduced [32]. The amount of total N present in the soil after addition of the broiler litter samples is shown in Table 3. The soil-broiler litter mixture was ground to pass through a 100-mesh (<149-μm) and a subsample was analyzed for organic C and N. Data obtained from this analysis were used in calculating the remaining organic N in the soils at the end of incubation period. The results showed an increase in organic C and N contents of the soil-broiler litter mixture. However, the increase varied among the ten soil samples analyzed. The total N content of the mixtures ranged from $2.91\,g\,kg^{-1}$ in Dothan soil to $4.62\,g\,kg^{-1}$ in Appling soil treated with broiler litter 1 (Table 3). The analysis also showed that for soils treated with broiler litter 1, the median for total N was $3.28\,g\,kg^{-1}$ with a mean of $3.40\,g\,kg^{-1}$. However, for the same soils treated with broiler litter 2, the median and the mean were 3.76 and $3.93\,g\,kg^{-1}$, respectively.

Among the ten soils used in this study, the cumulative ammonium-N (NH_4^+–N) produced in the nonamended soils were 21.2, 22.6, 23.7, 24.3, 26.5, 26.9, 27.0, 27, 35.8, and $50.5\,mg\,N\,kg^{-1}$ soil for Appling, Hartsells, Colbert, Linker, Decatur, Maytag, Troup, Dothan, Sucarnoochee, and Cecil soil, respectively, after 28 weeks of incubation (data not shown). On the other hand, soils amended with broiler litter 2 had significantly greater amounts of NH_4^+–N produced. For example, Maytag, Cecil, and Sucarnoochee soils amended with broiler litter 2 showed the highest amount of NH_4^+–N produced after 28 weeks of incubation with 325, 326, and $366\,mg\,kg^{-1}$ soil, respectively. However, the total amount of NO_3^-–N mineralized in the soils alone was 27.5 in Hartsells, 43.5 in Troup, 49.6 in Dothan, 62.2 in Appling, 58.1 in Colbert, 65.7 in Maytag, 69.3 in Decatur, 85.7 in Linker, 106 in Cecil, and $111\,mg\,N\,kg^{-1}$ soil in Sucarnoochee soil after 28 weeks of incubation (data not shown). In addition, the amount of NO_3^-–N mineralized also varied with soil types and broiler litter samples. For Appling soil amended with broiler litter 1, the total amount of NO_3^-–N released at 14 weeks of incubation was about $7.42\,mg\,N\,kg^{-1}$ soil while in that same soil amended with broiler litter 2, NO_3^-–N released was $8.31\,mg\,N\,kg^{-1}$ soil. In contrast, NO_3^-–N released in Cecil soil amended with broiler litter 1 at 14 weeks was $8.72\,mg\,N\,kg^{-1}$ soil while that released in Cecil soil amended with broiler litter 2 was only $4.03\,mg\,kg^{-1}$. The amount of NO_3^-–N released in the ten soils were significantly greater than those of NH_4^+–N released after 28 weeks of incubation. The cumulative amount of NO_3^-–N mineralized in soils amended with broiler litter 1 after 28 weeks of incubation varied from 131 in Troup to $269\,mg\,N\,kg^{-1}$ soil in Decatur soil, respectively. On the other hand, the total NO_3^-–N nitrified in soils amended with broiler litter 2 after 28 weeks of incubation ranged from 120 in Dothan soil to $248\,mg\,kg^{-1}$ soil in Appling and Sucarnoochee soil, respectively. In addition, Linker soil amended with broiler litter 1 or 2 nitrified the same amounts of N with $163\,mg\,N\,kg^{-1}$ soil, respectively. Only Sucarnoochee and Troup soils showed higher amounts of NO_3^-–N mineralized when amended with broiler litter 1 than with broiler litter 2. In those soils mineralization varied from 231 to 248 in Sucarnoochee amended with broiler litter 1 and 2, and from 131 to $138\,mg\,N\,kg^{-1}$ soil when Troup was amended with broiler litter 1 and 2, respectively. All remaining soils showed similar trends with broiler litter 1 mineralizing higher amounts of N as NO_3^-–N than those amended with broiler litter 2.

3.2. Nitrogen Mineralization Models. The trends of N mineralization from broiler litter added to ten Alabama soils were similar to those reported for Iowa soils amended with leguminous crops [33]. In this study, the differences in organic N mineralized may be attributed to the resistance of organic N fractions in the different broiler litter samples. In general, the amounts of N mineralized in the broiler litter-amended soils increased gradually but at a decreasing rate. Thus, mineralization of organic N added to soils starts with

Table 4: Regression equations (for curves in Figures 1–5) for organic N mineralized in selected Alabama soils.

Soil series	Treatment specified†		
	Soil alone	Soil + broiler litter 1	Soil + broiler litter 2
Appling	$N_m = 85.3\ (1 - e^{-0.1320t})$	$N_m = 252\ (1 - e^{-0.1730t})$	$N_m = 283\ (1 - e^{-0.0910t})$
Cecil	$N_m = 120\ (1 - e^{-0.0405t})$	$N_m = 251\ (1 - e^{-0.0494t})$	$N_m = 331\ (1 - e^{-0.0315t})$
Colbert	$N_m = 76.7\ (1 - e^{-0.406t})$	$N_m = 360\ (1 - e^{-0.0405t})$	$N_m = 369\ (1 - e^{-0.0406t})$
Decatur	$N_m = 86.9\ (1 - e^{-0.0627t})$	$N_m = 356\ (1 - e^{-0.063t})$	$N_m = 405\ (1 - e^{-0.0619t})$
Dothan	$N_m = 66.9\ (1 - e^{-0.0865t})$	$N_m = 131\ (1 - e^{-0.0877t})$	$N_m = 167\ (1 - e^{-0.0854t})$
Hartsells	$N_m = 47.9\ (1 - e^{-0.0620t})$	$N_m = 208\ (1 - e^{-0.0614t})$	$N_m = 294\ (1 - e^{-0.0625t})$
Linker	$N_m = 102\ (1 - e^{-0.0801t})$	$N_m = 195\ (1 - e^{-0.0819t})$	$N_m = 209\ (1 - e^{-0.0783t})$
Maytag	$N_m = 82.8\ (1 - e^{-0.0793t})$	$N_m = 221\ (1 - e^{-0.0764t})$	$N_m = 217\ (1 - e^{-0.0821t})$
Sucarnoochee	$N_m = 123\ (1 - e^{-0.0516t})$	$N_m = 569\ (1 - e^{-0.0230t})$	$N_m = 286\ (1 - e^{-0.0802t})$
Troup	$N_m = 59.7\ (1 - e^{-0.0640t})$	$N_m = 189\ (1 - e^{-0.0571t})$	$N_m = 185\ (1 - e^{-0.0709t})$

† N_m = organic N mineralized (mg kg^{-1}) at specific time (t).

Table 5: Comparison of calculated potentially mineralizable organic N pools (N_0) in soils alone and soils amended with broiler litter 1 or 2.

Soil series	Soil alone†	Soil + broiler litter 1††	Soil + broiler litter 2††
	mg NO_3^- kg^{-1}		
Appling	85.3c	252b	283a
Cecil	120a	251a	331a
Colbert	76.7cd	360a	368a
Decatur	86.9cb	356a	405a
Dothan	66.9ed	131a	167a
Hartsells	47.9f	208a	294a
Linker	102b	195a	209a
Maytag	82.8cd	221a	217a
Sucarnoochee	123a	569a	286b
Troup	59.7ef	189a	185a

† Means with the same letter in the soil alone column are not significantly different at $P < 0.05$.
†† Means with the same letter in the same row of the broiler litter-amended soils are not significantly different at $P < 0.05$.

a rapid mineralization of the easily mineralizable organic N, followed by mineralization of the intermediate fraction, and finally the most resistant organic fraction where the curve tends to plateau with increasing incubation time. In addition, this N mineralization pattern can be attributed to the differences in chemical properties of the soils. Previous studies showed that generally, the amounts of N mineralized correlate with total N, total C, and microbial N [34–36].

The differences in N mineralized from the same broiler litter sample in two different soils (Figures 1(a) and 1(b)) suggest that N mineralization is dependent not only on organic N fractions in a given broiler litter sample, but also on soil properties. Furthermore, mineralization of N in the broiler litter samples behaved differently in Decatur (Figure 2(b)), Hartsells (Figure 3(b)), and Sucarnoochee (Figure 5(b)) soils. However, in Cecil (Figure 1(b)), Colbert (Figure 2(a)), Linker (Figure 4(a)), Maytag (Figure 4(b)), and Troup (Figure 5(b)), N mineraliztion of the broiler litter samples followed much closer trends.

Initially the broiler litter samples mineralized rapidly during the first 10 weeks following their addition to soils, especially in Appling (Figure 1(a)) and Decatur (Figure 2(b)) soils. Nitrogen mineralization was slow in Troup soil (Figure 5(b)) containing 125 g kg^{-1} clay and 800 g kg^{-1} of sand, while the highest mineralization occurred in Decatur soil (Figure 2(b)) containing 900 g kg^{-1} sand and 100 g kg^{-1} silt. Mineralization of leguminous crops added to five Iowa soils incubated for 16 weeks at 30°C has been reported [33] and the results were similar to those discussed here.

The regression equations for organic N mineralized for the nonamended and amended soils are shown in Table 4. The potentially mineralizable (N_0) organic N mineralized in the nonamended soils ranged from 47.9 to 123 mg N kg^{-1} in Hartsells and Sucarnoochee soils, respectively. Hartsells soil showed the lowest N_0 mineralized probably because of its texture (high clay and moderate sand contents) that restricted access of soil microorganisms to organic residues. Conversely, in a sandy soil such as Sucarnoochee soil in which pore spaces are larger microorganisms have greater access to organic N [3]. The potentially mineralizable organic N (N_0) released in soils amended with broiler litter 1 or 2 were significantly higher than in those nonamended. The N_0 in soils amended with broiler litter 1 varied from 131 mg kg^{-1} in Dothan soil to 569 mg kg^{-1} in Sucarnoochee soil with a rate constant (k) of 0.0877 and 0.0230, respectively. Similarly, Dothan soil amended with broiler litter 2 had

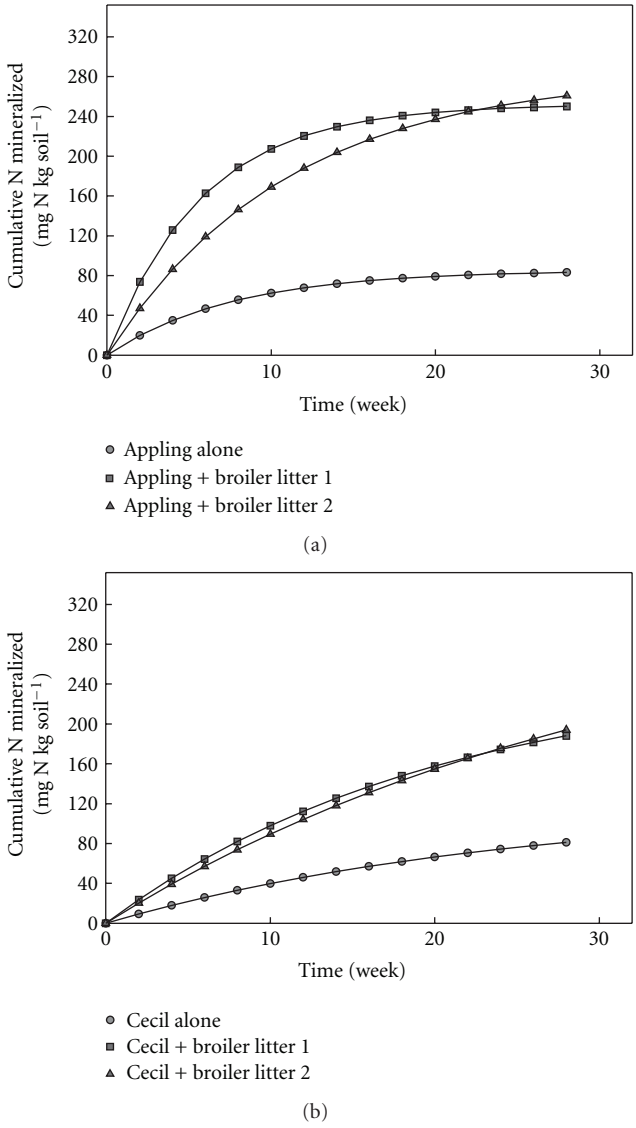


Figure 1: Cumulative organic nitrogen mineralized from Appling (a) or Cecil (b) soils amended with broiler litter 1 or 2 and incubated for 28 weeks under aerobic conditions.

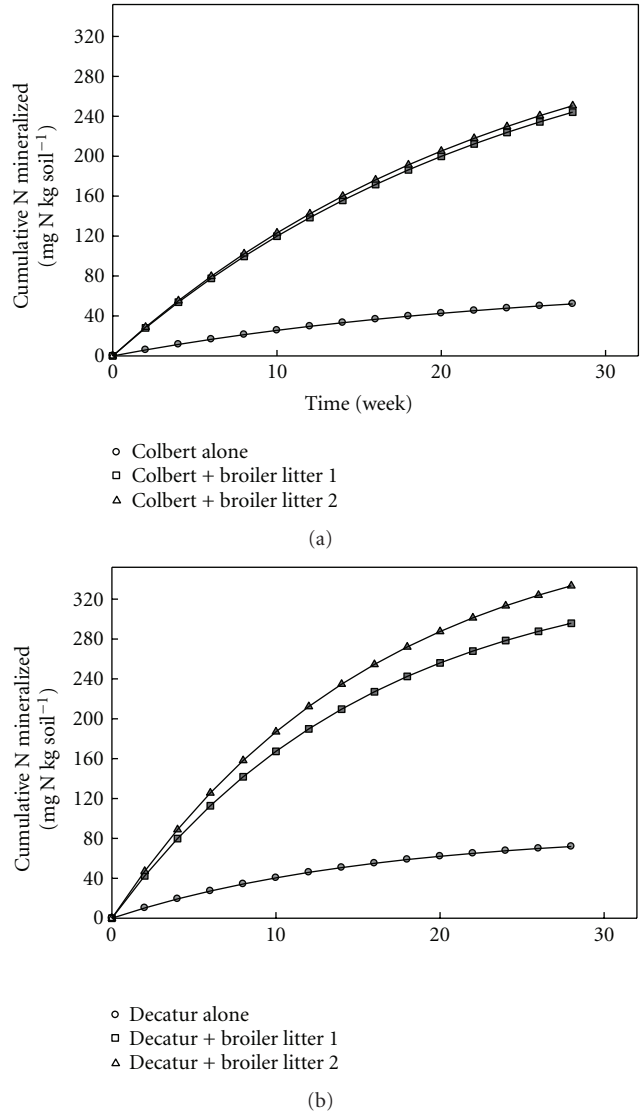


Figure 2: Cumulative organic nitrogen mineralized from Colbert (a) or Decatur (b) soils amended with broiler litter 1 or 2 and incubated for 28 weeks under aerobic conditions.

the lowest potentially mineralizable organic N releasing 167 mg kg^{-1} soil with a rate constant of 0.0854. However, Decatur soil amended with broiler litter 2 showed the highest potentially mineralizable N releasing 405 mg kg^{-1} soil with a rate constant of 0.0619. Among the ten soil samples studied, soils amended with broiler litter 2 had the highest N_0 as compared with those amended with broiler litter 1 with the exception of Maytag, Sucarnoochee, and Troup soils, which had greater N_0 when amended with broiler litter 1. Statistical analysis of the complied N_0 in Table 5 showed differences in the studied soils alone or amended with broiler litter. Among the nonamended soils, Appling, Colbert, Decatur, and Maytag soils showed no significant differences in N_0 at $P < 0.05$. Additionally, nonamended Cecil and Sucarnoochee soils ($N_0 = 120$ and $N_0 = 123$ mg N kg^{-1} soil, resp.) showed no significant difference at $P < 0.05$. Furthermore, for soils amended with broiler litter 1 or 2 there were no significant differences detected between Cecil, Colbert, Decatur, Dothan, Hartsells, Linker, Maytag, and Troup soils. However, Appling and Sucarnoochee soils treated with broiler litter 1 or 2 showed significant differences at $P < 0.05$. The broiler litter samples 1 and 2 added to the ten soils contained 27.5 and 46.1 g kg^{-1} of total N, respectively (Table 2). The bedding material also varied in the broiler litter samples; broiler litter 1 consisted of pine sawdust and broiler litter 2 peanut hulls. Therefore, the results suggest that the bedding material (peanut hulls or pine sawdust) may not have a significant difference on the N_0 pools.

3.3. Estimation of Mineralization Rate (k_i) of Organic N. To identify the various phases involved in the mineralization of organic N and to estimate the mineralization rate (k_i)

Table 6: First-order rate constants for decomposition of organic N in soil alone and broiler litter-amended soils.

Soil series	Broiler Litter Sample ID	Decomposition rate (week^{-1})†		Percentage of N mineralized at each Phase		Half-life of N Remaining (weeks)
		k_1	k_2	D_1	D_2	
Appling	none	0.00132	0.0003	6.73	1.38	75
Cecil	none	0.0127	0.0019	3.54	2.01	52
Colbert	none	0.004	0.002	2.27	1.34	50
Decatur	none	0.003	0.001	6.03	1.57	33
Dothan	none	0.006	0.002	3.80	1.78	50
Hartsells	none	0.0015	0.0006	1.85	0.78	66
Linker	none	0.007	0.003	5.10	0.37	33
Maytag	none	0.0105	0.0016	4.15	1.43	62
Sucarnoochee	none	0.012	0.001	4.68	2.31	38
Troup	none	0.0025	0.0026	2.49	0.95	40
Appling	1	0.0065	0.0023	18.8	1.10	15
Cecil	1	0.0026	0.0014	17.7	1.86	38
Colbert	1	0.0038	0.0025	18.2	3.32	26
Decatur	1	0.004	0.0018	11.3	2.40	25
Dothan	1	0.0028	0.0015	14.1	1.25	35
Hartsells	1	0.003	0.0014	20.7	2.29	33
Linker	1	0.0036	0.0012	20.4	2.32	28
Maytag	1	0.0044	0.0015	19.7	2.43	23
Sucarnoochee	1	0.003	0.0029	17.5	—	33
Troup	1	0.0023	0.00115	5.55	1.37	43
Appling	2	0.0039	0.0012	51.1	1.49	25
Cecil	2	0.0028	0.0014	29.9	—	35
Colbert	2	0.0031	0.0021	19.7	3.49	32
Decatur	2	0.0045	0.002	17.8	3.41	22
Dothan	2	0.0029	0.0018	33.7	1.56	34
Hartsells	2	0.0043	0.0021	28.6	2.69	23
Linker	2	0.0035	0.0014	26.6	2.58	28
Maytag	2	0.0044	0.0013	35.3	2.36	23
Sucarnoochee	2	0.0049	0.0018	28.7	2.93	20
Troup	2	0.0025	0.00095	18.5	1.29	40

† k_1 and k_2 were calculated from graphs prepared by plotting organic N remaining after each incubation time against time. No second phase was identified in Sucarnoochee and Cecil soils amended with broiler litter 2.

of the various organic N pools in the broiler litter, graphs were constructed by plotting the natural log of N remaining against incubation time (weeks) for the data collected. With the exception of Appling soil which showed three-phase decomposition, all other soils showed two-phase decomposition model and an example is provided in Figure 6 for two soils. In phase I (k_1), soil microorganisms mineralized quickly the easily mineralizable fraction in broiler litter; in phase II (k_2) a more resistant fraction of organic N is being mineralized, thus, mineralization rate slowed down. Finally, during phase III (k_3), soil microorganisms are mineralizing the most resistant fraction of organic N. A study conducted in three soil types concluded that soil type had a significant impact on broiler litter mineralization [37].

The half-life of N remaining corresponds to the amount of time required to mineralize half of the potentially mineralizable organic N (Table 6). Therefore, the higher the half-life of N remaining is, the slower the mineralization rate. The transformation of N mineralization data showed that the decomposition of organic N from broiler litter amended soils occurred in two phases as shown by D_1 and D_2. The percentage of N mineralized in the nonamended soils during phase I varied from 1.85% in Hartsells soil to 6.73% in Appling soil, respectively. However, in the same nonamended Hartsells and Appling soils, the percentages of organic N in phase II were only 0.78% and 1.38%, respectively. Again, in Appling soil amended with broiler litter 1, the organic N mineralized was 18.8% and 1.10% in phases I and II, respectively. With respect to soils amended with broiler litter 2, Appling soil mineralized 51.1% and 1.49%, of organic N in phases I, and II, respectively. Since the nonamended soils consist of a more resistant fraction of organic N, it was not easily mineralized by soil microbes; the values of organic N remaining in the nonamended soils were high. Similarly, the

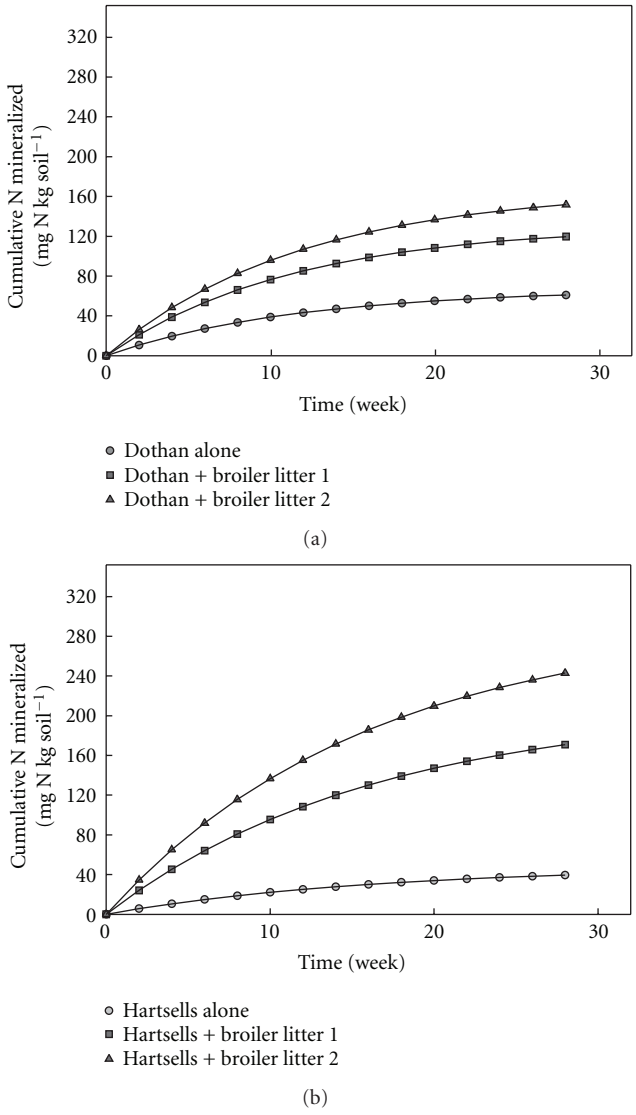


Figure 3: Cumulative organic nitrogen mineralized from Dothan (a) or Hartsells (b) soils amended with broiler litter 1 or 2 and incubated for 28 weeks under aerobic conditions.

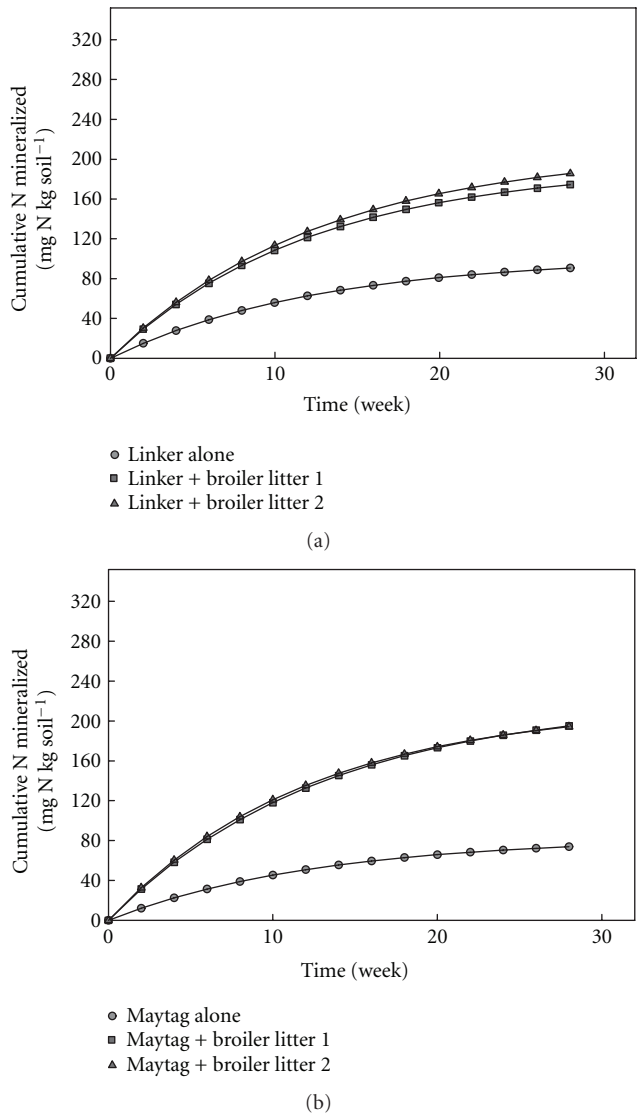


Figure 4: Cumulative organic nitrogen mineralized from Linker (a) or Maytag (b) soils amended with broiler litter 1 or 2 and incubated for 28 weeks under aerobic conditions.

half-life of N remaining in nonamended soils varied from 33 weeks in Decatur and Linker soils to 75 weeks in Appling soil. In addition, Appling and Maytag soils amended with broiler litter 1 and 2 showed significantly lower half-lives with 15 and 23 weeks when amended with broiler litter 1, and 25 and 23 weeks when amended with broiler litter 2, respectively. Moreover, the half-life of N remaining in the soils varied considerably with broiler litter samples and differences in soil type.

3.4. Relationships of Potentially Mineralizable Organic N (N_0) and Soil Properties. Linear regression lines show relationships between potentially mineralizable organic N (N_0) soil textures (Figure 7). There are a number of factors that affect the amount of available N in a soil for plant uptake. Some of these factors include soil properties such as texture, structure, temperature, pH, and organic matter content [38]. Other factors which affect N mineralization of broiler litter in soils include C/N ratio, particle size, pH of the litter, type of bedding material, and soluble N fractions of the litter [25]. The slopes of the linear curves are negative and imply that high silt and clay contents reduce N_0. However, high sand content accelerates mineralization of organic N in soils. A strong positive correlation ($r = 0.874^{***}$) between potentially mineralizable organic N (N_0) and sand content was observed. However, correlations between N_0 and both silt ($r = 0.780^{***}$) and clay ($r = 0.983^{***}$) were strongly negative. Influence of soil type and texture on N mineralization has been reported [39, 40]. These studies suggested that soils with relatively high silt and clay contents may have less ability to mineralize N than soils with high sand content, that promotes N mineralization. Nitrogen

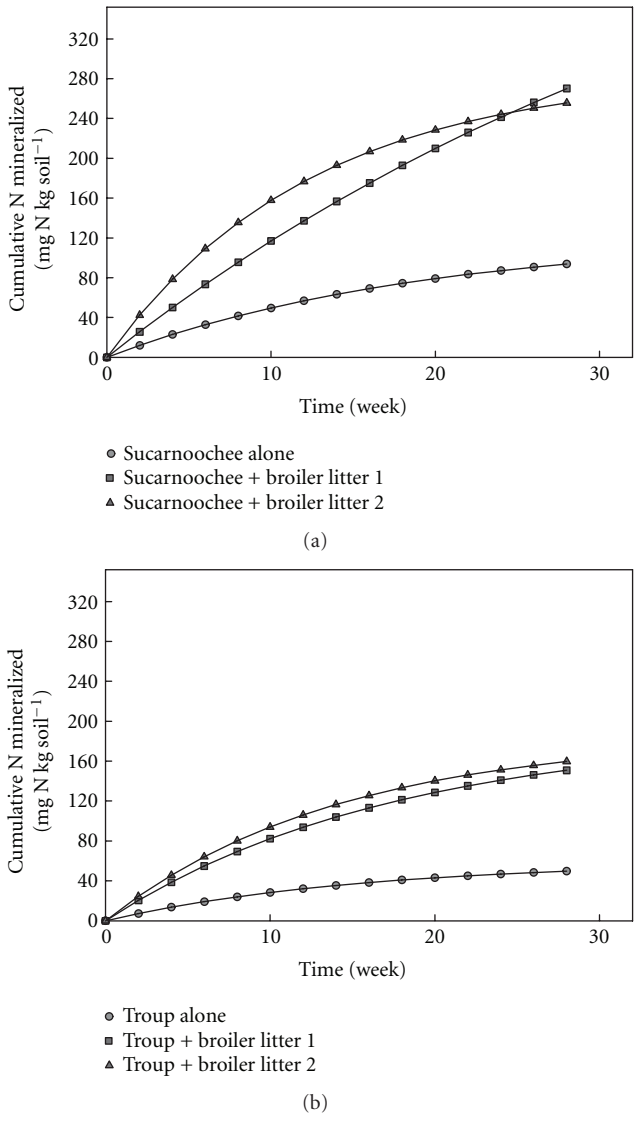


Figure 5: Cumulative organic nitrogen mineralized from Sucarnoochee soil (a) or Troup (b) soils amended with broiler litter 1 or 2 and incubated for 28 weeks under aerobic conditions.

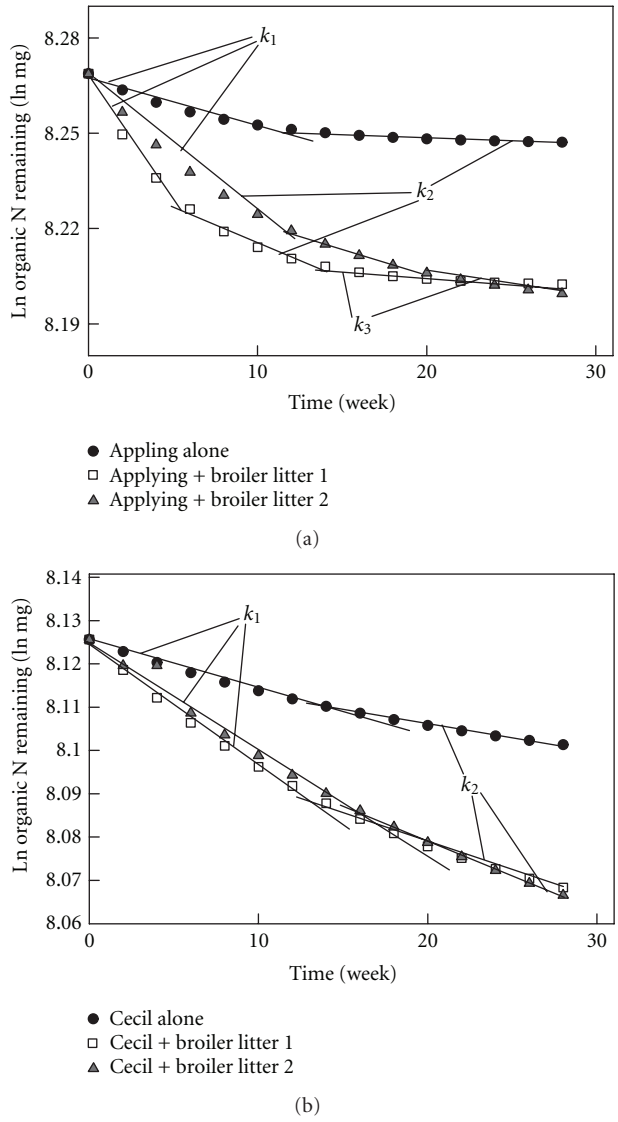


Figure 6: Natural log of organic N remaining in Appling (a) or Cecil (b) soils amended with broiler litter 1 or 2 as a function of time.

mineralization potential in five important agricultural soils of Hawaii showed that the amount and type of clay in a soil affects mineralization processes [3]. Finely textured soils with high clay content have many tiny micropores in which organic matter can find physical protection from microbial decomposition. These results also agree with those reported in nine soils amended with broiler litter that showed a strong positive correlation with sand and a negative correlation with silt and clay contents of the soils [41]. In addition, this study shows a positive correlation ($r = 0.782^{**}$) between N_0 and soil organic N (Figure 8(a)) and soil organic C ($r = 0.884^{***}$ Figure 8(b)). A positive correlation between C/N ratio and N mineralization ($r = 0.69$) was reported [42] but contradicted another study that reported no relationship between manure C/N ratios and N mineralization for a range of stored and fresh animal manures [43]. In this study also no significant relationship was found between N_0 and C/N ratio (data not shown).

4. Summary and Conclusions

Investigation of the mineralization of native N and added broiler litter as N source to soils clearly demonstrated that soil type impacts N mineralization. The mineralization of native or added broiler litter conformed to a first-order kinetic reaction but varied considerably with soil type and broiler litter samples. Broiler litter samples 1 and 2 mineralized different quantities of organic N from the ten soils studied. This indicates that organic N fractions in different broiler litter samples are not the same. Notably, the amounts of organic N mineralized from the same broiler litter sample in two different soil types suggest that organic N mineralized from the different

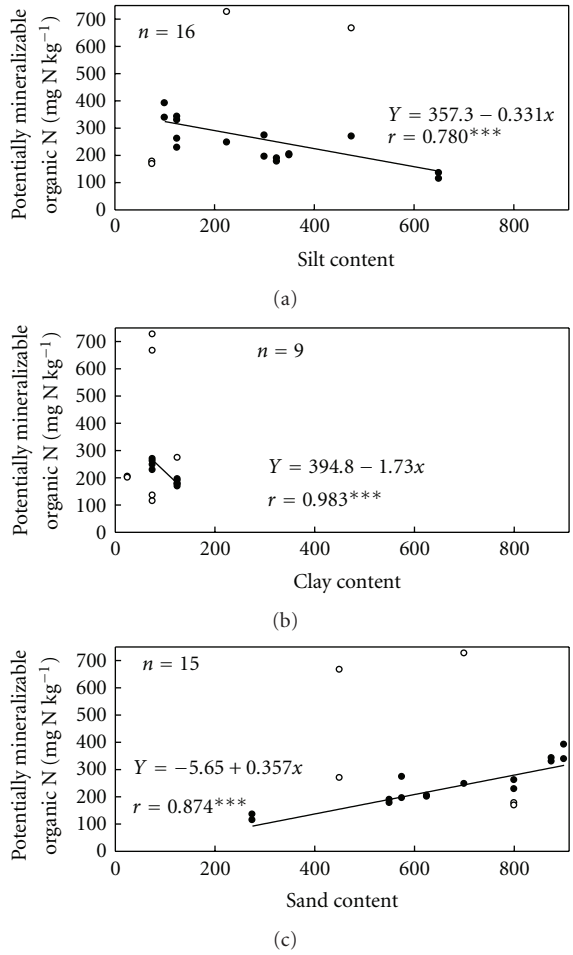


Figure 7: Relationships between potentially mineralizable organic nitrogen (N_0) and silt (a), clay (b), and sand (c) contents for soils amended with broiler litter 1 or 2. The open circles did not fit the relationships.

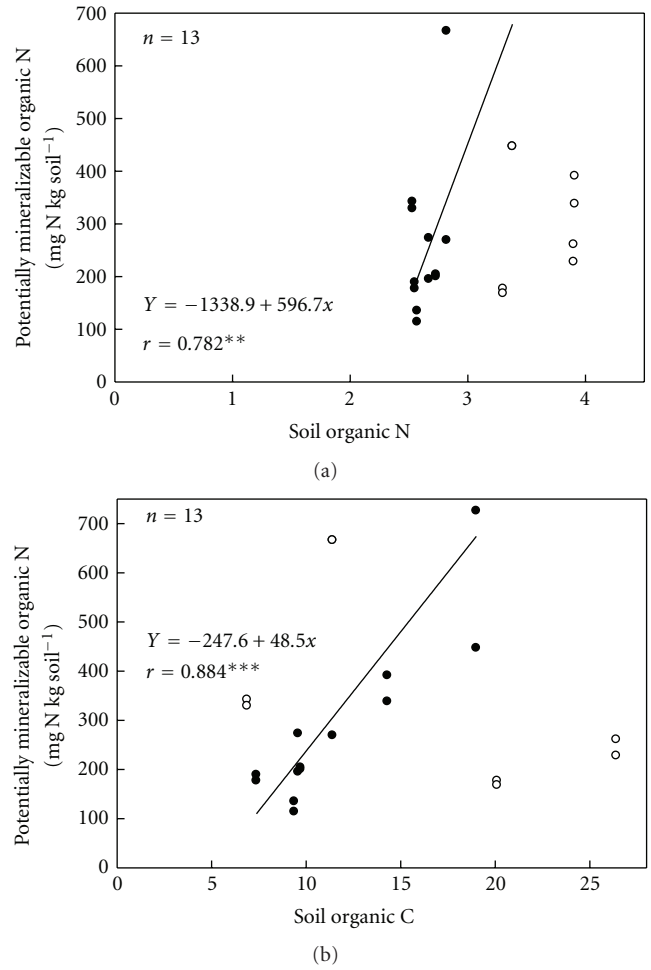


Figure 8: Relationships between potentially mineralizable organic nitrogen (N_0) and soil organic nitrogen (a) and soil organic carbon (b) in soils amended with broiler litter 1 or 2. The open circles did not fit the relationship.

broiler litter samples are dependent not only on organic N fractions in the broiler litter, but also on soil properties. Statistical analyses indicated that nonamended soil samples have differences in their ability to mineralize native organic N. However, among the ten soils studied only Appling and Sucarnoochee soils amended with broiler litter 1 or 2 showed significant differences at $P < 0.05$. The differences in the N_0 of the nonamended soils were obvious. The bedding materials varied in the broiler litter samples used in the study; broiler litter 1 consisted of pine sawdust and broiler litter 2 peanut hulls. The results suggested that the bedding material (peanut hulls or pine sawdust) may not have a significant difference on the N_0 pools. The decomposition of organic N from broiler litter amended soils occurred mainly in two phases represented by D_1, and D_2. Half-life of N remaining in the nonamended soils was significantly higher than that of soils amended with broiler litter 1 or 2 suggesting that the nonamended soils consisted of a very resistant organic N fraction that could not be easily mineralized by soil microbes. However, in the amended soils, the half-life was significantly lower and varied with broiler litter samples and soil types. The results also demonstrated that mineralization of broiler litter in soils is closely related to soil chemical and physical properties. Thus, decomposition of organic residues and animal waste in soils vary with waste and soils types and must be investigated in an effort to synchronize N release with crop demand and protect the environment from excess nitrate accumulation.

References

[1] P. A. Moore, T. C. Daniel, D. R. Edwards, and D. M. Miller, "Effect of chemical amendments on ammonia volatilization from poultry litter," *Journal of Environmental Quality*, vol. 24, no. 2, pp. 293–300, 1995.

[2] J. A. Delgado, *Potential Use of Innovative Nutrient Management Alternatives to Increase Nutrient Use Efficiency, Reduce Losses, and Protect Soil and Water Quality*, Special Issue of the Journal of Communications in Soil Science and Plant Analysis, Marcel Dekker, New York, NY, USA, 2001.

[3] J. Deenik, *Nitrogen Mineralization Potential in Important Agricultural Soils of Hawaii*, Soil and Crop Management, Cooperative Extension Service, 2006.

[4] Nutrition Business Journal, Organic Trade Association (OTA), Greenfield, Mass, USA, 2006, http://www.ers.usda.gov/publications/eib55/eib55ref.pdf.

[5] United States Department of Agriculture: Economic Research Services, 2009, http://www.nal.usda.gov/Briefing/Organic/Questions/farmersmarkets.htm/.

[6] K. P. Paudel and C. S. McIntosh, "Country report: Broiler industry and broiler litter-related problems in the southeastern United States," *Waste Management*, vol. 25, no. 10, pp. 1083–1088, 2005.

[7] National Agricultural Statistics Service, *Poultry-Production and Value 2007. Summary*, United States Department of Agriculture National Agricultural Statistics Service, 2008.

[8] J. S. Robinson and A. N. Sharpley, "Water quality: release of nitrogen and phosphorus from poultry litter," *Journal of Environmental Quality*, vol. 24, no. 1, pp. 62–67, 1995.

[9] United States Department of Agriculture: National Agriculture Statistic Service, 2010, http://www.nass.usda.gov/Charts_and_Maps/Poultry/brlmap.asp.

[10] K. Kpomblekou-A, "Relative proportion of inorganic and total nitrogen in broiler litter as determined by various methods," *Journal of the Science of Food and Agriculture*, vol. 86, no. 14, pp. 2354–2362, 2006.

[11] H. O. Liechty, M. A. Blazier, J. P. Wight, L. A. Gaston, J. D. Richardson, and R. L. Ficklin, "Assessment of repeated application of poultry litter on phosphorus and nitrogen dynamics in loblolly pine: implications for water quality," *Forest Ecology and Management*, vol. 258, no. 10, pp. 2294–2303, 2009.

[12] K. Kpomblekou-A, R. O. Ankumah, and H. A. Ajwa, "Trace and nontrace element contents of broiler litter," *Communications in Soil Science and Plant Analysis*, vol. 33, no. 11-12, pp. 1799–1811, 2002.

[13] C. C. Bitzer and J. T. Sims, "Estimating the availability of nitrogen in poultry manure through laboratory and field studies," *Journal of Environmental Quality*, vol. 17, no. 1, pp. 47–54, 1988.

[14] P. de Willigen, "Nitrogen turnover in the soil-crop system; comparison of fourteen simulation models," *Fertilizer Research*, vol. 27, no. 2-3, pp. 141–149, 1991.

[15] W. J. Wang, C. J. Smith, and D. Chen, "Predicting soil nitrogen mineralization dynamics with a modified double exponential model," *Soil Science Society of America Journal*, vol. 68, no. 4, pp. 1256–1265, 2004.

[16] N. G. Juma, E. A. Paul, and B. Mary, "Kinetic analysis of net nitrogen mineralization in soil.," *Soil Science Society of America Journal*, vol. 48, no. 4, pp. 753–757, 1984.

[17] F. E. Broadbent, "Empirical modeling of soil nitrogen mineralization.," *Soil Science*, vol. 141, no. 3, pp. 208–213, 1986.

[18] B. H. Ellert and J. R. Bettany, "Comparison of kinetic models for describing net sulfur and nitrogen mineralization," *Soil Science Society of America Journal*, vol. 52, no. 6, pp. 1692–1702, 1988.

[19] G. Stanford and S. J. Smith, "Nitrogen mineralization potentials of soils," *Soil Science Society of America Journal*, vol. 109, pp. 190–196, 1972.

[20] J. A. E. Molina, C. E. Clapp, and W. E. Larson, "Potentially mineraizable nitrogen in soil: the simple exponential model does not apply to the first 12 weeks of incubation," *Soil Science Society of America Journal*, vol. 44, pp. 442–443, 1980.

[21] L. J. Mebius, "A rapid method for the determination of organic carbon in soil," *Analytica Chimica Acta*, vol. 22, no. C, pp. 120–124, 1960.

[22] J. M. Bremmer and C. S. Mulvaney, "Nitrogen-total," in *Agronomy*, A. L. Page, H. R. Miller, and D. R. Keeney, Eds., vol. 9, part 2, pp. 595–624, American Society of Agronomy, Madison, Wis, USA, 2nd edition, 1982.

[23] D. R. Keeny and D. W. Nelson, "Nitrogen-Inorganic forms," in *Agronomy*, A. L. Page, R. H. Miller, and R. D. Keeney, Eds., vol. 9, part 2, pp. 642–698, American Society of Agronomy, Madison, Wis, USA, 2nd edition, 1982.

[24] V. J. Kilmer and J. T. Alexander, "Method of making mechanical analysis of soils," *Soil Science Society of America Journal*, vol. 48, pp. 753–757, 1949.

[25] A. Sissoko and K. Kpomblekou-A, "Carbon decomposition in broiler litter-amended soils," *Soil Biology and Biochemistry*, vol. 42, no. 4, pp. 543–550, 2010.

[26] J. L. Smith, R. R. Schobel, B. L. McNeal, and G. S. Campbell, "Potential errors in the first-order model for estimating soil nitrogen mineralization potentials," *Soil Science Society of America Journal*, vol. 44, pp. 996–1000, 1980.

[27] A. J. Barr, J. H. Goodnight, J. P. Sall, and J. I. Helwig, *A User's Guide to SAS*, SAS Institute, Raleigh, NC, USA, 1976.

[28] C. M. Gilmour, F. E. Broadbent, and S. M. Beck, "Recycling of carbon and nitrogen through land disposal of various wastes," in *Soils for Management of Organic Wastes and Waste Waters*, L. E. Elliot and F. J. Stevenson, Eds., Soil Science Society of America, 1977.

[29] SAS Institute, *SAS User's Guide*, SAS Institute, New York, NY, USA, 1990.

[30] D. W. Marquardt, "An algorithm for least squares estimation of non-linear parameters," *Journal of Society of Applied Mathematic*, vol. 45, pp. 431–441, 1963.

[31] F. J. Stevenson, "Origin and distribution of nitrogen in soil," in *Nitrogen in Agricultural Soils*, F. J. Stevenson, Ed., pp. 1–42, Agronomy Society of America, Madison, Wis, USA, 1982.

[32] M. S. Coyne, *Soil Microbiology: An Exploratory Approach*, Delmar, New York, NY, USA, 1999.

[33] L. N. Nakhone and M. A. Tabatabai, "Nitrogen mineralization of leguminous crops in soils," *Journal of Plant Nutrition and Soil Science*, vol. 171, no. 2, pp. 231–241, 2008.

[34] G. M. Marion, J. Kummerow, and P. C. Miller, "Predicting nitrogen mineralization in chaparral soils," *Soil Science Society of America Journal*, vol. 45, pp. 956–961, 1981.

[35] I. J. Manguiat, I. Watanabe, G. B. Mascariña, and J. G. Tallada, "Nitrogen mineralization in tropical wetland rice soils: I. Relationship with temperature and soil properties," *Soil Science and Plant Nutrition*, vol. 42, no. 2, pp. 229–238, 1996.

[36] V. Z. Antonopoulos, "Comparison of different models to simulate soil temperature and moisture effects on nitrogen mineralization in the soil," *Journal of Plant Nutrition and Soil Science*, vol. 162, no. 6, pp. 667–675, 1999.

[37] K. R. Sistani, A. Adeli, S. L. McGowen, H. Tewolde, and G. E. Brink, "Laboratory and field evaluation of broiler litter nitrogen mineralization," *Bioresource Technology*, vol. 99, no. 7, pp. 2603–2611, 2008.

[38] K. H. Nahm, "Factors influencing nitrogen mineralization during poultry litter composting and calculations for available nitrogen," *World's Poultry Science Journal*, vol. 61, no. 2, pp. 238–255, 2005.

[39] P. Sorensen, E. S. Jensen, and N. E. Nielsen, "The fate of ^{15}N-labelled organic nitrogen in sheep manure applied to soils of

different texture under field conditions," *Plant and Soil*, vol. 162, no. 1, pp. 39–47, 1994.

[40] C. W. Honeycutt, T. S. Griffin, and Z. He, "Manure nitrogen availability: dairy manure in northeast and central U.S. soils," *Biological Agriculture and Horticulture*, vol. 23, no. 2, pp. 199–214, 2005.

[41] R. M. Gordillo and M. L. Cabrera, "Mineralizable nitrogen in broiler litter: II. Effect of selected soil characteristics," *Journal of Environmental Quality*, vol. 26, no. 6, pp. 1679–1686, 1997.

[42] M. D. Serna and F. Pomares, "Comparison of biological and chemical methods to predict nitrogen mineralization in animal wastes," *Biology and Fertility of Soils*, vol. 12, no. 2, pp. 89–94, 1991.

[43] J. Z. Castellanos and P. F. Pratt, "Mineralization of manures nitrogen correlation with laboratory indexes," *Soil Science Society of America Journal*, vol. 45, pp. 354–357, 1981.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 978528, 8 pages
doi:10.1155/2012/978528

Research Article

Nitrogen Immobilization in Plant Growth Substrates: Clean Chip Residual, Pine Bark, and Peatmoss

Cheryl R. Boyer,[1] H. Allen Torbert,[2] Charles H. Gilliam,[3] Glenn B. Fain,[3] Thomas V. Gallagher,[4] and Jeff L. Sibley[3]

[1] *Department of Horticulture, Forestry and Recreation Resources, Kansas State University, 2021 Throckmorton Plant Sciences Center, Manhattan, KS 66506, USA*
[2] *USDA-ARS National Soil Dynamics Laboratory, 411 South Donahue Drive, Auburn, AL 36832, USA*
[3] *Department of Horticulture, Auburn University, 101 Funchess Hall, Auburn, AL 36849, USA*
[4] *School of Forestry & Wildlife Sciences, Auburn University, 602 Duncan Drive, Auburn, AL 36849, USA*

Correspondence should be addressed to Cheryl R. Boyer, crboyer@k-state.edu

Received 6 January 2012; Accepted 5 March 2012

Academic Editor: Mark Reiter

Rising costs of potting substrates have caused horticultural growers to search for alternative, lower-cost materials. Objectives of this study were to determine the extent of nitrogen immobilization and microbial respiration in a high wood-fiber content substrate, clean chip residual. Microbial activity and nitrogen availability of two screen sizes (0.95 cm and 0.48 cm) of clean chip residual were compared to control treatments of pine bark and peatmoss in a 60-day incubation experiment. Four rates (0, 1, 2, or 3 mg) of supplemental nitrogen were assessed. Peatmoss displayed little microbial respiration over the course of the study, regardless of nitrogen rate; followed by pine bark, 0.95 cm clean chip residual, and 0.48 cm clean chip residual. Respiration increased with increasing nitrogen. Total inorganic nitrogen (plant available nitrogen) was greatest with peatmoss; inorganic nitrogen in other treatments were similar at the 0, 1, and 2 mg supplemental nitrogen rates, while an increase occurred with the highest rate (3 mg). Clean chip residual and pine bark were similar in available nitrogen compared to peatmoss. This study suggests that nitrogen immobilization in substrates composed of clean chip residual is similar to pine bark and can be treated with similar fertilizer amendments during nursery production.

1. Introduction

Pine bark (PB) and peatmoss (PM) have traditionally been used as nursery and greenhouse substrates in the US. These materials are becoming more costly to use in horticultural industries due to increasing fuel costs, reduced availability of PB [1], and environmental concerns over the use of PM for growing crops [2, 3]. Finding alternative substrates as a way to reduce costs has become an important issue for growers.

One promising alternative substrate is CCR, a forest by-product of the 'clean chip' industry. The 'clean chip' industry processes small caliper pine trees into uniform, bark-free material for making paper products. This procedure is conducted on site at pine plantations with in-field harvesting equipment. This equipment delimbs, debarks, and chips the material into the back of a chip van/truck for shipment to a pulp mill. The remaining material, composed of approximately 40% wood, 35% bark, 10% needles, and 15% indistinguishable fine material, is either spread back across the harvested area or processed once more through a grinder with 10.2 to 15.2 cm screens and sold to the pulp mills for boiler fuel. Currently, this leftover material composes around 25% of the site biomass and represents an income loss for forest landowners.

Clean chip residual has been evaluated (in a fresh state) for the production of several types of horticultural crops [4–7]. The residual material is obtained from loggers and further processed through a swinging hammer mill in order to produce material with reasonable particle size for horticultural use. Since this material is processed before use,

it can be hammer milled to pass several different screen sizes, producing substrates that are suitable for a variety of crop types and container sizes. Boyer et al. [4] evaluated perennials, buddleia (*Buddleja davidii* 'Pink Delight'), and verbena (*Verbena canadensis* 'Homestead Purple') in CCR and reported similar results among all treatments. A further study indicated that the use of supplemental N (in addition to standard control release fertilizer) was not necessary for growth of buddleia as compared to PB [5]. Later, Boyer et al. [6] demonstrated that annual plants, ageratum (*Ageratum houstonianum* 'Blue Hawaii'), and salvia (*Salvia superba* 'Vista Purple') grown in CCR or in combinations of CCR and PM produced similarly sized plants when compared to a traditional PB substrate treated with the same fertilizer regime. Woody plants such as loropetalum (*Loropetalum chinensis* var. *rubrum*) were also shown to have adequate growth in several screen sizes of CCR (compared to PB) over the course of one year [7].

While crop growth in CCR has been equal to that displayed by plants grown in traditional substrates, questions remain regarding the high wood content of forest residuals (especially among growers). Since PB has a high lignin content, it is slow to decompose, and producing crops over a short-term growing season (and some long-term seasons) has not caused problems due to shrinkage of substrate during decomposition [8].

Gruda et al. [9] reported significant N-immobilization resulting in less tomato (*Lycopersicon lycopersicum*) plant growth in substrates composed of a 100% wood fiber product. N-immobilization was calculated on the basis of N-balance including N-uptake by plants and residual mineral N in the substrates. Higher N-immobilization was found by increasing N-application rates. They determined that it is necessary to supply wood fiber substrates with nutrient solutions or fertilizer from the beginning of plant culture. Also, substrates without plants in this study (wood fiber and white PM) exposed to the same environmental conditions showed the same tendencies in N-immobilization as substrates with plants.

Concern has arisen over whether the high wood content of CCR will immobilize N to an extent that plants experience a reduction in growth early in the crop cycle. This is especially important in greenhouse crops where the first few days and weeks are critical to long-term crop growth. The objective of this study was to determine the extent of N-immobilization in CCR, PB, and PM in order to make recommendations regarding how to overcome such a production problem.

2. Materials and Methods

Clean chip residual used in this study was obtained from a 10- to 12-year-old loblolly pine (*Pinus taeda*) plantation near Atmore, AL, which was thinned and processed for clean chips using a total tree harvester (Peterson DDC-5000-G Portable Chip Plant, Peterson Pacific Corp., Eugene, OR) and a horizontal grinder with 10.2 cm screens (Peterson 4700B Heavy Duty Horizontal Grinder, Peterson Pacific Corp., Eugene, OR). The material was further processed through a swinging hammer mill (no. 30; C.S. Bell, Tifton, OH) with either a 0.95 cm or 0.48 cm screen to produce two CCR products for testing. These two CCR particle sizes were compared with PB and PM (Table 1). Pine bark used in this study was obtained from Pineywoods Mulch Company (Alexander City, AL). Peatmoss was obtained from Premier Horticulture, Inc. (Quakertown, PA) and was tested to confirm that no supplemental N had been added prior to use in this study.

Table 1: Physical properties of clean chip residual, pine bark, and peatmoss substrates.[z]

Substrates[y]	Air space[x]	Container capacity[w] (% Vol)	Total porosity[v]	Bulk density ($g \cdot cm^{-3}$)[u]
0.48 cm CCR	28 b[t]	57 b	85 b	0.22 a
0.95 cm CCR	48 a	42 d	90 b	0.19 b
PB	31 b	48 c	79 c	0.18 b
PM	11 c	87 a	98 a	0.11 c

[z]Analysis performed using the North Carolina State University porometer.
[y]CCR: clean chip residual, PB: pine bark, and PM: sphagnum peatmoss.
[x]Air space is the volume of water drained from the sample/volume of the sample.
[w]Container capacity is (wet weight − oven dry weight)/volume of the sample.
[v]Total porosity is container capacity + air space.
[u]Bulk density after forced-air drying at 105°C (221.0°F) for 48 h.
[t]Means within column followed by the same letter are not significantly different based on Waller-Duncan k ratio t-tests at $\alpha = 0.05$ ($n = 3$).

Substrate air space (AS), container capacity (CC), and total porosity (TP) were determined following procedures described by Bilderback et al. [10]. Substrate bulk density (BD) ($g \cdot cm^{-3}$) was determined from 347.5 $g \cdot cm^{-3}$ samples dried in a 105°C forced-air oven for 48 h. Substrates were analyzed for particle size distribution (PSD) by passing a 100 g air dried sample through 12.5, 9.5, 6.35, 3.35, 2.36, 2.0, 1.4, 1.0, 0.5, 0.25, and 0.11 mm sieves with particles passing the 0.11 mm sieve collected in a pan. Sieves were shaken for 3 min with a Ro-Tap (Ro-Tap RX-29, W.S. Tyler, Mentor, OH) sieve shaker (278 oscillations/min, 159 taps/min). Substrate samples (four reps per treatment) were further evaluated for soilless media nutrient analysis (Brookside Laboratories, Inc., New Knoxville, OH). The material was ground and screened before saturated media extracts (water based) were prepared from the samples. Substrate pH, EC, NO_3, NH_4, and all other nutrients were measured using this water extract. Plant available NO_3 and NH_4 were determined using flow injection analysis (FIAlab-2500, FIAlab Instruments, Bellevue, WA), and Ca, Mg, P, K, Na, SO_4, B, Mn, Zn, Fe, and Cu were determined by microwave digestion with inductively coupled plasma-emission spectrometry (ICP) (Thermo Jarrell Ash 6500 ICAP series, Offenbach, Germany).

An incubation study was conducted at the USDA-ARS National Soil Dynamics Laboratory in Auburn, AL to determine N mineralization/immobilization and microbial activity of each of the four substrate materials. A completely randomized design (CRD) with 272 experimental units was conducted. Treatment structure was factorial with four substrates by four N rates by four sample dates, each with four

replications. There were also 16 units (four for each collection date) without substrate, serving as ambient CO_2 traps. The incubation procedure consisted of weighing 20 g (dry weight basis) of substrate into plastic containers. Deionized water was added to adjust samples to consistent moisture content. Moisture content was determined by saturating 20 g of each substrate with deionized water and recording wet weight (after draining to simulate CC) and dry weight (after drying in an oven at 105°C for 48 h). The change in weight (wet minus dry) divided by the wet weight multiplied by 100 equals the percent (%) of moisture content (average of three subsamples). Each substrate had different percent of moisture contents, and subsequently an appropriate amount of deionized water was added to each sample in order to bring the moisture of the substrate up to CC. Container capacity is the amount of water in a just-drained container substrate. Four rates of supplemental N (0, 1, 2, and 3 mg of N added by the addition of 0, 0.5, 1.0, or 1.5 mL of 2000 ppm stock solution of NH_4NO_3) were added to each of the four substrates in the study. The containers were placed in sealed glass jars with 10 mL of water for humidity control and a vial containing 10 mL of 1 M NaOH as a CO_2 trap. The jars were incubated in the dark at 25°C and removed after 7, 15, 30, and 60 days. Carbon mineralization, which is a direct measurement of total microbial respiration, was measured in this study. Carbon dioxide in the NaOH traps was determined by titrating the excess base with 1 M HCl in the presence of $BaCl_2$. All traps were measured at each sampling date, and fresh NaOH traps were placed in the jars remaining to be sampled at later dates. At each sampling date, a set of samples were measured for inorganic N concentration. Samples were extracted with a 2 N KCl solution and measured for NH_4-N and NO_3-N using a Model 680 Microplate Reader (Bio-Rad Laboratories Inc., Hercules, CA). Inorganic N was calculated as the sum of NH_4 and NO_3. Potential N mineralization was the difference between final and initial inorganic N contents.

Data were analyzed using Waller-Duncan k ratio t-tests ($P \leq 0.05$) and a statistical software package (SAS Institute, Cary, NC). Data were analyzed separately for each sampling date.

3. Results and Discussion

3.1. Substrate Physical and Chemical Properties. Physical properties of the four substrates tested varied (Table 1). Each substrate had significantly different AS with 0.95 cm CCR having the greatest (48%) and PM having the least (11%). Container capacity was also different for each substrate; however, PM had the greatest CC (87%), as expected, and PB had the least CC (48%). Both CCR treatments were similar in TP but were between of 98% for PM and 79% for PB. Bulk density was greatest for 0.48 cm CCR (0.22 g·cm^{-3}) and least for PM (0.11 g·cm^{-3}).

Particle size analysis revealed that 0.48 cm CCR had the least amount of coarse particles (0.8%), while PM had the greatest amount of coarse particles (38%) (Table 2). Pine bark and 0.95 cm CCR were similar (30% and 27%) for coarse particles. Both CCR substrates had the highest amount of medium-sized particles (48% for 0.48 cm and 50% for 0.95 cm). Pine bark had 38% medium-sized particles and PM had 31%. The greatest percentage of fine particles was measured in 0.48 cm CCR (51%) followed by PM (31%), PB (32%), and 0.95 cm CCR (24%).

Substrate pH was significantly different for each substrate (Table 3). Clean chip residual screened at 0.95 cm had the highest pH (5.5) while 0.48 cm had a pH of 5.0. Peatmoss had the next highest pH (4.8) while PB had the lowest pH (4.1). Peatmoss and PB generally require lime addition to raise the pH for adequate plant culture. This may not be needed for substrates composed of CCR as its pH is already in an acceptable range for plant growth. Electrical conductivity was in the typical range for plant production, though each substrate was different (from 0.15 to 0.29 mS·cm^{-1}).

Substrate chemical analysis revealed that PM had a significantly higher amount of NO_3-N (39.0 ppm) than all other treatments (0.1-0.2 ppm) (Table 3). Values for NH_4-N were all low (from 0.1 to 0.4 ppm). Potassium was high in all substrates except PM (48.2–84.6 ppm versus 6.9 ppm). Calcium was greatest in PM (27.7 ppm) and least in the CCR treatments (from 5.3 to 9.1 ppm). Magnesium was also greatest in PM (28.3 ppm) and least in CCR (2.6 to 7.0 ppm). Sulfur was high in PB (50.9 ppm) and low in 0.95 cm CCR (7.7%). Iron, Mn, and Zn were higher in 0.48 cm CCR than all other treatments; however, these differences are not meaningful in a production context.

3.2. Microbial Respiration. Microbial respiration (MR) was evaluated at each sampling date (Table 4). Peatmoss consistently had the least microbial respiration regardless of date or supplemental N rate. The greatest microbial respiration occurred with the CCR treatments. As particle size decreased (0.48 cm), microbial respiration increased. Also, as N rate increased, microbial respiration increased in CCR and PB.

Microbial respiration at 7 days after treatment (DAT) showed that at each N rate, 0.48 cm CCR had the greatest microbial respiration followed by 0.95 cm CCR, PB, and PM (Table 4). For 0.48 cm and 0.95 cm CCR, microbial respiration was highest with 2 mg N and decreased significantly as N rate decreased. Pine bark had significantly higher microbial respiration at 2 mg N than at 0 and 1 mg N (each was different from each other).

At 15 DAT, 0.48 cm CCR (0 and 1 mg N rates) had the greatest microbial respiration followed by 0.95 cm CCR, PB, and PM (Table 4). At 2 and 3 mg N, the CCR treatments switched with 0.95 cm CCR having more microbial respiration than 0.48 cm. For 0.48 cm and 0.95 cm CCR, MR was highest with 2 mg N and decreased significantly as N rate decreased. Pine bark microbial respiration was similar at the three highest N rates, and although 0 and 1 mg N had less microbial respiration they were similar to each other. There were no differences in microbial respiration for PM at any N rate.

The greatest microbial respiration at 30 DAT for 0 mg N was with 0.48 cm CCR followed by 0.95 cm CCR, PB, and PM (Table 4). At 1 and 2 mg N both CCR treatments were similar, but PB followed by PM had less microbial

Table 2: Particle size analysis of clean chip residual, pine bark, and peatmoss substrates.

U.S. standard sieve no.	Sieve opening (mm)	Substrate[z]			
		0.48 cm CCR	0.95 cm CCR	PB	PM
1/2	12.50	0.0 a[y]	0.0 a	0.0 a	2.2 a
3/8	9.50	0.0 b	0.0 b	0.1 b	8.2 a
1/4	6.35	0.0 d	2.7 c	6.0 b	11.0 a
6	3.35	0.8 c	23.9 a	24.0 a	17.0 b
8	2.36	8.8 c	20.0 a	12.6 b	9.1 c
10	2.00	7.9 a	8.1 a	5.0 b	3.5 c
14	1.40	19.0 a	13.2 b	11.3 c	9.0 d
18	1.00	12.4 a	8.2 b	9.1 b	9.0 b
35	0.50	13.0 b	7.7 c	13.8 ab	15.1 a
60	0.25	12.1 a	7.8 b	8.4 b	9.4 b
140	0.11	15.1 a	7.0 b	5.1 b	5.0 b
270	0.05	5.8 a	0.9 c	2.5 b	1.1 c
pan	0.00	5.1 a	0.5 c	2.1 b	0.4 c
		Texture[x]			
Coarse		0.8 c	26.6 b	30.0 b	38.3 a
Medium		48.0 a	49.5 a	38.0 b	31.0 c
Fine		51.2 a	23.9 c	32.0 b	30.7 b

[z]CCR: clean chip residual, PB: pine bark, and PM: sphagnum peatmoss.
[y]Percent weight of sample collected on each screen, means within row followed by the same letter are not significantly different based on Waller-Duncan k ratio t-tests at $\alpha = 0.05$ ($n = 3$).
[x]Coarse: >3.35 mm, Medium: >1.00–<3.35 mm and Fine: <1.0 mm.

Table 3: Chemical properties of clean chip residual, pine bark, and peatmoss substrates.

Substrate[z]	pH	EC ($mS \cdot cm^{-1}$)[y]	Substrate micronutrient content[x]				
			B (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
0.48 cm CCR	5.0 b[w]	0.21 b	0.23 a	3.7 a	2.3 a	0.01 b	0.28 a
0.95 cm CCR	5.5 a	0.15 c	0.17 b	2.1 b	0.6 b	0.02 ab	0.06 c
PB	4.1 d	0.23 b	0.16 b	1.3 c	1.0 b	0.03 a	0.16 b
PM	4.8 c	0.29 a	0.13 c	0.2 d	0.2 c	0.01 b	0.05 c
			Substrate macronutrient content				
	NO_3-N (ppm)	NH_4-N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	SO_4 (ppm)
0.48 cm CCR	0.1 b	0.2 b	4.1 a	84.6 a	9.1 c	7.0 c	15.8 c
0.95 cm CCR	0.2 b	0.4 a	1.4 b	48.2 b	5.3 d	2.6 d	7.7 d
PB	0.1 b	0.1 b	4.0 a	52.5 b	16.2 b	11.5 b	50.9 a
PM	39.0 a	0.1 a	1.6 b	6.9 c	27.7 a	28.3 a	29.5 b

[z]CCR: clean chip residual, PB: pine bark, PM: sphagnum peatmoss.
[y]$1\ mS \cdot cm^{-1} = 1$ mmho/cm.
[x]Substrate analysis performed on nonamended samples; N: nitrogen, P: phosphorous, K: potassium, Ca: calcium, Mg: magnesium, S: sulfur, B: boron, Fe: iron, Mn: manganese, Cu: copper, Zn: zinc, and $1\ ppm = 1\ mg \cdot kg^{-1}$.
[w]Means within column followed by the same letter are not significantly different based on Waller-Duncan k ratio t-tests ($\alpha = 0.05$, $n = 3$).

respiration. At the highest N rate, 0.95 cm CCR had the greatest microbial respiration followed by 0.48 cm CCR, PB, and PM. For 0.48 cm CCR the greatest microbial respiration was with 1 and 2 mg N, while 0 and 3 mg N had less microbial respiration. For 0.95 cm CCR and PB only 0 mg N was significantly less than other N rates. There were no differences in microbial respiration for PM at any N rate.

Microbial respiration at 60 DAT showed that at 0 and 3 mg N rate, both CCR treatments had the highest microbial respiration, followed by PB and PM (Table 4). At 1 and 2 mg N rates, 0.95 cm CCR had the greatest microbial respiration followed by 0.48 cm CCR, PB, and PM. For 0.48 cm CCR and PM there were no differences across N rates. For 0.95 cm CCR, only the highest rate of N had less microbial respiration

Table 4: Microbial respiration in clean chip residual, pine bark and peatmoss substrates.

Substrate[z]	Carbon mineralization (mg/kg)				
	0 mg N	1 mg N[y]	2 mg N	3 mg N	MSD N-rate[x]
			0–7 days		
0.48 cm CCR	2370	3236	3584	3882	113
0.95 cm CCR	1757	2593	2987	3414	154
PB	1918	2311	2367	2478	111
PM	143	94	614	258	335
MSD Substrate	84	121	284	170	
			8–15 days		
0.48 cm CCR	2615	2827	2888	3358	183
0.95 cm CCR	2200	2620	3105	3706	209
PB	1625	1986	1944	2021	329
PM	356	384	353	371	117
MSD Substrate	283	140	172	149	
			16–30 days		
0.48 cm CCR	3479	3615	3631	3436	134
0.95 cm CCR	3229	3556	3728	3714	225
PB	2254	2485	2585	2586	129
PM	751	591	578	641	166
MSD Substrate	195	139	123	126	
			31–60 days		
0.48 cm CCR	4133	3812	3721	3837	459
0.95 cm CCR	4082	4211	4041	3767	324
PB	2950	3173	3356	3417	190
PM	1723	1682	1581	1582	258
MSD Substrate	355	166	238	248	
			Total: 0–60 days		
0.48 cm CCR	12360	13414	13778	14108	609
0.95 cm CCR	11016	13110	14377	14624	799
PB	8954	10097	10313	10484	422
PM	2989	2922	2762	2781	440
MSD Substrate	668	405	662	409	

[z]CCR: clean chip residual, PB: pine bark, and PM: sphagnum peatmoss.
[y]2000 ppm stock solution of NH_4NO_3 (0, 0.5, 1.0, 1.5 mL).
[x]MSD based on Waller-Duncan k ratio t-tests ($\alpha = 0.05$).

than the other rates, though the values were similar to 0 and 2 mg N.

Clean chip residual consistently had the greatest amount of microbial respiration among the substrates over the course of the incubation period (0–60 DAT) (Table 4). At 0 mg N rate, 0.48 cm CCR had greater microbial respiration than 0.95 cm, but at 1 and 2 mg N it was similar. At 3 mg N, 0.95 cm CCR had greater microbial respiration than 0.48 cm CCR. Pine bark and PM were different from each other and less than CCR treatments for microbial respiration. Across the N rates for 0.48 cm CCR, microbial respiration increased with increasing N rate. For 0.95 cm CCR, microbial respiration increased with increasing N rate, though 2 and 3 mg N were similar. Pine bark was similar at 1, 2, and 3 mg N rates, only 0 mg N had less microbial respiration. There were no differences in microbial respiration across N rates for PM.

3.3. Total Inorganic N (Plant Available N). At 7 DAT, PM had more N than all other treatments; all other treatments were similar (Table 5). At 2 and 3 mg N, PM had the most N followed by PB and the CCR treatments, which were similar to each other. Across N rates for 0.48 cm CCR and PB, 3 mg N had more N than other rates, which were similar. For 0.95 cm CCR, N increased with increasing N rate, though 0 and 2 mg N were similar. Peatmoss had greater available N as N rate increased.

Total inorganic N at 15 DAT showed that at 0 and 1 mg N PM had the greatest amount of available N followed by PB and CCR treatments, which were all similar to each other

Table 5: Total inorganic nitrogen (NH_4 and NO_3) mineralization in clean chip residual, pine bark and peatmoss substrates.

Substrate[z]	Nitrogen mineralization (mg/kg)				
	0 mg N	1 mg N[y]	2 mg N	3 mg N	MSD N-rate[x]
			0–7 days		
0.48 cm CCR	31	28	37	128	16
0.95 cm CCR	44	77	262	515	174
PB	44	56	440	1458	459
PM	1753	2549	3359	5264	790
MSD Substrate	130	174	250	850	
			0–15 days		
0.48 cm CCR	58	61	42	50	55
0.95 cm CCR	53	52	64	168	79
PB	5	88	293	1572	442
PM	1619	2937	3610	5869	588
MSD Substrate	169	281	169	630	
			0–30 days		
0.48 cm CCR	101	148	99	80	67
0.95 cm CCR	114	116	102	310	119
PB	75	108	152	1061	82
PM	2530	3591	4043	6149	768
MSD Substrate	342	429	356	402	
			0–60 days		
0.48 cm CCR	63	87	91	117	44
0.95 cm CCR	91	142	121	142	34
PB	33	39	58	761	94
PM	1806	2632	3533	5404	783
MSD Substrate	44	150	205	734	

[z]CCR: clean chip residual, PB: pine bark, and PM: sphagnum peatmoss.
[y]2000 ppm stock solution of NH_4NO_3 (0, 0.5, 1.0, 1.5 mL).
[x]MSD based on Waller-Duncan k ratio t-tests ($\alpha = 0.05$).

(Table 5). At 2 and 3 mg N, PM had the highest N followed by PB and the two CCR treatments, which were similar to each other. There was no significant difference in available N for 0.48 cm CCR across N rates. For 0.95 cm CCR and PB, the greatest amount of available N was with 3 mg N, all other N rates had less N and were similar to each other. Peatmoss had increasingly available N as N rate increased.

At 30 DAT, results for total available N were similar to results at 15 DAT, with one exception: among substrates treated with 2 mg supplemental N, PM had the greatest amount of N and all other treatments were similar (Table 5). Also for PM, 1 and 2 mg N were similar, though the trend continued toward having more available N as N rate increased.

Peatmoss had the most available N at all N rates among substrates at 60 DAT (Table 5). At 0 mg N, 0.95 cm CCR had greater N than PB. For all other N rates, CCR and PB had similar available N, though less than PM. Across N rates, there were no differences for 0.48 cm CCR. For the remainder of the substrates, available N increased with increasing N rates.

4. Discussion

Incubation studies have previously been used to evaluate N-immobilization for horticultural purposes. A study by Hartz and Giannini [11] reported short-term net N-immobilization (2-week aerobic incubation) in samples of composted municipal yard and landscape wastes from three locations, with an overall trend toward decreased immobilization with increased compost age. At least 9–12 weeks of composting were required to minimize the undesirable characteristics of immature compost. Compost materials generally provide enough N to negate the use of supplemental fertilizer; however, materials such as PB, PM, and CCR do not contain adequate N to support plant growth and require fertilization before use in plant production.

A subsequent study by Hartz et al. [12] determined the N and C mineralization rates of 19 manure and compost samples for use as soil amendments in vegetable production in 1996 and 12 additional samples in 1997. Net N mineralization was measured at 4- or 8-week intervals while

C mineralization was measured at 4-week intervals. An average of 16%, 7%, and 1% of organic N was mineralized after 12 weeks in 1996, and an average of 15%, 6%, and 2% was mineralized after 24 weeks of incubation for 1997, in manure, manure compost, and plant residue compost, respectively. Mineralization of manure C averaged 35% of initial C content after 24 weeks, while compost averaged only 14%. Within 4 (compost) or 16 weeks (manure), the rate of mineralization of amendment C had declined to a level similar to soil organic C.

Waste paper as a substitute for PM displays significant N-immobilization and high pH [13]. An incubation study was conducted to define the N status of the paper medium. Initial results indicated diminished plant growth in pure paper substrate. The amount of additional N needed was difficult to predict during cultivation. A composting process was deemed necessary to overcome N-immobilization and lower pH and to improve water-conducting properties of the substrate.

When manure compost is used, N-immobilization will eventually stop and N-mineralization will begin. In the previous cases, N was provided to the plant instead of being removed, resulting in competition between plants and microbes for N. Our results indicate that N became available for 0.95 cm CCR during the course of our study, but the change was small relative to the loss of N from PM.

While CCR exhibits high microbial respiration (particularly with smaller particle sizes), microbial activity and N-immobilization were generally similar to PB. Clean chip residual screened to 0.48 cm was more microbially active, most likely due to increased surface area resulting from smaller particle size. Since CCR has an inherently high percentage of PB (35%), it tends to perform similarly; the addition of 40% wood fiber does not seem to inhibit plant growth or require amendment changes in nursery crop production. This study indicated that almost no management differences should be expected for crops produced in CCR compared to PB.

Peatmoss had virtually no microbial activity as measured by respiration in this study. However, unlike PB and CCR this was not due to N limitation as there was no indication that respiration was impacted at all by the addition of N. While it is clear that the remaining materials produced significant immobilization of the fertilizer N, there was little or no difference between PB and CCR. While PB did have increased N levels at the highest supplemental rate, this was miniscule compared to PM and does not imply a need to change management. In fact, N levels in PB decreased over time compared to CCR, which showed slow increases in N. For example, at 2 mg supplemental N PB had 440 $mg \cdot kg^{-1}$ available N at 7 DAT, which was reduced to 58 $mg \cdot kg^{-1}$ by day 60. On the other hand, CCR had 37 $mg \cdot kg^{-1}$ at 7 DAT but increased to 91 $mg \cdot kg^{-1}$ by 60 DAT. This indicates that while the immobilization of N was similar in PB and CCR, it may have increased in PB over time. This is reflected in respiration where there initially was much lower microbial respiration from PB, but by 31–60 DAT the respiration rate was similar to CCR and much more responsive to changes in supplemental N rate. This indicates that PB became more inclined toward N-immobilization as time progressed.

In the current study, pH and EC of CCR were determined to be acceptable for plant culture. In fact, CCR may not require a limestone amendment to raise pH. Since 0.95 cm CCR and PB have similar particle size distributions, we recommend this screen size for 1-gal. containers on outdoor beds, while 0.48 cm CCR is more suitable for greenhouse production. Since many PM suppliers premix amendments into shipped products, it is essential to determine the correct fertilization amendments so that substrates composed of CCR can perform similarly to PM. The results of this study indicate that growers should not be concerned about short-term negative impacts from N-immobilization in CCR (compared to PB).

References

[1] W. Lu, J. L. Sibley, C. H. Gilliam, J. S. Bannon, and Y. Zhang, "Estimation of U.S. bark generation and implications for horticultural industries," *Journal of Environmental Horticulture*, vol. 24, pp. 29–34, 2006.

[2] W. R. Carlile, "Growing media and the environment lobby in the UK 1997–2001," *Acta Horticulturae*, vol. 644, pp. 107–113, 2004.

[3] S. Holmes, "Peat and peat alternatives: their use in commercial horticulture in England and Wales in 2003," A Report for Horticulture and Potatoes Division, Department for Environment, Food and Rural Affairs, ADAS Horticulture, Boxworth, UK, 2004.

[4] C. R. Boyer, G. B. Fain, C. H. Gilliam, T. V. Gallagher, H. A. Torbert, and J. L. Sibley, "A new substrate for container-grown plants: Clean Chip Residual," *Procedures of the International Plant Propagators Society*, vol. 56, pp. 553–559, 2006.

[5] C. R. Boyer, G. B. Fain, C. H. Gilliam, T. V. Gallagher, H. A. Torbert, and J. L. Sibley, "Clean chip residual (CCR) substrate for container-grown perennials: Effect of supplemental nitrogen rates," *HortScience*, vol. 42, p. 439, 2007.

[6] C. R. Boyer, G. B. Fain, C. H. Gilliam, T. V. Gallagher, H. A. Torbert, and J. L. Sibley, "Clean chip residual: a substrate component for growing annuals," *HortTechnology*, vol. 18, no. 3, pp. 423–432, 2008.

[7] C. R. Boyer, C. H. Gilliam, G. B. Fain, T. V. Gallagher, H. A. Torbert, and J. L. Sibley, "Production of woody nursery crops in clean chip residual substrate," *Journal of Environmental Horticulture*, vol. 27, pp. 56–62, 2009.

[8] S. W. Kenna and C. E. Whitcomb, "Hardwood chips as an alternative medium for container plant production," *HortScience*, vol. 20, pp. 867–869, 1985.

[9] N. Gruda, S. V. Tucher, and W. H. Schnitzler, "N-immobilization of wood fiber substrates in the production of tomato transplants," *Journal of Applied Botany*, vol. 74, no. 1-2, pp. 32–37, 2000.

[10] T. E. Bilderback, W. C. Fonteno, and D. R. Johnson, "Physical properties of media composed of peanut hulls, pine bark and peatmoss and their effects on azalea growth," *Journal of the American Society for Horticultural Science*, vol. 107, pp. 522–525, 1982.

[11] T. K. Hartz and C. Giannini, "Duration of composting of yard wastes affects both physical and chemical characteristics of compost and plant growth," *HortScience*, vol. 33, no. 7, pp. 1192–1196, 1998.

[12] T. K. Hartz, J. P. Mitchell, and C. Giannini, "Nitrogen and carbon mineralization dynamics of manures and composts," *HortScience*, vol. 35, no. 2, pp. 209–212, 2000.

[13] H. D. Molitor and U. Brűckner, "Waste paper—a substitute for peat in horticulture," *Acta Horticulturae*, vol. 450, pp. 47–55, 1997.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 120429, 12 pages
doi:10.1155/2012/120429

Research Article

Nitrogen Availability and Uptake by Sugarbeet in Years Following a Manure Application

Rodrick D. Lentz and Gary A. Lehrsch

North West Irrigation and Soils Research Laboratory, USDA-ARS, 3793 N 3600 W, Kimberly, ID, 83341-5076, USA

Correspondence should be addressed to Rodrick D. Lentz, rick.lentz@ars.usda.gov

Received 16 November 2011; Accepted 20 February 2012

Academic Editor: Mark Reiter

The use of solid dairy manure for sugarbeet production is problematic because beet yield and quality are sensitive to deficiencies or excesses in soil N, and soil N availability from manure varies substantially depending on the year of application. Experimental treatments included combinations of two manure rates (0.33 and 0.97 Mg total N ha^{-1}) and three application times, and non-manure treatments (control and urea fertilizer). We measured soil net N mineralization and biomass, N uptake, and yields for sprinkler-irrigated sugarbeet. On average, the 1-year-old, low-rate manure, and 1- and 2-year-old, high-rate manure treatments produced 1.2-fold greater yields, 1.1-fold greater estimated recoverable sugar, and 1.5-fold greater gross margins than that of fertilizer alone. As a group the 1-year-old, low-rate manure, and 2- and 3-year-old, high-rate-manure treatments produced similar cumulative net N mineralization as urea fertilizer; whereas the 1-year-old, high-rate manure treatment provided nearly 1.5-fold more N than either group. With appropriate manure application rates and attention to residual N and timing of sugarbeet planting, growers can best exploit the N mineralized from manure, while simultaneously maximizing sugar yields and profits.

1. Introduction

An estimated 20 million Mg manure is produced annually by the 9-million-cow US dairy herd. The regional dairy center in southern Idaho comprises 5.6% of the US total dairy herd and produces approximately 1.11 million Mg manure annually. In Idaho, much of the dairy manure is soil applied to supply crop nutrients and as a means of rebuilding soil organic carbon. The latter is particularly important for eroded soils, which are common in this historically furrow irrigated region [1]. To maximize their use of manure and minimize losses of nitrogen (N) to the environment, growers need to know how much N becomes available to crops from manure applications [2]. In addition, as competition increases for cropland in the region, farmers who rent acreage can expand the pool of land available to them if they are willing to utilize manured ground.

This is particularly important for sugarbeet (*Beta vulgaris* L.) growers because yield and beet quality parameters, sugar, and brei nitrate concentration are sensitive to both insufficient N [3, 4] and excess soil N [5, 6]. In addition, sugarbeet tends to incorporate soil residual N preferentially over fertilizer N, that is, sugarbeet will utilize more soil residual N and less applied fertilizer N than corn or tomato crops [7]. Applying excess N fertilizer early in the season or applying an optimal N application after June can divert photosynthate sugars normally used for beet root growth and sucrose accumulation to excess top growth [8]. By contrast, multiple small feedings of N to the sugarbeet from May through July can increase sucrose accumulation in roots [9]. Early research showed a positive influence of manure on beet yield and sucrose concentration [3, 5]. Still, planting sugarbeet in recently manured fields is not always recommended because N availability from the manure is not well quantified and is believed to occur too late in the season to improve yield and quality [10]. However, Lentz et al. [11] reported that (1) peak net N mineralization in manure-amended, irrigated soils coincided with maximum N uptake by beet and (2) first-year manure applications $\leq 20\,Mg\,ha^{-1}$ (dry wt.) had no significant adverse effect on beet yield or quality.

Much of the N in dairy manure is in the organic form and only becomes available for uptake by crops via the time-dependent microbial-mediated process of mineralization.

Several studies have examined crop N uptake after multiple dairy manure applications, for barley (*Hordeum vulgare* L.) [12–14], corn (*Zea mays* L.) [15], wheat (*Triticum aestivum* L.) [16], and orchard grass (*Dactylis glomerata* L.) [17], or in the first year after a single manure application, for corn [18, 19], sugarbeet [11], and orchard grass (*Dactylis glomerata* L.) [17]. Relative to the total N applied in dairy manure, N recovery by corn, sugarbeet, and orchard grass in the first year after a single application ranged from −5 to 40% and averaged 21% [11, 17–19]. Crop N uptake from dairy manure amendments is influenced by type of crop [15], manure characteristics [20], organic amendment history [21], soil and location factors [18, 22, 23], application timing [12], and cropping management [24].

Far fewer studies have assessed N uptake by crops two and three or more years after a single manure application. For corn, the crop N recovery in the 2nd year after manure application was reported to be 9% by Klausner et al. [25], 8 to 15% by Ma et al. [15], and 15% by Eghball and Power [19], and N recovery for corn in years 3 through 4 was reported by Klausner et al. [25], being 2 or 3%. Similar studies for sugarbeet are lacking. One of the difficulties encountered when measuring crop N recovery from a manure application in successive years is the obfuscation caused by climatic variations between years [11]. Our objective was to (1) determine the effect of a single dairy manure application on sugarbeet yields and quality, N uptake, and N recovery for one, two, and three years after applying manure to a calcareous, irrigated, southern Idaho soil, and (2) employ an experimental approach that would reduce the confounding effects of climate between years.

2. Materials and Methods

We conducted the experiment at a site located 1.7 km southwest of Kimberly, ID (42E 31.12′N, 114E 22.47′W, elevation of 1190 m). The field plots were prepared in Portneuf silt loam soils (coarse silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid). The experimental site had a history of maznure applications, receiving 40 to 75 Mg ha^{-1} dry wt. every 3 yr between 1969 and 1986. In 1991 the uppermost 0.3 m (Ap horizon) of the Portneuf silt loam's profile was removed to expose the underlying Bk horizon and simulate an eroded profile [26]. For noneroded soil, the Ap horizon was left undisturbed. The site last received manure in 1994, 10 yrs before field plot preparations began for the current study. The eroded Portneuf soil profile is deep and calcareous, with textures ranging from silt loam to very fine sandy loam. Its surface soil (0 to 15 cm), that is, the Bk horizon, is a silt loam and contains on average 184 g kg^{-1} clay, 609 g kg^{-1} silt, 207 g kg^{-1} sand, has a pH of 7.8 (H_2O saturated paste), electrical conductivity (EC) of 0.08 S m^{-1}, and includes 4.1 mg kg^{-1} organic carbon, and 221 mg kg^{-1} calcium carbonate equivalent. A silica and calcium carbonate-cemented horizon (20–60% cementation) occurs between depths of 33 to 130 cm in the eroded Portneuf. The soil has a mean cation exchange capacity of 190 mmol$_c$ kg^{-1} and exchangeable sodium percentage of 1.5.

2.1. Experimental Design. Comparing sugarbeet yield and N uptake from a soil in years following a one-time manure application is problematic. Comparisons between annual sugarbeet measurements would be influenced not only by the treatment, but also by pest problems related to the continuous beet plantings and by climatic factors, which vary from year to year. To limit the effect of these confounding factors, we applied manure treatments at a 1x rate (average bulk application rate of 21.7 Mg ha^{-1} dry wt. or 0.31 Mg total N ha^{-1}), and a nominal 3x rate (average bulk application of 68.9 Mg ha^{-1} dry wt. or 0.97 Mg total N ha^{-1}) once only to a different set of field plots in the fall of each year 2004, 2005, and 2006. Thus, when sugarbeet was grown in 2007, the field plots included a set of two manure-rate treatments that were 1, 2, or 3 years old and were exposed to the same climatic conditions.

The experimental design was a randomized complete block with nine treatments and 4 replicates (Table 1). The experiment included the six manure treatments, with a manure-1x (m1) and manure-3x (m3) applied once to different plots of "eroded" Portneuf silt loam in 2004, 2005, and 2006. Three no-manure treatments were also included, urea fertilized (Fert) and control (no fertilizer or manure) treatments on eroded Portneuf soil, and a fertilized (urea) treatment on noneroded Portneuf soil (NE-Fert). No inorganic N fertilizer was applied to manure treatments. The Fert and NE-Fert treatments received 135 kg N ha^{-1} as urea-N, based on a sugarbeet yield goal of 63 Mg ha^{-1} [27] and a spring preplant soil test, which determined residual inorganic N present in the root zone (0–90 cm). The manure-1x application rate was a commonly applied rate in the region. At application time, we estimated the m1 manure would provide an average 107 kg N ha^{-1} to crops in the first year after application, based upon earlier reports that 32% of total manure N was available to crops in the first year [28]. Since a soil test indicated that no P or K fertilizer was needed on our site, we applied none. Plots were 9.1 m wide × 21.3 m long and accommodated 16 rows of beets.

For each year that manure treatments were applied, we obtained solid dairy cattle (*Bos* species) manure that had been stockpiled at a local dairy through the summer. The manure's average total C concentration (standard error) was 217 g kg^{-1} (58 g kg^{-1}), total N was 14.1 g kg^{-1} (2.6 g kg^{-1}), and C : N ratio was 15.9 (1.5).

2.2. Field Operations. Manure was applied to designated plots on 18 Nov. 2004, 22 Dec. 2005, and 19 Oct. 2006 using a commercial spreader truck equipped with rooster-comb beaters. Two to four 0.15 m^2 trays were randomly placed in each plot prior to spreading to quantify application rate. The manure collected in each tray was weighed, mixed, subsampled for moisture, C, and N analyses, and then returned to the soil surface from which it had been collected. The field was disked to a depth of 0.1 m within 48 hours of manure application. Plots were not fertilized in 2005 prior to planting spring barley. Barley was harvested in mid-July 2005. In fall 2005 prior to manure application, surface residue was burned to destroy weedy growth that had occurred after harvest.

Table 1: Description of treatments.

Treatment name	Treatment ID	Soil type	Added N source	Bulk applic. rate, dry wt. Mg ha^{-1}	Year of application†	Treatment age (y) at time of measurement
Noneroded fertilizer	NE-Fert	Noneroded	Urea	0.29	Each year	1
Control	Control	Eroded	None	0	N/A	1
Fertilizer	Fert	Eroded	Urea	0.29	Each year	1
Manure-1x						
2006	m1-y1	Eroded	Dairy manure	17.4	2006	1
2005	m1-y2	Eroded	Dairy manure	32.5	2005	2
2004	m1-y3	Eroded	Dairy manure	23.0	2004	3
Manure-3x						
2006	m3-y1	Eroded	Dairy manure	56.7	2006	1
2005	m3-y2	Eroded	Dairy manure	78.4	2005	2
2004	m3-y3	Eroded	Dairy manure	71.7	2004	3

† All fertilizer was applied in spring 2007 while all manure was applied in fall of the year shown.

In March 2006 soil samples were taken from plots at 0-to-30 cm and 30-to-60 cm depths and analyzed for soil N, P, and K (described below). Levels of P and K in the soils were adequate for small grain. On 13 Apr. 2006 the Fert and NE-Fert treatments received 134 kg N ha^{-1} as urea via hand-held spreader, while the control and manure plots received none. The field was disked to 0.1 m depth and roller-harrowed prior to planting barley in late April 2006. Barley residue and volunteer growth was burned on 13 Oct. 2006 before manure was applied to the designated plots.

On 15 Mar. 2007 soil samples were collected from plots in 30 cm increments down to 90 cm. We applied urea to the Fert and NE-Fert treatments and immediately incorporated the material with a roller harrow. Sugarbeet seed was planted (cv. BETA 4023R) on 20 Apr. 2007 in rows 0.56 m apart, with an in-row spacing of 55 mm and later thinned (30 May 2007) to a population of 117,000 plants ha^{-1} (manufacturer or trade names are included for the readers' benefit. The USDA-ARS neither endorses nor recommends such products). Insect control was accomplished using a Poncho seed treatment (CropScience LP, Research Triangle Park, NC, USA). Standard commercial procedures were used to control weeds and diseases. A single cultivation was performed on 26 June 2007. Irrigation through the growing season was supplied via sprinkler to meet the crop's evapotranspiration requirements. The beet crop was harvested on 10 Oct. 2007. Meteorological data required to calculate crop evapotranspiration (ET) were acquired from a weather station located 5.6 km northeast of the experimental plots. A rain gauge located near the field plot measured growing season precipitation. Crop ET was estimated from the maximum reference ET calculated using the Kimberly-Penman ET model [29], adjusted with the appropriate daily crop coefficient. Mean monthly air temperature, and total monthly precipitation, and irrigation during the 36-month study (including the plot preparation period, Fall, 2004 through 2006, and 2007 growing season) are reported in Figure 1.

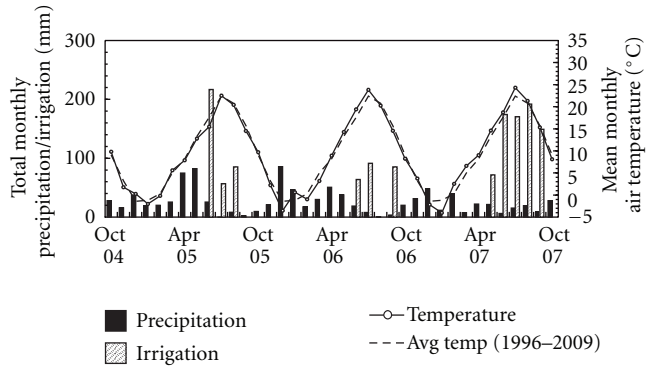


Figure 1: Total monthly precipitation and irrigation amounts, and mean monthly air temperature at the study site from fall 2004, when the first manure treatment was applied, through the 2007 growing season, when sugarbeet was planted on the experimental plots.

2.3. Sampling and Analyses. We measured N uptake in sugarbeet four times (1 June, 13 July, 20 Aug., and 27 Sep.) during the 2007 growing season by sampling total biomass of plant tops (aboveground tissue) and roots from 2 m of two adjacent rows (4 m total). The shredded sugarbeet roots and other aboveground plant tissue were dried at 65°C for dry matter determination. After grinding the dried tissue in a Thomas Wiley mill (Swedesboro, NJ) to pass an 865 μm screen, its total-N concentration was determined on a Thermo-Finnigan FlashEA1112 CNS analyzer (CE Elantech Inc., Lakewood, NJ, USA).

Sugarbeet yields were determined on 10 Oct. 2007 from two samples in each plot, each consisting of two adjacent 7.6 m long rows. Beet root subsamples collected for each of the two plot samples were analyzed for soil tare, as well as quality factors such as brei nitrate, brei conductivity, and sugar concentration, by the Amalgamated Sugar Company laboratory (Paul, ID, USA). Plot values were computed as

the arithmetic mean of the two samples. The projected gross margin for each plot was computed as gross revenue minus operating costs. The gross revenue was calculated as the product of beet yield (tons, wet wt.) and the 2007 grower beet payment, which varied from $33.47 to $39.23 per ton of beets (wet wt.) depending on beet sugar percentage. Operating costs [30] were assumed equal for all treatments except for differences related to fertilizer and manure application. Manure costs included only transport and spreading fees based on a 3.2 km one-way haul distance. Manure application costs were amortized across two years, hence the manure cost in the 3rd year after application was zero. Costs associated with annual fertilizer treatments included the price of product and its application.

A buried bag technique [31] was used to measure net N mineralization in plot soils during the 2007 growing season. Briefly, three 5.7 cm diameter soil cores, 0-to-30 cm deep were collected on 25 Apr. 2007 in each plot (one from three of the plot's four quadrants), composited, and passed through a 0.4 cm screen. A subsample of the composited soil was collected to determine baseline (or initial) inorganic N and soil water content. The remaining soil was placed in 10 μm thick, 5 cm diam. polyethylene tubes sealed on one end. After being filled, the tubes were sealed on the remaining end, resulting in three 30 cm long soil columns that were inserted into the sample holes created previously. The bag's polyethylene film was only slightly permeable to water vapor but allowed good gas exchange between the enclosed soil and that surrounding it [31, 32]. A single bag was pulled from each plot on 15 June, 1 Aug., and 2 Oct. 2007. The net N mineralization during the period between burial and retrieval was calculated by subtracting the baseline inorganic N concentration (NO_3-N + NH_4-N) of the initial soil (collected 25 Apr.) from that of the retrieved bag. A positive difference indicated net N mineralization, while a negative value indicated net N immobilization during the period. We measured net N mineralization using buried bags for 25 Apr. to 15 June, 15 June to 1 Aug., and 1 Aug. to 2 Oct. The latter two period values were computed by difference relative to the previously retrieved buried bag sample. In addition, we estimated the net N mineralization in the not-yet-planted plots from 15 Mar. to 25 Apr. as the difference in soil inorganic N concentration (0–30 cm) between the two dates. We reported the net N mineralized as mg N kg^{-1} soil. Cumulative available soil N (0-to-30 cm depth) during the growing season was computed as the sum of the initial soil inorganic N present on 15 Mar., added fertilizer N (if any), and net N mineralized across the four periods.

The March 2006 and 2007 field soil samples and buried bag soil samples were air dried at 35°C and ground to pass a 2 mm screen. Soil N was extracted using a 2 *M* KCl solution. Within 6 h of extraction, the NO_3-N concentration in each extract was determined using an automated flow injection analyzer (Lachat Instruments, Loveland, CO, USA) after cadmium reduction (Method 12-107-04-1-B) while NH_4-N concentration was determined using a salicylate-hypochlorite method (Method 12-107-06-2-A). The soil's inorganic N concentration was calculated as the sum of the NO_3-N and NH_4-N concentrations (mg N kg^{-1} of dry soil). Bicarbonate extractable P [33] and exchangeable K [34] (except without the addition of charcoal) were determined on field soil samples using ICP-OES. Manure C and N concentrations were determined on a freeze-dried sample with the CNS analyzer described above.

2.4. Statistical Analysis. Crop yield, biomass, N uptake, and quality factors for sugarbeet (brei nitrate, brei conductivity) were examined separately for each reporting interval via analysis of variance (ANOVA), PROC Mixed [35]. The statistical model included treatment as the fixed effect and block as the random effect. Treatment means were separated using the Tukey option [35]. We also included several single-degree-of-freedom orthogonal contrasts in the analysis. These included up to five class comparisons, where a class represents a combination of treatments: (1) no-manure versus manure treatments, where the no-manure class is control + Fert + NE-Fert and manure is m1 + m3; (2) manure-1x versus manure-3x treatments, averaged across all years; (3) manure-1x versus manure-3x treatments for years 2 and 3 only; (4) manure only treatments (m1 + m3) in year-1 versus years 2 and 3; (5) m1-y1 + m3-y1 + m3-y2 versus NE-Fert + Fert. The last contrast (number 5) tested the hypothesis that, relative to fertilizer applications, the effect of manure on the crop was influenced by the interaction between the factors, manure rate and age of application. Since the manure-1x added less C and N to the soil, its influence on the crop would diminish more rapidly than the manure-3x applications. All analyses were conducted using a $P = 0.05$ significance level. An identical statistical approach was used to analyze treatment effects on cumulative available soil N.

Since the experiment was conducted at a single location, findings pertain principally to that location. With judicious foresight, however, inferences made and conclusions drawn may apply to other locations with similar climatic conditions and crop management practices.

3. Results and Discussion

Meteorological data presented in Figure 1 portray the climatic conditions that prevailed during the years when the experimental plots were being developed and for 2007, the year that sugarbeet was grown on the site. The 2007 growing season was warmer than average, specifically during the February–July period, which was on average 1.5°C warmer than the 1996-to-2009 mean. The plots received 175 mm of annual rainfall in 2007, or 70% of the 1996-to-2009 mean value. The increased early-summer heat units coupled with abundant irrigation water supplies and the delay of hard frost until after October (instead of late September) contributed to near optimal 2007 sugarbeet yields in southern Idaho [36].

3.1. Sugarbeet Biomass and N Uptake. Several treatment effects were significant for the sugarbeet cumulative biomass (Table 2) and N uptake (Table 3) within each measurement period. The contrast tests identified several relationships with respect to treatment classes. First, the no-manure and manure treatments on the whole produced similar cumulative biomass and N uptake in sugarbeet tops, roots, and whole

Table 2: The influence of treatment on the total cumulative biomass for 2007 sugarbeet plant components. Table gives *P* values for treatment effects, and single-degree-of-freedom orthogonal comparisons derived from an analysis of variance.

Source of variation	Accumulated sugarbeet biomass											
	Tops				Roots				Whole plant			
	1 June	13 July	20 Aug	27 Sept	1 June	13 July	20 Aug	27 Sept	1 June	13 July	20 Aug	27 Sept
	P values											
Treatment (TRT)	**	***	***	**	0.36	**	***	**	0.02	***	***	***
Orthogonal contrasts†												
No manure versus manure	0.64	0.26	0.7	0.5	0.9	0.6	0.8	0.25	0.7	0.4	0.9	0.23
Man: m1 versus m3	**	**	***	0.06	**	0.07	***	0.13	**	*	***	*
Man: y1 versus y2 & y3	0.9	***	**	0.29	0.82	**	**	*	0.9	***	**	*
Man y2 & y3: m1 versus m3	**	**	***	*	**	*	***	*	**	**	***	**

† No manure: NEFert + control + Fert; Man: manure = m1 + m3 where m1: manure-1x; m3: manure-3x; y1, y2, y3: fall manure applied 1, 2, and 3 years in the past, respectively.
$^{*}P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001$.

Table 3: The influence of treatment on N uptake in 2007 sugarbeet plant components. Table gives *P* values for treatment effects, and single-degree-of-freedom orthogonal comparisons derived from an analysis of variance.

Source of variation	N uptake by beet biomass components											
	Tops				Roots				Whole plant			
	1 June	13 July	20 Aug	27 Sept	1 June	13 July	20 Aug	27 Sept	1 June	13 July	20 Aug	27 Sept
	P values											
Treatment (TRT)	*	***	***	***	*	**	***	***	*	***	***	***
Orthogonal contrasts†												
No manure versus manure	0.26	0.25	0.78	0.52	0.38	0.35	0.75	0.7	0.65	0.26	0.9	0.6
Man-1x versus Man-3x	***	*	***	*	**	0.08	***	**	***	*	***	*
Man: y1 versus y2 & y3	0.9	***	*	0.15	0.87	***	**	0.11	0.9	***	*	0.09
Man y2 & y3: m1 versus m3	**	**	***	*	*	0.06	***	**	**	**	***	**

† No manure: NEFert + control + Fert; Man: manure = m1 + m3 where m1: manure-1x; m3: manure-3x; y1, y2, y3: fall manure applied 1, 2, and 3 years in the past, respectively.
$^{*}P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001$.

plants. This result was partly due to substantial variability among treatment responses within each class, for example, the control treatment values were about half those of other no-manure treatments, and the m1-y3 values were about half that of other manure treatments (Table 4). However, the result shown in Table 3 suggests that no-manure treatments provided similar quantities of soil N to sugarbeet on average as did the manure treatments. As a consequence, season-long, total biomass production, and N uptake were similar between the groups (Table 4). See the discussion later in this section.

Second, compared to the manure-1x treatments, the manure-3x in general resulted in 1.12x greater season-long cumulative biomass production and 1.37x greater N uptake (Table 4). In addition, the relative difference between manure-3x and manure-1x responses was greater in the 2nd and 3rd years after manure application (i.e., comparing results of the m1 versus m3 contrast in y2, y3 with that of the m1 versus m3 contrast averaged across all years in Table 4). The disproportionately smaller increase in both biomass and N uptake in response to a tripling of the manure rate indicated that the manure-3x treatment supplied excess N, and/or crop utilization of manure N decreased with increasing manure application [37]. As time since application increased (comparing the m1 versus m3 contrast in y2, y3), the N supplied by manure-1x apparently was less able to support beet growth than the manure-3x, causing a greater difference in biomass production and N uptake between the manure rate classes.

Third, the year of manure application affected total sugarbeet biomass and N uptake more during the early-June to mid-July period than at season's end (Tables 2 and 3). By 13 July 2007 the y1 manure treatments produced 1.4x greater sugarbeet biomass with 1.7x greater N uptake than the average for y2 and y3 manure treatments (Table 4). These disparities declined from that date onward. Thus by season's end, the sugarbeet in 2-year-old and 3-year-old manure plots had largely caught up to those of the 1-year-old manure treatments, such that differences were no longer significant. Thus y1 manure treatments generally provided greater

Table 4: Accumulated total biomass and N uptake in sugarbeet (tops and roots) at four times during the 2007 growing season.

Treatment†	Biomass				N uptake			
	1 June	13 July	20 Aug	27 Sept	1 June	13 July	20 Aug	27 Sept
	Mg ha^{-1} (dry wt.)				Kg ha^{-1}			
No manure								
NE-Fert	0.15a‡	7.3ab	17.5a	25.0bc	6.57a	124.6ab	183.8ab	257.4ab
Control	0.06b	4.2b	10.4c	17.1c	2.81b	52.7b	103.1b	152.5b
Fert	0.10ab	8.4a	20.9a	30.6a	4.85ab	164.6a	288.4a	383.3a
Manure-1x								
m1-y1	0.07ab	8.0a	18.2ab	25.3ab	3.18ab	140.3a	201.6ab	268.4ab
m1-y2	0.08ab	4.8b	12.1bc	21.8ab	3.6ab	62.6b	119.4b	211.0b
m1-y3	0.07b	4.1b	11.7bc	17.3c	2.98ab	56.4ab	123.2b	151.4b
Manure-3x								
m3-y1	0.13ab	7.6a	19.6a	24.8abc	5.89ab	140.5a	251.8a	300.2ab
m3-y2	0.13ab	7.0ab	18.4a	25.3ab	5.86ab	118.6ab	236.8a	315.4ab
m3-y3	0.13ab	6.0ab	17.3a	22.7b	5.44ab	92.4ab	211.4a	252.6ab
Treatment classes for orthogonal contrasts								
No manure	0.10	6.6	16.2	24.1	4.7	114.0	189.4	262.9
Manure	0.10	6.3	16.2	22.8	4.5	101.8	191.1	246.2
Manure-1x	0.07b	5.6b	14.0b	21.5b	3.3b	86.4b	146.9b	207.6b
Manure-3x	0.13a	6.9a	18.4a	24.1a	5.7a	117.2a	235.2a	284.8a
Manure Year-1	0.10	7.8a	18.9a	24.8	4.5	140.4a	227.8a	273.4
Manure Year 2 & 3	0.10	5.5b	14.9b	21.8	4.5	82.5b	172.7b	232.6
Year 2 & 3: m1	0.08b	4.5b	11.9b	19.6b	3.3b	59.5b	121.3b	181.2b
Year 2 & 3: m3	0.13a	6.5a	17.85a	24.0a	5.65a	105.5a	224.1a	284.0a

† NE-Fert: noneroded fertilizer (all other treatments on eroded soil); m1: manure-1x; m3: Manure-3x; y1, y2, y3: fall manure applied 1, 2, and 3 years in the past, respectively; manure: m1 + m3; no manure: NEFert + Control + Fert.
‡ Within a given plant component and sample date, treatment means followed by the same lower case letter are not significantly different ($P < 0.05$). Not displayed if effect was not significant in the ANOVA (Table 5).

available soil N than y2 and y3 manure during the June-July sugarbeet growth period, but in later months, either soil N availability declined or some factor interfered with the growth and N uptake in y1 manure beets. We hypothesize that the former was the case, resulting from increased N immobilization for y1 beets during the June and July. The release of abundant, readily metabolized C from manure in y1 may have stimulated microbial growth [38, 39]. Lentz et al. [11] showed that immobilization in manure-amended soils was greater in y1 after application compared to y2 and y3 (see later discussion).

Fourth, when y1 manure treatments as a class were compared with y2 and y3 manure treatments, y1 had 1.14x greater season-long total biomass production and 1.18x greater total N uptake (Table 4). Within a manure treatment and measurement period, however, the magnitude and significance of the differences between y1 manure treatments and y2 or y3 manure treatments were greater and more common for manure-1x than for manure-3x treatments (Table 4). This suggests that manure-3x treatments, regardless of age, provided adequate N for the crop. Furthermore, the m1-y1 treatment resulted in similar sugarbeet biomass production and N uptake as any manure-3x treatment no matter the year applied. This indicates that the m1-y1 treatment also provided adequate N for the sugarbeet.

The Fert and NE-Fert treatments consistently produced greater season-long crop biomass and N uptake than the control, although the difference was significant only for Fert after 1 June (Table 4, Figure 2), reflecting the greater N availability in the two fertilized treatments compared to the control. The NE-Fert produced greater sugarbeet biomass and N uptake than Fert on 1 June, day of year (DOY) 152, whereas the opposite tendency was observed at later dates. This likely resulted because seedlings emerged later and stand counts were 15% smaller (after thinning) in Fert plots relative to NE-Fert (data not presented). Later in the season, the lesser plant density for Fert compared to NE-Fert and other treatments (after thinning) may have rendered it less susceptible to a powdery mildew outbreak [40], which was identified in the field in midsummer and subsequently treated with fungicide and sulfur.

3.2. Sugarbeet Yield, Quality, and Profitability. Clean beet yields for all treatments ranged from 56.4 to 101.1 Mg ha^{-1} and averaged 83.0 Mg ha^{-1} (Table 5). These yields compare favorably with the average 2007 sugarbeet yield for southern

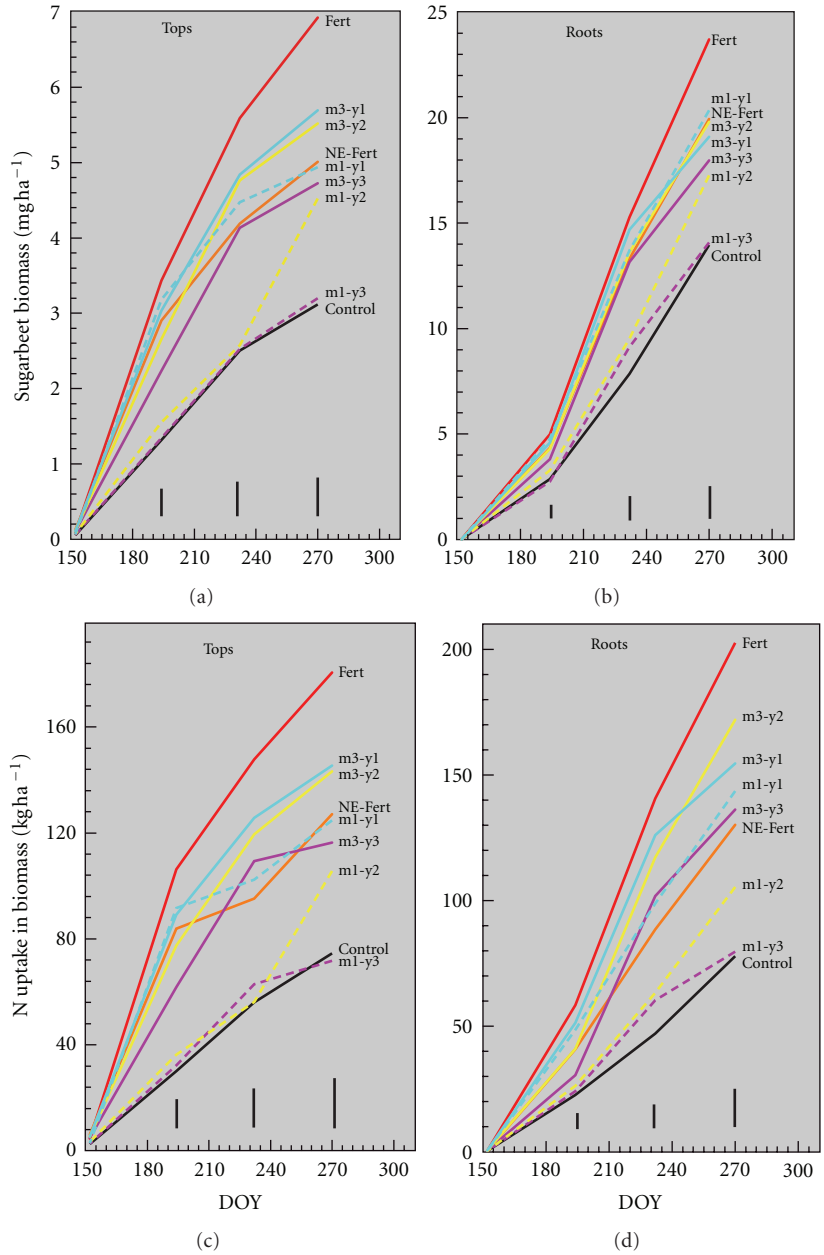


FIGURE 2: The effect of treatments on biomass accumulation in sugarbeet tops, that is, aboveground tissue (a) and root (b) components, and on N uptake in sugarbeet tops (c) and roots (d) in 2007. (Measured on dates (DOY) 1 June (152), 13 July (194), 20 Aug. (232), and 27 Sep. (270). Bar length represents the mean standard error ($n = 4$) for the 9 treatments at the given measurement date.

Idaho growers, 76.6 Mg ha^{-1} [36]. Sugarbeet yield and quality were affected by treatments, whether considered individually or when compared as classes (contrasts). The m1-y1, m3-y1, and m3-y2 treatments produced 1.3 to 1.8 times greater root yields than NE-Fert, control, m1-y2, and m1-y3 treatments (Table 5). Contrast tests showed that yields increased about 1.2-fold when (1) manure instead of fertilizer or no amendment was added to soil; (2) the manure amendment rate was increased from 1x to 3x; (3) sugarbeet was planted in the first year after fall manure application instead of waiting until the 2nd or 3rd year after application (Table 5).

Sugar concentration in beets ranged from 15.6 to 17.7% and averaged 16.7% (Table 5) with concentrations being generally greater in lower-yielding treatments, as expected. Our study's mean sugar concentration was nearly equivalent to the average sugar concentration obtained by southern Idaho growers in 2007, that is, 16.8% [36]. The NE-Fert and control treatments produced greater beet sugar concentrations than Fert, m3-y2, and m3-y3 treatments (mean 17.6 versus 16.0). Beet sugar concentrations decreased slightly (3–6% on average) when (1) manure was applied instead of fertilizer or no amendment; (2) manure application was

Table 5: Treatment and orthogonal contrast mean values for sugarbeet yield, quality, and gross margin parameters.

Treatment[†]	Clean beet root yield[‡]	Sugar	Est. Recov. sugar[‡]	Brei nitrate	Brei conductivity	Gross margin[§]
	Mg ha^{-1}	%	Mg ha^{-1}	mg kg^{-1}	dS m^{-1}	$US ha^{-1}
No manure						
NE-Fert	75.2c[§]	17.7a	11.6ab	59.8c	0.58d	979bc
Control	56.4c	17.6a	8.5b	106.8c	0.68cd	292 d
Fert	90.2ab	16.4b	12.3ab	187.3bc	0.88bc	1272ab
Manure-1x						
m1-y1	101.0a	16.7ab	14.0a	147.1bc	0.91b	1884a
m1-y2	72.8bc	16.8ab	10.4ab	143.6bc	0.77bcd	731bcd
m1-y3	64.5c	16.8ab	9.1b	185.4bc	0.81bc	484cd
Manure-3x						
m3-y1	97.7a	16.8ab	13.6a	149.1bc	0.95b	1676a
m3-y2	101.1a	16.0b	13.2a	259.6ab	0.96b	1510ab
m3-y3	88.5ab	15.6b	11.2ab	308.5a	1.02a	1138b
Treatment classes for contrasts						
No manure	73.9b	17.2a	10.8	118.0b	0.71a	848b
Manure	87.6a	16.5b	11.9	198.9a	0.90b	1237a
Manure-1x	79.4b	16.8a	11.2b	158.7b	0.83b	1033b
Manure-3x	95.8a	16.1b	12.7a	239.1a	0.98a	1441a
Manure y1	99.4a	16.8a	13.8a	148.1b	0.93	1780a
Manure y2 & y3	81.7b	16.3b	11.0b	224.3a	0.89	966b
Year 2 & 3: m1	68.7b	16.8a	9.8b	164.5b	0.79b	607b
Year 2 & 3: m3	94.8a	15.8b	12.2a	284.1a	0.99a	1324a
m1-y1, m3-y1, m3-y2	99.9a	16.5b	13.6a	185.3	0.9a	1690a
NE-Fert, Fert	82.7b	17.1a	12.0b	123.6	0.7b	1126b

[†] NE-Fert: noneroded fertilizer (all other treatments on eroded soil); m1: manure-1x; m3: manure-3x; y1, y2, y3: fall manure applied 1, 2, and 3 years in the past, respectively; manure: m1 + m3; no manure: NEFert + Control + Fert.
[‡] Clean yield: yield minus soil tare; Est. Recov. Sugar: estimated amount of sugar extractable from beets per unit area.
[§] Gross margin: gross revenue minus operating costs.
[¶] For a given yield or quality parameter, treatment means or means for individual orthogonal contrasts followed by the same lower case letter are not significantly different ($P < 0.05$). Not displayed if effect was not significant in the ANOVA.

increased from 1x to 3x; or (3) sugarbeet was planted in the first year after fall manure application instead of waiting until the 2nd or 3rd year after application. These results are consistent with the concept that increasing N availability decreases beet root sugar concentration [8, 9].

Increased nitrate and soluble impurity (conductivity) concentrations in sugarbeet brei (fresh macerated beet root) are associated with a decrease in the quantity of sugar recovered from the sugarbeet and increased sugar extraction costs [4, 27]. When the manure application rate increased from 1x to 3x, brei nitrate increased an average 1.6-fold (from 158.7 to 239 mg kg^{-1}) and conductivity increased 1.2-fold on average. Brei conductivity of manure treatments in year 1 did not differ from the mean value for year 2 and year 3. The m3-y3 treatment produced the greatest brei nitrate concentrations in beet roots, 309 mg kg^{-1}. While this value exceeded the 250 mg kg^{-1} target level recommended for southern Idaho [27], it was still well below the mean value for the 2007, southcentral Idaho sugarbeet crop, 351 mg kg^{-1} (S. Camp, Amalgamated Sugar Co., personal communication, 2010).

The control produced the least estimated recoverable sugar, 8.5 Mg ha^{-1} (Table 5). The treatments m1-y1, m3-y1, and m3-y2 produced the greatest estimated recoverable sugar values (mean 13.6 Mg ha^{-1}), which were 1.5x greater than that of the two least performing treatments, m1-y3 and control (mean 8.8 Mg ha^{-1}), and 1.1x that of the two fertilizer treatments (mean 12.0 Mg ha^{-1}). In addition, the estimated recoverable sugar in beets increased 1.22-fold, on average, when manure application was increased from the 1x to 3x rate or sugarbeet was planted in the first year after fall manure application instead of waiting until the 2nd or 3rd year after application.

The gross margins listed in Table 5 integrate treatment effects on beet yield and quality and fertilizer or manure costs, and provide a measure of treatment effects on profitability. An examination of individual manure treatments revealed that all except m1-y3 produced similar or greater gross margins than either the Fert or NE-Fert. Contrast tests showed that 1) the average gross margin for m1-y1, m3-y1, and m3-y2 manure treatments was 1.5-fold greater

Table 6: Treatment and contrast class mean values for soil (0–30 cm) inorganic N concentrations in spring (before and after planting), cumulative net N mineralization, and cumulative available N during the growing season.

Treatment†	Soil N 15 Mar.	Soil N 25 Apr.	Cum. net N mineralized 25 Apr.–27 Sept.	Cum. available N 15 Mar.–27 Sept.
			mg kg^{-1}	
No manure				
NE-Fert	8.3b‡	39.0a	18.0bc	57.0bc
Control	10.9b	12.6b	14.6c	27.1d
Fert	8.9b	33.6a	19.6bc	62.8bc
Manure-1x				
m1-y1	13.5b	31.8a	32.8ab	64.6b
m1-y2	10.0b	17.9b	23.7bc	41.6c
m1-y3	8.3b	14.1b	22.5bc	36.7cd
Manure-3x				
m3-y1	24.1a	45.9a	41.4a	87.3a
m3-y2	12.7b	27.8ab	28.5bc	56.3bc
m3-y3	13.7b	28.7ab	29.1b	57.8bc
Treatment classes for contrasts				
No manure	9.4	28.4	17.1b	49.0b
Manure	13.7	27.7	29.7a	57.4a
Manure-1x	10.6	21.3b	26.3b	47.6b
Manure-3x	16.8	34.1a	32.9a	67.1a
Manure y1	18.8	38.9a	37.1a	76.0a
Manure y2 & y3	11.2	22.1b	26.0b	48.1b
Year 2 & 3: m1	9.2b	16.0b	23.1b	39.2b
Year 2 & 3: m3	13.2a	28.3a	28.8a	57.1a

† NE-Fert: noneroded fertilizer (all other treatments on eroded soil); m1: manure-1x; m3: manure-3x; y1, y2, y3: fall manure applied 1, 2, and 3 years in the past, respectively; manure: m1 + m3; No manure: NEFert + Control + Fert.

‡ For a given yield or quality parameter, treatment means or means for individual orthogonal contrasts followed by the same lower case letter are not significantly different ($P < 0.05$). Not displayed if effect was not significant in the ANOVA.

than that for NE-fert and Fert treatments; 2) manure-3x treatments as a whole produced 1.4-fold greater gross margin than manure-1x treatments; 3) the mean gross margin for y1 manure treatment class was 1.8-fold greater than the y2 and y3 manure mean value; and 4) in the 2nd and 3rd year after manure application the manure-3x treatments on average resulted in a 2.2-fold greater gross margin than the manure-1x treatments.

Thus, fall manure applications 1 or 2 years prior to growing sugarbeet can potentially widen profit margins relative to conventional fertilizers applied preplant in the spring. While the m1-y1 manure treatment produced the greatest mean gross margin, use of greater manure application rates might be advisable. Manure quality often varies and the 1x rate leaves less margin for error. A greater application rate every two years, rather than one, halves application costs. Moreover, a high N-demand crop such as corn could be grown the year before sugarbeet to efficiently and profitably use the N mineralized in the first 12 months after the manure was applied [11]. On the other hand, mineralized N (as NO_3^-) could be leached below the sugarbeet's root zone before or during the beet growing season. Note that our margin analysis does not account for extra costs that may arise due to manure use, for example, additional management costs associated with increased weed pressure.

The influence of increasing manure applications on sugarbeet yields and estimated recoverable sugar in year 1 were also investigated by Lentz et al. [11] in 2003 for similar soils in southern Idaho. Lentz et al. [11] reported that, in contrast to the results of this study, sugarbeet root yields and recoverable sugar decreased as manure application rates increased. This difference was likely due to less initial residual soil N and less C and total N in the manure used in the current study relative to those in 2003. In sum, these factors decreased the N available in the 2007 soils which in turn reduced the possibility that excessive N mineralized from manure amendments would limit beet yields and recoverable sugar values [2, 8].

3.3. N Mineralization and Availability. The contrast tests for the season-long (25 Apr. to 27 Sept.) cumulative net N mineralization (Table 6) established that (1) manure treatments taken as a class produced 1.7x greater N than no-manure treatments; (2) manure-3x treatments on average produced 1.3x greater N than manure-1x treatments; (3) N

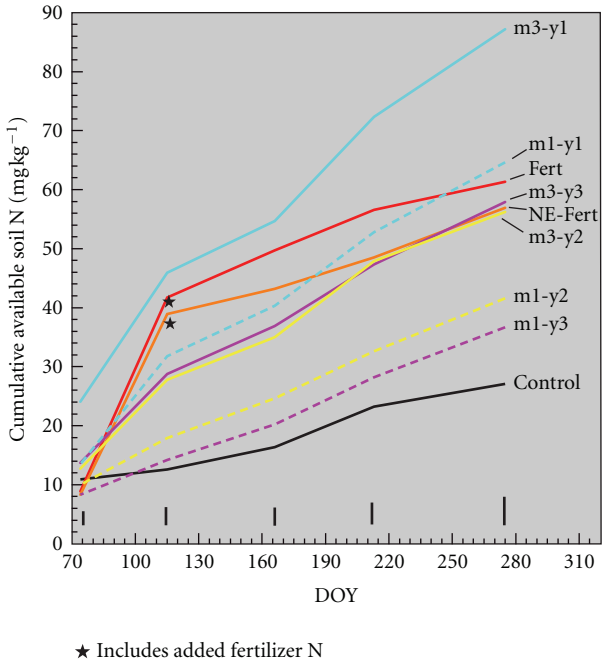


Figure 3: Cumulative net soil N available through mineralization and any fertilizer addition in the 0-to-30 cm soil during the 2007 sugarbeet growing season (measured on dates (DOY) 15 Mar. (74), 25 Apr. (115), 15 June (166), 1 Aug. (213), and 2 Oct. (275). Bar length represents the mean standard error ($n = 4$) for the 9 treatments at the given measurement date.

mineralized from y1 manure treatments as a class was 1.4x greater than that for the y2 and y3 manure treatment class mean. Hence, the cumulative available soil N from manure amendments generally declined as application rate decreased and time since application increased (Table 6, Figure 3).

The net N mineralized in the uppermost 0.3 m soil profile during the growing season (25 Apr. to 27 Sept.) for year-1 manure treatments was 32.8 mg kg^{-1} for manure-1x, or 2.2 times the control value, and 41.4 mg kg^{-1} for manure-3x, or 2.8 times that of the control (Table 6). These net N mineralization values for the year-1 treatments were similar to those reported by Lentz et al. [11] for comparable treatments in 2003, that is, 32.6 (manure-1x) and 48.7 mg kg^{-1} (manure-3x). Net N mineralized during the growing season for year-2 and year-3 manure treatments was reduced an average 30% in comparison to year-1 manure (Table 6). Findings from the 2007 growing season showed that fertilizer and the m1-y1, m3-y2, and m3-y3 treatments supplied similar amounts of cumulative N. In contrast m3-y1 provided nearly 1.5x more N ($P < 0.0001$), and m1-y2 and m1-y3 provided 37% less N ($P < 0.0001$) than the mean fertilizer treatment value (Table 6, Figure 3). The control and m1-y3 treatments provided the least cumulative available soil N, produced the least biomass, and led to the least N being incorporated into crop tissue (Figures 2 and 3).

For manure treatments, mineralized N accumulated at a slower rate in the interval from 25 Apr. to 15 June (DOY 115 to 166) than for other intervals (revealed as a decrease in slope in Figure 3). This slowing of the rate was most pronounced for the larger and more recent manure applications. This corroborates observations made by Lentz et al. [11], who described an identical phenomenon in their experiment conducted on similar soils at Kimberly, ID. The slowing rate of net N mineralization was likely due to immobilization of manure N that occurred after soils warmed during this early summer period. Mean soil temperatures at the 10 cm depth exceeded 21°C by mid-June (data not shown). Seasonal N mineralization data from an Ontario, Canada, experiment also showed a subtle dip in mineralization rate during this period, but the researchers described a more substantial decrease in N mineralization rate after DOY 227 [15]. The researchers attributed the substantial decreases to the release of carbonaceous root exudates and subsequent N immobilization [15]. Similar declines in N mineralization during the early summer period were reported for coastal Alabama soils amended with composted dairy manure [41].

The pattern of crop biomass accumulation and N-uptake in sugarbeet tops and roots (Figure 2) generally followed that of soil N availability (Figure 3). There were two exceptions. First, while Fert and NE-Fert treatments provided similar soil N, Fert produced substantially greater season-long crop biomass and N uptake than the NE-Fert (Figure 2, Table 4). This may be related to the differences in stand density and mildew pathology, as discussed previously. Second, though the net N mineralized for the m3 treatment was greater in y1 than for y2 or y3 (Figure 3), the extra N mineralized in y1 did not result in greater season-long crop N uptake (Figure 2, Table 4). This reveals that the N derived from the 3x manure (applied in the previous fall) was not utilized efficiently, presumably because it exceeded crop needs. Moreover, the excess soil mineral N in the 3x treatments was subject to leaching losses.

4. Conclusions

This study quantifies the effects of stock-piled dairy manure applications made 1, 2, or 3 years previously on sugarbeet. Results of this and a previous, related study [11] on calcareous, southern Idaho soil indicate that the influence of manure N applications on soil N availability, N uptake, and sugarbeet yield and quality was a function of residual inorganic soil N at the start of the growing season, the amount of Fall-applied manure added, and the year in which the manure was applied. A Fall manure application alone, when applied at an appropriate rate and planted to sugarbeet in either the first or second year after application provided adequate N nutrition for the production of a high quality sugarbeet crop. Furthermore, these manure treatments (m1-y1, m3-y1, and m3-y2) increased estimated recoverable sugar yields an average of 1.1-fold and increased gross margins an average of 1.5-fold relative to conventional fertilizer treatments. The increases in recoverable sugar and gross margins documented in this study are likely to vary from one site to another as a function of soil type, climate, and growing conditions. Our results illustrate nonetheless how proper manure management can increase sugarbeet yields and producer profit margins.

Abbreviations

Fert:	Fertilizer on eroded soil
NE-Fert:	Fertilizer on noneroded soils
EC:	Electrical conductivity
y1, y2, y3:	Fall manure applied 1, 2, and 3 years in the past, respectively.

References

[1] C. W. Robbins, B. E. Mackey, and L. L. Freeborn, "Improving exposed subsoils with fertilizers and crop rotations," *Soil Science Society of America Journal*, vol. 61, no. 4, pp. 1221–1225, 1997.

[2] J. N. Carter, D. T. Westermann, and M. E. Jensen, "Sugarbeet yield and quality as affected by nitrogen level," *Agronomy Journal*, vol. 68, no. 1, pp. 49–55, 1976.

[3] R. Gardner and D. W. Robertson, "Comparison of the effects of manures and commercial fertilizers on the yield of sugar beets," *Proceedings American Society of Sugar Beet Technology*, vol. 4, pp. 27–32, 1946.

[4] A. P. Draycott and D. R. Christenson, *Nutrients for Sugarbeet Production: Soil Plant Relationships*, CABI Publishing, Cambridge, Mass, USA, 2003.

[5] A. D. Halvorson and G. P. Hartman, "Long-term nitrogen rates and sources influence sugarbeet yield and quality," *Agronomy Journal*, vol. 67, pp. 389–393, 1975.

[6] J. N. Carter, "Effect of nitrogen and irrigation levels, location and year on sucrose concentration of sugarbeets in Southern Idaho," *Journal of the American Society of Sugar Beet Technologists*, vol. 21, no. 3, pp. 286–306, 1982.

[7] F. J. Hills, F. E. Broadbent, and O. A. Lorenz, "Fertilizer nitrogen utilization by corn, tomato, and sugarbeet," *Agronomy Journal*, vol. 75, pp. 423–426, 1983.

[8] J. N. Carter and D. J. Traveller, "Effect of time and amount of nitrogen uptake on sugarbeet growth and yield," *Agronomy Journal*, vol. 73, pp. 665–671, 1981.

[9] F. N. Anderson and G. A. Peterson, "Effect of incrementing nitrogen application on sucrose yield of sugarbeet," *Agronomy Journal*, vol. 80, pp. 709–712, 1988.

[10] J. G. Davis and D. G. Westfall, "Fertilizing sugar beets," Colorado State University Cooperative Extension Fact Sheet No. 0.542, Colorado State University, Fort Collins, Colo, USA, 2010.

[11] R. D. Lentz, G. A. Lehrsch, B. Brown, J. Johnson-Maynard, and A. B. Leytem, "Dairy manure nitrogen availability in eroded and noneroded soil for sugarbeet followed by small grains," *Agronomy Journal*, vol. 103, pp. 628–643, 2011.

[12] E. M. Hansen, I. K. Thomsen, and M. N. Hansen, "Optimizing farmyard manure utilization by varying the application time and tillage strategy," *Soil Use and Management*, vol. 20, no. 2, pp. 173–177, 2004.

[13] J. J. Miller, B. W. Beasley, C. F. Drury, and B. J. Zebarth, "Barley yield and nutrient uptake for soil amended with fresh and composted cattle manure," *Agronomy Journal*, vol. 101, no. 5, pp. 1047–1059, 2009.

[14] E. B. Mallory, T. S. Griffin, and G. A. Porter, "Seasonal nitrogen availability from current and past applications of manure," *Nutrient Cycling in Agroecosystems*, vol. 88, no. 3, pp. 351–360, 2010.

[15] B. L. Ma, L. M. Dwyer, and E. G. Gregorich, "Soil nitrogen amendment effects on nitrogen uptake and grain yield of maize," *Agronomy Journal*, vol. 91, no. 4, pp. 650–656, 1999.

[16] B. Gagnon, R. R. Simard, R. Robitaille, M. Goulet, and R. Rioux, "Effect of composts and inorganic fertilizers on spring wheat growth and N uptake," *Canadian Journal of Soil Science*, vol. 77, no. 3, pp. 487–495, 1997.

[17] V. R. Kanneganti and S. D. Klausner, "Nitrogen recovery by orchard grass from dairy manure applied with or without fertilizer nitrogen," *Communications in Soil Science and Plant Analysis*, vol. 25, no. 15-16, pp. 2771–2781, 1994.

[18] P. P. Motavalli, K. A. Kelling, and J. C. Converse, "First-year nutrient availability from injected dairy manure," *Journal of Environmental Quality*, vol. 18, no. 2, pp. 180–185, 1989.

[19] B. Eghball and J. F. Power, "Phosphorus- and nitrogen-based manure and compost applications: corn production and soil phosphorus," *Soil Science Society of America Journal*, vol. 63, no. 4, pp. 895–901, 1999.

[20] T. S. Griffin, Z. He, and C. W. Honeycutt, "Manure composition affects net transformation of nitrogen from dairy manures," *Plant and Soil*, vol. 273, no. 1-2, pp. 29–38, 2005.

[21] E. B. Mallory and T. S. Griffin, "Impacts of soil amendment history on nitrogen availability from manure and fertilizer," *Soil Science Society of America Journal*, vol. 71, no. 3, pp. 964–973, 2007.

[22] R. Xie and A. F. Mackenzie, "Urea and manure effects on soil nitrogen and corn dry matter yields," *Soil Science Society of America Journal*, vol. 50, no. 6, pp. 1504–1509, 1986.

[23] G. Wen, J. J. Schoenau, J. L. Charles, and S. Inanaga, "Efficiency parameters of nitrogen in hog and cattle manure in the second year following application," *Journal of Plant Nutrition and Soil Science*, vol. 166, no. 4, pp. 490–498, 2003.

[24] T. B. Parkin, T. C. Kaspar, and C. Cambardella, "Oat plant effects on net nitrogen mineralization," *Plant and Soil*, vol. 243, no. 2, pp. 187–195, 2002.

[25] S. D. Klausner, V. R. Kanneganti, and D. R. Bouldin, "An approach for estimating a decay series for organic nitrogen in animal manure," *Agronomy Journal*, vol. 86, no. 5, pp. 897–903, 1994.

[26] G. A. Lehrsch and D. C. Kincaid, "Sprinkler irrigation effects on infiltration and near-surface unsaturated hydraulic conductivity," *Transactions of the ASABE*, vol. 53, no. 2, pp. 397–404, 2010.

[27] A. Moore, J. Stark, B. Brown, and B. Hopkins, "Southern Idaho fertilizer guide: sugar beets," CIS 1174, University of Idaho Extension, Idaho, Moscow, 2009.

[28] B. Eghball, B. J. Wienhold, J. E. Gilley, and R. A. Eigenberg, "Mineralization of manure nutrients," *Journal of Soil and Water Conservation*, vol. 57, no. 6, pp. 470–473, 2002.

[29] J. L. Wright, D. T. Westermann, and G. A. Lehrsch, "Studying nitrate-N leaching with a bromide tracer in an irrigated silt loam soil," in *Best Management Practices for Irrigated Agriculture and the Environment*, J. Schaack, A. W. Freitag, and S. S. Anderson, Eds., pp. 229–242, U.S. Committee on Irrigation and Drainage, Denver, Colo, USA, 1998, Proceedings of the Irrigation and Drainage Water Management Conference, Fargo, ND, USA, 1997.

[30] P. Patterson, The economics of growing sugarbeets in southern Idaho: a short run gross margin analysis. AEES No 09-01, US Department of Agricultural Economics and Rural Sociology, University of Idaho Extension, 2009, http://www.amalgamatedsugar.com/articles/SugarbeetPacket.pdf.

[31] K. S. Balkcom, A. M. Blackmer, and D. J. Hansen, "Measuring soil nitrogen mineralization under field conditions," *Communications in Soil Science and Plant Analysis*, vol. 40, no. 7-8, pp. 1073–1086, 2009.

[32] C. F. Eno, "Nitrate production in the field by incubating the soil in polyethylene bags," *Soil Science Society Of America Proceedings*, vol. 24, no. 4, pp. 277–279, 1960.

[33] S. R. Olsen, C. V. Cole, F. S. Watatanabe, and L. A. Dean, "Estimation of available phosphorus in soils by extraction with sodium bicarbonate," USDA Circ. 939, U.S. Government Printing Office, Washington, DC, USA, 1954.

[34] J. J. Schoenau and R. E. Karamanos, "Sodium bicarbonate-extractable P, K, and N," in *Soil Sampling and Methods of Analysis*, M. R. Carter, Ed., pp. 51–58, Lewis Publishers, Boca Raton, Fla, USA, 1993.

[35] SAS Institute Inc., *SAS Online Documentation, Version 9.2 [CD-ROM]*, SAS Institute, Inc., Cary, NC, USA, 2009.

[36] S. Kraus, "Beet growers enjoy record yields," Ag Weekly, p. 29, 2007.

[37] R. D. Lentz and G. A. Lehrsch, "Net nitrogen mineralization from past year's manureand fertilizer applications," *Soil Science Society of America Journal*. In press, https://www.soils.org/publications/sssaj/view/76-3/s11-0282-6-4-23-2012.pdf.

[38] B. C. Liang, E. G. Gregorich, M. Schnitzer, and H.-R. Schulten, "Characterization of water extracts of two manures and their adsorption on soils," *Soil Science Society of America Journal*, vol. 60, no. 6, pp. 1758–1763, 1996.

[39] B. Marschner and K. Kalbitz, "Controls of bioavailability and biodegradability of dissolved organic matter in soils," *Geoderma*, vol. 113, no. 3-4, pp. 211–235, 2003.

[40] J. J. Burdon and G. A. Chilvers, "Host density as a factor in plant disease ecology," *Annual Review of Phytopathology*, vol. 20, pp. 143–166, 1982.

[41] D. B. Watts, H. A. Torbert, and S. A. Prior, "Soil property and landscape position effects on seasonal nitrogen mineralization of composted dairy manure," *Soil Science*, vol. 175, no. 1, pp. 27–35, 2010.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 101074, 10 pages
doi:10.1155/2012/101074

Research Article

Comparison of Raw Dairy Manure Slurry and Anaerobically Digested Slurry as N Sources for Grass Forage Production

Olivia E. Saunders,[1] Ann-Marie Fortuna,[1,2] Joe H. Harrison,[3] Elizabeth Whitefield,[3] Craig G. Cogger,[1] Ann C. Kennedy,[4] and Andy I. Bary[1]

[1] *Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164, USA*
[2] *Department of Soil Science, North Dakota State University, 1301 12th Avenue North, Fargo, ND 58105, USA*
[3] *Department of Animal Sciences, Washington State University, Puyallup, WA 98371, USA*
[4] *Land Management and Water Conservation Research Unit, USDA-ARS, Washington State University, Pullman, WA 99164, USA*

Correspondence should be addressed to Ann-Marie Fortuna, annmarie.fortuna@ndsu.edu

Received 29 November 2011; Accepted 23 January 2012

Academic Editor: H. Allen Torbert

We conducted a 3-year field study to determine how raw dairy slurry and anaerobically digested slurry (dairy slurry and food waste) applied via broadcast and subsurface deposition to reed canarygrass (*Phalaris arundinacea*) affected forage biomass, N uptake, apparent nitrogen recovery (ANR), and soil nitrate concentrations relative to urea. Annual N applications ranged from 600 kg N ha^{-1} in 2009 to 300 kg N ha^{-1} in 2011. Forage yield and N uptake were similar across slurry treatments. Soil nitrate concentrations were greatest at the beginning of the fall leaching season, and did not differ among slurry treatments or application methods. Urea-fertilized plots had the highest soil nitrate concentrations but did not consistently have greatest forage biomass. ANR for the slurry treatments ranged from 35 to 70% when calculations were based on ammonium-N concentration, compared with 31 to 65% for urea. Slurry ANR calculated on a total N basis was lower (15 to 40%) due to lower availability of the organic N in the slurries. No consistent differences in soil microbial biomass or other biological indicators were observed. Anaerobically digested slurry supported equal forage production and similar N use efficiency when compared to raw dairy slurry.

1. Introduction

There is a need for a set of best management practices that addresses how to utilize the growing quantity of reactive nitrogen (N) produced by livestock operations. Animal agriculture in the United States has become more specialized with farms consolidating and growing in size [1]. The number of dairy farms has decreased by 94% since 1960, but the number of animals has remained constant [2]. Animal consolidation has created challenges with respect to on-farm N surplus, waste management and nutrient loading in the environment [3, 4]. Annually in the United States, more than 5800 Mg of manure N is produced [5]. One approach to ameliorate negative environmental impacts associated with animal manures is through adoption of anaerobic digestion technologies to treat farm-generated manures and food processing wastes [6–9]. Digestion of wastes can provide a stable and consistent source of nutrients comparable to inorganic fertilizers such as urea.

Anaerobic digestion converts organic carbon into methane used to generate electricity, and it also converts organic N to plant available ammonium (NH_4^+), increasing the ratio of NH_4^+/total N in the effluent [10]. Carbon is removed during both the methane production and fiber removal processes, resulting in a smaller C : N ratio of the effluent [11]. Therefore, digested effluent can serve as low-cost source of readily available nutrients for crop production. Some studies [12] have found increased yield and N availability with application of anaerobically digested material as compared to nondigested material, possibly due to increased N availability and reduced carbon (C) content. Anaerobically digested manure can provide sufficient nutrients to support biomass and crop yields equivalent to synthetic fertilizers and raw manures [13, 14]. The apparent mineral nitrogen recovery

(ANR_M) of tall fescue (*Festuca* spp.) receiving raw dairy manure slurry was reported by Bittman et al. [13] as 55 and 51% at early and late applications, respectively, using the drag shoe method (band applied directly to soil, under plant canopy). When surface applied, the ANR_M was 37% applied early and 40% when applied late. Similar results were presented by Cherney et al. [15] with orchardgrass (*Dactulis glomerata* L.) and tall fescue.

Perennial systems that contain living plants year round tend to remove more reactive N than annual systems. Mean reed canarygrass biomass measured in trials in Minnesota was 13 Mg ha^{-1} under modest N applications (168 kg N ha^{-1} yr^{-1}) [16]. Bermudagrass (*Cynodon* spp.) fertilized with 89–444 kg manure N ha^{-1} yielded a mean of 7.92 Mg dry matter ha^{-1} over four or five cuttings per year [17]. However, the forage crop recovered only 25% of the N applied over the four years included in the study. Reed canarygrass (*Phalaris arundinacea*) is an ideal candidate for N removal because of its ability to store any left-over N applied during the growing season in rhizomes overwinter, providing a significant advantage to the forage in early spring when soil-N may be limited [18].

As with any N source, application of manure N in excess of crop uptake can result in NO_3^- leaching [19]. Up to 46% of applied manure N may persist in the soil, increasing the potential for loss of N after multiple applications in a growing season [20]. Some studies indicate that manure N poses less of a risk to leaching than the same amount of N in the form of synthetic fertilizer [21] due to immobilization of N that often occurs as humic materials build up in soil. Other researchers have determined manure increases NO_3^- leaching [22]. Irrespective of the source and properties of the N fertilizer applied during winter months when plants are dormant, NO_3^- leaching can be the main source of N loss [23].

Manure additions can enhance soil fertility and quality through their short and long-term contribution to soil C and N [24–27]. Current research demonstrates that long-term manure applications increase soil organic matter, basal respiration, microbial biomass, and enzymatic activity (measures of soil quality), while mineral fertilizers can decrease pH, enzymatic activity, and microbial biomass C [28]. Organic amendments such as manure also have an effect on microbial community structure in addition to enhancing the activity, C content, and size of soil microbial biomass [29, 30]. A study by Zhong et al. [31] demonstrated total phospholipid fatty acid (PLFA), gram-negative, and actinobacterial PLFA were highest in treatments of organic matter and organic matter + mineral NPK fertilizer. Functional diversity from organic manure and organic manure + mineral NPK fertilizers increased over time far more than with additions of synthetic fertilizers alone. We anticipate that long-term application of raw dairy slurry and digested slurry will enhance soil quality affecting microbial community structure and activity overtime.

The goal of this study was to determine the fate of applied N in anaerobically digested slurry (derived from mixed dairy slurry and food waste), raw dairy slurry, and urea during forage production. Specifically, we compare the biomass, ANR and N uptake of forages to determine which N source(s) has the potential to maintain forage biomass and reduce reactive N. In addition, we evaluated the effectiveness of subsurface deposition versus broadcast application of raw slurry and anaerobically digested slurry to improve forage biomass production, ANR and N uptake of forages, as well as reduce residual reactive N. Our hypotheses were (1) digested slurry would have more available N and generate a greater forage response than raw slurry, (2) subsurface deposition would conserve more N than surface application, resulting in greater forage response, particularly for the raw manure with higher solids content, and (3) application of digested slurry would reduce soil nitrate-N concentrations relative to urea.

2. Materials and Methods

2.1. Site Description. A field-based experiment, located on a commercial dairy in Monroe, Washington, was established in 2009. The field was mapped as 90% Puget silty clay loam (fine-silty, mixed, superactive, nonacid, mesic Fluvaquentic Endoaquepts) and 10% Sultan silt loam soils (fine-silty, isotic, mesic, Aquandic Dystrochrepts) [32] and had a history of manure applications. The site had climatic conditions typical of the Maritime Pacific Northwest with cool wet winters and dry summers. The 2009 growing season had a drier than normal summer, while 2011 had a cool spring and dry summer (Table 1). The 2010 season had the best growing conditions, with a warm spring and more summer rainfall than 2009 or 2011.

The experimental design included six treatments in a randomized complete block design with four replicates (3.6 m × 18 m). Treatments included two dairy manure slurries (raw and anaerobically digested), two slurry application methods (broadcast and subsurface deposition), inorganic N (pelletized urea), and a zero-fertilizer treatment that received 0 kg N ha^{-1}.

The raw dairy slurry was flushed from the barn floor and obtained fresh from a holding tank. Digested slurry was produced in an anaerobic digester with a plug-flow design, operating within mesophilic (23.5°C) conditions, with an approximate 17-day retention time, and storage capacity of ~6,100,000 liters. Liquid slurry from a single dairy consisting of 1,000 lactating cows was codigested with pre-consumer food-waste substrates. Food-waste consisted of no more than 30% of the total digester input and included whey, egg byproduct, processed fish, ruminant blood, biodiesel byproduct, and Daf grease (dissolved air flotation). After digestion, materials were passed through a rotating drum screen solid separator where solids were removed for composting and liquids pumped to a storage lagoon. The digested slurry applied to plots was obtained just after liquids-solids separation and prior to lagoon storage. A 250 mL subsample of each slurry was taken during each application (Table 2), cooled, and analyzed for total-nitrogen, nitrate-N, ammonium-N, total-phosphorus, and total solids [33] (Table 3).

Table 1: Average air temperature and total precipitation by month beginning at the start of plot implementation through the 3rd growing season.

Year	Month	Average air temp (°C)	Total precipitation (mm)
2009	April	9.2	61
	May	12.8	73
	June	**16.8**	**19**
	July	**20.0**	**6**
	August	**18.0**	**13**
	September	15.5	54
	October	9.9	100
	November	7.6	137
	December	1.4	35
2010	January	7.3	89
	February	7.0	44
	March	7.8	54
	April	9.4	55
	May	11.2	66
	June	**14.4**	**61**
	July	**17.0**	**7**
	August	**17.1**	**22**
	September	15.3	85
	October	10.6	85
	November	5.7	107
	December	5.3	184
2011	January	5.1	120
	February	3.4	80
	March	7.1	119
	April	7.2	79
	May	10.6	74
	June	**14.1**	**39**
	July	**15.7**	**18**
	August	**16.4**	**3**
	September	15.5	23
	October	10.2	71

Data from Washington State University AgWeatherNet, 21-Acres Station.

Table 2: Dates of forage harvest and fertilizer (slurry and urea) applications for field study in Monroe, WA for 2009–2011.

2009		2010		2011	
Forage Harvest	Fertilizer application[a]	Forage Harvest	Fertilizer application[a]	Forage harvest	Fertilizer application[a]
	17-Apr-09[b]		4-Mar-10		
7-May-09[c]	14-May-09	26-Apr-10	11-May-10	5-May-11	19-May-11
2-Jun-09	8-Jun-09	10-Jun-10	22-Jun-10	10-Jun-11	22-Jun-11
1-Jul-09	20-Jun-09[d]	7-Jul-10	15-Jul-10	14-Jul-10	4-Aug-11
28-Jul-09	11-Aug-09	12-Aug-10		22-Aug-10	31-Aug-11
31-Aug-09		15-Sep-10	30-Sep-10	20-Sep-10	
29-Sep-09		2-Dec-10		18-Oct-11	
30-Nov-09					

[a] Soil samples taken 1-day prior to fertilizer application.
[b] Early season manure application by grower, prior to plot establishment.
[c] Harvest prior to plot establishment, yield data from this harvest does not include replicates.
[d] Unintended slurry application from grower.

Table 3: Annual mean N and P concentrations of raw and anaerobically digested slurries applied to pasture plots.

	2009		2010		2011	
	Raw dairy Slurry	Digested slurry	Raw dairy slurry	Digested slurry	Raw dairy slurry	Digested slurry
Percent Total Solids (%)	2.8	1.9	3.4	2.0	3.4	1.4
Total N, mg kg^{-1}[a]	1441	1617	1653	2672	1475	2000
NH_4–N, mg kg^{-1}	707	1038	776	1253	760	930
Organic N, mg kg^{-1}[b]	734	578	877	1419	715	1070
Total P, mg kg^{-1}	350	300	331	292	330	210

[a]N and P concentrations reported as is.
[b]Organic nitrogen (N) = total N − NH_4–N.

A mix of reed canarygrass (*Phalaris arundinacea*) cv. "Palaton" and white clover (*Trifolium repens*) was overseeded into the field at 62 kg ha^{-1} in May 2006, three years before the start of this experiment. Plots were sprayed with broad leaf herbicides on 18 June 2009, 10 July 2010, and 8 August 2011 with 1.17 L ha^{-1}, 2, 4-Dichlorophenoxy acetic acid, 73 mL ha^{-1} Carfentrazone-ethyl (*Aim*), and 410 mL ha^{-1}, dicamba (*Banvel*) to control the clover.

2.2. Slurry Application Method. Slurries were applied via two application methods, subsurface deposition and surface broadcast application. Subsurface deposition was accomplished with a 4169-liter capacity manure tank fitted to a National Volume Equipment pump (model MEC 4000/PALD) with a 3.05 meter Aerway Sub-Surface Deposition (Model AW1000-2B48-D) and custom Banderator attachment for application of manure through eight PVC pipes attached directly behind the Banderator tines. Tines were set 19 cm apart on the roller and allowed to drop 10 cm below the soil surface creating intermittent slices 12.5 cm in length at the surface. Visual observation of the plots suggested that the tines created slices at random locations throughout the growing season. Surface broadcast of raw and anaerobically digested slurries were accomplished using an Aerway system with the tines raised above the soil surface.

Application rates for the raw and anaerobically digested slurry were projected to be equal in total N and allowed to vary in ammonia-N, for a total yearly application of approximately 600 kg N ha^{-1} in 2009, 500 kg N ha^{-1} in 2010, and 300 kg N ha^{-1} in 2011 (Table 4). We reduced the amount of N applied on urea and slurry treatments each year of the study from 2009 to 2011 based on the fall soil nitrate concentrations. When soil nitrate-N is above 35 mg N kg^{-1}in the fall, it is recommended that applications be eliminated after August 1st, N application rates be reduced in the subsequent year by 25–40 percent and sidedress N at planting be eliminated [34]. An early season raw dairy slurry application (Table 2, application 1 in 2009) was applied by the grower to all plots prior to establishment of the field plots and is not included in the statistical analyses. An inadvertent application of 143 kg N ha^{-1} across all plots by the grower in June of 2009 (Table 2, application 4) is included in the analysis. This accounts for the higher annual application rate in 2009. Application rates were lowest in 2011 because wet conditions prevented an early season application (Table 4), and the slurries had lower mean N concentrations. Plots were fertilized no more than five days after grass harvest. There were a total of five manure applications per year during 2009-2010 and four in 2011 (Table 2).

2.3. Field Management and Analysis. The aboveground biomass from grass swaths, 0.6 × 0.6 m, was harvested from the center of each plot every 28–35 d (Table 2) using hand-held hedge clippers. Three subsamples were taken within each of the four plot replicates for each treatment. The three subsamples were divided into grasses, clover, and weeds to adjust the aboveground biomass and ANR measurements. Due to herbicide applications, weeds were minimal all years. White clover biomass was significant in two of the cuttings in 2011. Samples were bagged, and weighed immediately. Forage was then dried at 55°C for 24 hrs, weighed, and ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) with a 1 mm screen. Ground samples were analyzed for forage nitrogen content with a Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI; AOAC, 2001) by Cumberland Valley Analytical Services Inc. (Hagerstown, MD).

Soil samples were collected from each plot and analyzed for Bray-1 P, exchangeable K, and pH at the beginning of the experiment 2009 and again at the end of 2010. Six soil cores per plot were taken to a 30-cm depth using a 2.54 cm diameter soil sampling probe and composited. Additional soil samples were collected for nitrate-N analysis monthly throughout the growing season using the same method, except for biweekly from mid-September through the end of November. Nitrate-N below the 30 cm depth was not measured. Soil chemical properties were analyzed by Soiltest Farm Consultants (Moses Lake, WA) using the methods of Gavlak et al. [33]. Ammonium-N was determined using a salicylate-nitroprusside method and nitrate-N using the cadmium reduction method. Soil samples for gravimetric water content were homogenized by mixing, and a subsample was dried at 38°C for 72 hrs [35].

Whole-soil phospholipid fatty acid (PLFA) procedures generally followed Bligh and Dyer [36] as described by Petersen and Klug [37] and modified by Ibekwe and Kennedy [38]. Fatty acid methyl esters were analyzed on a gas chromatograph (Agilent Technologies GC 6890, Palo Alto, CA)

Table 4: Application rate of fertilizer source at each application period, and seasonal total N and P inputs, 2009–2011.

Application	1	2	3	4	5	1	2	3	4	5	Seasonal total		
	Total N kg ha^{-1}					NH_4^+–N kg ha^{-1}					Total N kg ha^{-1}	NH_4^+–N kg ha^{-1}	Total P kg ha^{-1}
2009													
Control	111[a]	0	0	143[b]	0	51	0	0	83[b]	0	254	135	0
Urea	111[a]	112	112	143[b]	112	51	112	112	83[b]	112	590	471	0
Raw	111[a]	121	168	143[b]	47	51	64	78	83[b]	23	590	300	130
Digested	111[a]	176	115	143[b]	81	51	114	93	83[b]	47	626	389	121
2010													
Control	0	0	0	0	0	0	0	0	0	0	0	0	0
Urea	112	112	112	112	0	112	112	112	112	0	448	448	0
Raw	92	112	113	99	84	53	24	58	53	53	500	240	92
Digested	86	121	63	67	129	55	40	35	40	98	466	268	54
2011													
Control	0	0	0	0	0	0	0	0	0	0	0	0	0
Urea	0	112	112	112	0	0	112	112	112	0	336	336	0
Raw	0	52	60	84	63	0	34	24	29	47	258	135	51
Digested	0	33	136	69	70	0	23	52	33	27	308	134	26

[a]Early season slurry application by grower, prior to plot establishment.
[b]Application 4 in 2009 was an unintended application from the grower to all plots. Urea fertilizer considered equal to NH_4^+ in plant availability.

with a fused silica column and equipped with a flame ionizer detector and integrator. ChemStation (Agilent Technologies) operated the sampling, analysis, and integration of the samples. Extraction efficiencies were based on the nonadecanoic acid peak as an internal standard. Peak chromatographic responses were translated into mole responses using the internal standard and responses were recalculated as needed. Microbial groups were calculated based on the procedure of Pritchett et al. [39].

2.4. Slurry Analysis. Slurries were analyzed for total N, ammonium-N, and total P (Table 3). Nitrogen was extracted via the Kjeldahl method [33]. Phosphorus was analyzed using a Thermo IRIS Advantage HX Inductively Coupled Plasma (ICP) Radial Spectrometer (Thermo Instrument Systems, Inc., Waltham, MA) by the Dairy One Forage Analysis Laboratory (Ithaca, NY).

2.5. Statistical Analyses and Calculations. An analysis of variance (ANOVA) was run using SAS PROC MIXED on the aboveground forage biomass, nitrogen content in forage, soil nitrate-N, and soil biological groups for all treatments across the three years [40]. Data were analyzed as a randomized complete block design with each of the six treatments analyzed independently. Crop biomass and crop-nitrogen content from each year were analyzed separately using ANOVA with treatment and sample day as fixed effects. Significance is indicated with a $\rho < 0.05$ [40].

Forage apparent N recovery (ANR%) was calculated in 2010 and 2011 as a percentage of N (total and inorganic) applied during the season based on the work of Cogger et al. [41] and Bittman et al. [13]:

$$100 \times (\text{annual grass N uptake, treated}) - (\text{annual grass N uptake, control})/\text{applied N}. \quad (1)$$

Estimates of N fixed in white clover were set to 80% of total N in clover biomass based on ^{15}N studies conducted in a pasture of similar forages and N fertilizer management [42]. Using the above correction, 80% of clover N was subtracted from the forage N uptake values used in the ANR calculations for the two cuttings in 2011 with significant amounts of clover.

3. Results

3.1. Baseline Soil Data. Soil data sampled in May 2009 prior to the start of the field experiment (Table 5) indicated that fertility was not different across the field site. Organic matter, a source of inorganic-N, averaged 5.5 percent (55 g kg^{-1}). Bulk density of soils in the field ranged from 1.14 to 1.30 g cm^{-3}, with a mean average density of 1.21 g cm^{-3}.

3.2. Forage Biomass, N Uptake and ANR. Analysis of variance results for cumulative forage biomass in 2009 to 2011 are presented in Table 6. Total yield was greatest in 2010 (14.1–18.0 Dry Mg ha^{-1}) and lowest in 2011 (9.2–11.1 Dry Mg ha^{-1}). The 2009 data (8.08–9.5 Dry Mg ha^{-1}) did not include the first cutting of the year (7.7 Mg ha^{-1}) because it was harvested before plots and treatments were established. The growing conditions in 2010 were the most favorable of the three seasons. Forage biomass in 2011 was reduced by cool spring temperatures and low summer rainfall (Table 1). Urea had the highest yield in 2009, (Table 6). In 2010, urea and digested broadcast slurry had higher yield than the digested slurry applied subsurface. Slurry type and application method did not affect yield in 2009 or 2011.

Similar trends occurred when comparing crop N uptake in the forage grasses (Table 6). Urea-treated plots accumulated the most plant N, ranging from 296 to 655 kg N ha^{-1} removed per year. Uptake of N in forage grasses was greatest in 2010 (Table 6). Slurry type and application method did not have a significant effect on N uptake any year.

Table 5: Soil pH, Bray-P, and exchangeable K at start of experiment and after two years of slurry applications.

Plot	pH	Bray P	NH_4OAc K
		mg kg^{-1}	
	Baseline, 12 May 2009		
Control	6.0	173	591
Urea	6.0	176	608
Raw-subsurface	6.0	160	598
Raw-broadcast	6.0	165	632
Digested-subsurface	6.1	140	616
Digested-broadcast	6.0	168	612
	2 December 2010		
Control	6.2a	173	379b
Urea	6.0b	176	286c
Raw-subsurface	6.3a	186	479a
Raw-broadcast	6.2a	185	465a
Digested-subsurface	6.2a	173	447ab
Digested-broadcast	6.2a	162	440ab

Letters in a column within a year indicate significant differences at $\rho = 0.05$, letters are not included when no significant differences were found. Samples from different dates were analyzed separately using an ANOVA.

Table 6: Annual forage yield and N uptake, 2009 to 2011.

Treatment	Forage yield Dry Mg ha^{-1}			Nitrogen uptake N kg ha^{-1}		
	2009[a]	2010	2011	2009[b]	2010	2011
Control	8.0[b]	14.1[c]	9.2[b]	283[c]	362[cd]	192[c]
Urea	9.5[a]	18.0[a]	11.1[a]	389[a]	655[a]	296[a]
Raw- subsurface	8.6[b]	16.6[ab]	10.5[a]	330[b]	507[b]	263[b]
Raw- broadcast	7.9[b]	17.0[ab]	10.8[a]	308[bc]	531[b]	254[b]
Digested-subsurface	8.6[b]	16.1[b]	10.9[a]	332[b]	501[b]	239[b]
Digested-broadcast	8.7[b]	17.8[a]	10.9[a]	338[b]	550[ab]	255[b]

Letters within a column indicate significant differences at $\rho = 0.05$.
[a]Values for forage yield from the first harvest prior to implementation of nitrogen fertilizer treatments and application method were 7.7 Mg ha^{-1}.
[b]The N content in forage yield from the first harvest prior to implementation of nitrogen fertilizer treatments and application method was 253 kg N ha^{-1}.

Nitrogen uptake was lowest in 2011, likely a result of lower N application rates (Table 4) and poorer weather during the spring and summer. Forages in 2011 also contained significant amounts of clover, an N fixer (27% of the dry mass of forage yield at harvest 1 and 34% at harvest 2). Less than 10% of the forage biomass was clover in 2009 and 2010.

In the first full season of the study (2010), the recovery of applied N in the forage (ANR) was higher than in 2011 (Table 7). More favorable weather patterns for growth in 2010 compared with 2011 probably increased ANR in 2010. Urea treatments had an ANR of 65% in 2010 and 31% in 2011. Calculations based on total N applied in slurries were lower, ranging from 29 to 40% in 2010 and 15 to 24% in 2011, and similar between the two types of slurry. ANR calculations based only on the amount of total NH_4^+–N applied in slurries were 52 to 70% in 2010 and 35 to 53% in 2011, similar to ANR observed for urea.

3.3. Soil Nitrate-N. Plots receiving urea had the highest concentration of soil nitrate-N over the three seasons, while there were few differences among the slurry treatments (Table 8). Soil nitrate-N concentrations were highest in all fertilized treatments from July to the start of the fall rainy season, when the potential for leaching increases. Soil nitrate-N levels were greatest in 2009, likely because of the high rates of N applied that year. Lower soil nitrate-N in 2010 reflected the high N uptake during the favorable growing conditions that year. Soil nitrate-N increased again in 2011, particularly in the fall. This was despite a lower N application rate and may reflect the reduced yield and N uptake by the forages during the less favorable growing season in 2011.

3.4. Microbial Groups. Microbial groups in general did not vary with treatment, but rather varied by year (Table 9). The control and urea treatments varied from the other treatments most consistently for most groups, while no consistent differences were observed among the slurry treatments. By 2011, the control treatment had significantly lower bacteria and anaerobic markers than the other treatments, but similar levels of overall microbial biomass and fungi.

Table 7: Apparent nitrogen recovery (ANR) in harvested forage as percentage of total and ammonium N applied, 2010 and 2011.

	ANR 2010		ANR 2011	
Treatment	% of Total N	% of NH_4^+–N	% of Total N	% of NH_4^+–N
Urea	65	65	31	31
Raw-subsurface	29	60	15	35
Raw-broadcast	34	70	24	47
Digested-subsurface	30	52	23	53
Digested-broadcast	40	70	20	46

Urea fertilizer considered equal to NH_4^+ in plant availability.

Table 8: Soil NO_3^-–N (mg kg^{-1}) at 0 to 30 cm depth, 2009–2011.

	Soil NO_3^-–N (mg kg^{-1})					
	Treatment					
Sample Date	Control	Urea	Raw subsurface	Raw broadcast	Digested subsurface	Digested broadcast
				2009		
12-May	20gh	21gh	18gh	19gh	19gh	20gh
4-Jun	18gh	28fg	24fg	23 g	30fg	24fg
6-Jul	35fe	80b	71bc	76bc	68c	65cd
3-Aug	34f	86ab	80b	76bc	86ab	71bc
9-Sep	20gh	91a	66cd	82ab	72bc	67c
21-Sep	20gh	81b	53de	62cd	78bc	55d
1-Oct	17gh	62cd	52de	50de	56d	45de
19-Oct	14gh	91a	35fe	54de	44e	45de
3-Nov	11h	54de	23g	30fg	29fg	23g
19-Nov	10h	22gh	9.8h	12h	11h	10h
30-Nov	11h	11gh	12h	12h	10h	12h
				2010		
26-Feb	12fg	11fg	15ef	15ef	13f	14ef
11-May	13f	23d	20de	20de	20de	18ef
16-Jun	6.1g	9.2fg	7.9g	7.0g	7.4g	7.3g
13-Jul	10fg	25cd	18e	18de	16ef	14ef
17-Aug	13fg	61a	23cd	22de	18ef	22de
30-Sep	18de	36b	23cd	28c	22de	19de
12-Oct	12fg	28bc	22de	22de	27cd	24cd
26-Oct	7.2g	12fg	15ef	17ef	21de	16ef
2-Dec	6.9g	8.2g	11fg	9.5fg	10fg	10fg
				2011		
4-Apr	6.1g	6.2g	7.0g	6.5g	7.0g	8.0g
21-Jun	7.9g	18ef	11fg	11fg	12fe	12fg
4-Aug	8.7g	21ef	15fg	17ef	18ef	18ef
30-Aug	12fg	43cd	22ef	16f	23ef	17ef
16-Sept	17ef	48bc	39cd	48bc	48bc	32d
29-Sept	17ef	46c	36d	42cd	55b	36d
13-Oct	19ef	66a	45c	44c	50bc	44c
4-Nov	8.4g	32d	28de	24e	30de	23ef

Letters within a year indicate significant differences at $\rho = 0.05$.

4. Discussion

4.1. Forage Biomass, N Uptake and ANR. Forage biomass, plant N-uptake, and nitrate concentrations during the 2009–2011 growing seasons were affected by seasonal and long-term N management (a history of manure applications) that resulted in high N uptake from the control treatments. Also, favorable growing conditions in 2010 allowed for a more productive field season in this year. For this study, total harvest yield during each season was within the range of other published work where animal manures were applied to forages harvested multiple times over a season [16, 17, 41].

While other studies have shown incorporation of manure to increase yield and crop N content by reducing gaseous losses [13], we did not see an improvement in crop N content from incorporation of slurries in this system. Forages grown

Table 9: Soil microbial analyses from field plots in the spring, 2009–2011.

	Biomass g kg^{-1}	Bacteria Mole percent[a]	Fungi Mole percent	Bacteria to fungi ratio	Anaerobe Mole percent	Mono-unsaturated Mole percent
			May 2009			
Control	535 ab	0.246	0.098	3.01	0.091	0.338
Urea	433 c	0.246	0.092	3.23	0.092	0.348
Raw-B	538 ab	0.243	0.093	3.18	0.094	0.335
Raw-SSD	454 bc	0.237	0.094	3.07	0.091	0.330
Digested-B	473 bc	0.242	0.092	3.18	0.093	0.324
Digested-SSD	623 a	0.238	0.083	3.45	0.091	0.322
			May 2010			
Control	610 a	0.243 b	0.071 abc	4.18 ab	0.115 ab	0.328 b
Urea	333 b	0.215 c	0.074 ab	3.48 c	0.101 b	0.322 b
Raw-B	401 a	0.266 ab	0.084 a	4.04 bc	0.116 ab	0.357 ab
Raw-SSD	297 b	0.268 a	0.066 bc	4.91 a	0.127 a	0.414 a
Digested-B	258 b	0.259 ab	0.071 abc	4.65 ab	0.123 a	0.398 a
Digested-SSD	279 b	0.267 ab	0.066 c	4.97 a	0.125 a	0.406 a
			April 2011			
Control	512	0.221 b	0.087 ab	3.11 d	0.082 c	0.341 b
Urea	447	0.250 a	0.078 c	3.96 a	0.094 ab	0.380 a
Raw-B	489	0.257 a	0.093 ab	3.35 bcd	0.092 b	0.335 b
Raw-SSD	428	0.253 a	0.095 a	3.25 cd	0.100 ab	0.345 b
Digested-B	441	0.258 a	0.085 bc	3.68 ab	0.101 ab	0.357 ab
Digested-SSD	491	0.255 a	0.085bc	3.61 abc	0.102 a	0.359 ab

Letters within a column within a year indicate significant differences at $\rho = 0.05$. No letters indicate no significant differences within that column.
[a]Mole percent = (mole substance in a mixture)/(mole mixture) %.

in plots with broadcast applied slurries took up the same amount of N or more N than with subsurface deposition, which may have been caused by plant-growth disturbance from the airway banderator when subsurface applying effluent. Additionally, the infiltration rate of the anaerobically digested slurry may have been rapid enough that gaseous losses in the field were not different among subsurface deposition and broadcast applications. From an agronomic perspective, the two slurry types performed equally well as urea over the three growing seasons. Anaerobically digested slurry was suitable for forage production when applied at rates equal to raw dairy slurry. Moller and Stinner [8] also reported no differences in N uptake between digested and undigested slurry. How the system will respond after many years of anaerobically digested slurry application is unclear as the quantity of organic N applied is less than that of raw dairy slurry, supplying less recalcitrant N to the pool of soil organic matter.

4.2. Soil Nitrate-N and Microbial Groups. We found few differences between slurry treatments in seasonal soil NO_3^- concentrations. There was, however, significantly more nitrate-N in urea-treated plots on many dates, even though there was slightly less total N applied to the urea plots in some years. The spike in nitrate concentration in October on soils where urea was applied in place of slurries indicates a greater potential for N leaching from urea compared with the slurries. All treatments declined in NO_3^- concentrations to levels that were not significantly different from control treatments after the fall rains began. Lower soil nitrate-N during the growing season of 2010 compared with 2009 may be due in part to a lower amount of total nitrogen applied. Also, little rainfall during the 2009 growing season may have caused a buildup of soil nitrate in the surface layers. Higher late-season nitrate in 2011 compared with 2010 may have been the result of poorer growing conditions reducing N uptake.

Postharvest soil nitrate-N is a measure of residual plant-available N subject to leaching loss, and an indicator of excess applied N and/or poor yield. The recommended timing of postharvest soil nitrate testing in forage systems that utilize animal manure as a source of fertility in the Maritime Pacific Northwest is prior to October 15 [34]. Nitrate concentrations from soil samples collected from our site in mid-October showed that all treatments except the control exceeded 30 mg NO_3–N kg ha^{-1} in 2009 and 2011, with NO_3–N levels highest in the urea treatment. Fall nitrate-N levels above 30 mg kg^{-1} are considered excessive in manured pastures, and reduced rates and adjusted timing of applications are recommended [34].

While soil nitrate concentrations decreased during the fall 2009 months, it is likely that some of this nitrate was not entirely leached from the system, but stored in the canary grass rhizomes over winter as described by Partala et al. [18].

This is evident in the significantly higher yields and nitrogen content of forages during the early season harvest on 26 April 2010.

While the focus of this study is N, dairy manure also contains high levels of P. Runoff from high-P soils can lead to eutrophication in fresh water. Soil P levels were already excessive at the start of this study, because of the history of dairy manure applications at the site, and P tended to increase in the slurry-treated plots during the study (Table 5). The anaerobically digested slurry contained less P than the raw dairy slurry, probably because it had a lower solids content, which would lead to less P accumulation over time.

Microbial groups varied with year more than treatment in these field studies. Urea treatments varied from the other treatments to the greatest extent. The raw and anaerobically digested materials did not alter the soil microbial components as determined by PLFA. Our results may partially be the result of past manure applications.

5. Conclusions

Subsurface deposition did not increase yield or N uptake compared with surface broadcast application, possibly because the slurries were low enough in solids to infiltrate readily into the soil, and because the subsurface injectors could have disrupted plant growth. Anaerobically digested dairy slurry was shown to provide adequate soil fertility and N availability for crop uptake and forage production over the three field seasons. In the short term, anaerobically digested slurry did not significantly increase yield or N uptake compared with similar rates of raw slurry.

This study indicated that soil nitrates measured to a 30 cm depth were fairly consistent across slurry treatments and application methods during each of the field seasons. Soil nitrate-N was lower in 2010 due to favorable growing conditions and lower total applied N relative to 2009. Although urea treatments had the highest apparent N recovery value, the potential for nitrate leaching was also greatest under this management. Anaerobically digested slurry did not increase soil NO_3^- concentrations or alter the microbial composition and provided equal forage production and similar N use efficiency when compared to undigested dairy slurry.

Acknowledgments

Funding for this project was provided by WA Dairy Prod. Commission 113547-001, USDA-NRCS 68-3A 75-5-178 and Hatch Project 0711. The authors would like to thank our student aides: Jeni Maakad, Taylor Graves, Mike Pecharko, Jacob Turner, Clayton Waller, and Allison Wood. They would also like to welcome Vivienne Marie Whitefield to their work group born November 21, 2011.

References

[1] P. Gerber, H. A. Mooney, J. Dijkman, S. Tarawali, and C. DeHaan, *Livestock in a Changing Landscape: Experiences and Regional Perspectives*, vol. 2, Island Press, Washigton, DC, USA, 2010.

[2] T. J. Centner, "Regulating concentrated animal feeding operations to enhance the environment," *Environmental Science and Policy*, vol. 6, no. 5, pp. 433–440, 2003.

[3] T. J. Centner, "Evolving policies to regulate pollution from animal feeding operations," *Environmental Management*, vol. 28, no. 5, pp. 599–609, 2001.

[4] A. N. Hristov, W. Hazen, and J. W. Ellsworth, "Efficiency of use of imported nitrogen, phosphorus, and potassium and potential for reducing phosphorus imports on Idaho dairy farms," *Journal of Dairy Science*, vol. 89, no. 9, pp. 3702–3712, 2006.

[5] R. L. Kellogg, C. H. Lander, D. C. Moffett, and N. Gollehon, *Manure Nutrients Relative to the Capacity of Cropland and Pastureland to Assimilate Nutrients: Spatial and Temporal Trends for the United States*, United States Department of Agriculture, Natural Resources Conservation Service, Washigton, DC, USA, 2000.

[6] AgSTAR Program, "Managing manure with biogas recovery systems improved performance at competitive costs," Environmental Protection Agency, Office of Air and Radiation. 430-F02-004, 2002.

[7] H. Hou, S. Zhou, M. Hosomi et al., "Ammonia emissions from anaerobically-digested slurry and chemical fertilizer applied to flooded forage rice," *Water, Air, and Soil Pollution*, vol. 183, no. 1–4, pp. 37–48, 2007.

[8] K. Moller and W. Stinner, "Effects of organic wastes digestion for biogas production on mineral nutrient availability of biogas effluents," *Nutrient Cycling in Agroecosystems*, vol. 87, no. 3, pp. 395–413, 2010.

[9] M. A. Moser, "Anaerobic digesters control odors, reduce pathogens, improve nutrient manageability, can be cost competitive with lagoons, and provide energy too!," The AgSTAR Program, 2010, http://www.epa.gov/agstar/resources/man_.html.

[10] J. Michel, A. Weiske, and K. Moller, "The effect of biogas digestion on the environmental impact and energy balances in organic cropping systems using the life-cycle assessment methodology," *Renewable Agriculture and Food Systems*, vol. 25, no. 3, pp. 204–218, 2010.

[11] K. Moller, W. Stinner, A. Deuker, and G. Leithold, "Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems," *Nutrient Cycling in Agroecosystems*, vol. 82, no. 3, pp. 209–232, 2008.

[12] H. C. de Boer, "Co-digestion of animal slurry can increase short-term nitrogen recovery by crops," *Journal of Environmental Quality*, vol. 37, no. 5, pp. 1968–1973, 2008.

[13] S. Bittman, C. G. Kowalenko, D. E. Hunt, and O. Schmidt, "Surface-banded and broadcast dairy manure effects on tall fescue yield and nitrogen uptake," *Agronomy Journal*, vol. 91, no. 5, pp. 826–833, 1999.

[14] E. R. Loria, J. E. Sawyer, D. W. Barker, J. P. Lundvall, and J. C. Lorimor, "Use of anaerobically digested swine manure as a nitrogen source in corn production," *Agronomy Journal*, vol. 99, no. 4, pp. 1119–1129, 2007.

[15] D. J. R. Cherney, J. H. Cherney, and E. A. Mikhailova, "Orchardgrass and tall fescue utilization of nitrogen from dairy manure and commercial fertilizer," *Agronomy Journal*, vol. 94, no. 3, pp. 405–412, 2002.

[16] C. C. Sheaffer, G. C. Marten, D. L. Rabas, N. P. Martin, and D. W. Miller, *Reed Canarygrass*, vol. 595, Minnesota Agricultural Experiment Station, 1990.

[17] M. A. Sanderson and R. M. Jones, "Forage yields, nutrient uptake, soil chemical changes, and nitrogen volatilization from bermudagrass treated with dairy manure," *Journal of Production Agriculture*, vol. 10, no. 2, pp. 266–271, 1997.

[18] A. Partala, T. Mela, M. Esala, and E. Ketoja, "Plant recovery of ^{15}N-labelled nitrogen applied to reed canary grass grown for biomass," *Nutrient Cycling in Agroecosystems*, vol. 61, no. 3, pp. 273–281, 2001.

[19] J. S. Angle, C. M. Gross, R. L. Hill, and M. S. McIntosh, "Soil nitrate concentrations under corn as affected by tillage, manure, and fertilizer applications," *Journal of Environmental Quality*, vol. 22, no. 1, pp. 141–147, 1993.

[20] G. R. Munoz, J. M. Powell, and K. A. Kelling, "Nitrogen budget and soil N dynamics after multiple applications of unlabeled or (15) nitrogen-enriched dairy manure," *Soil Science Society of America*, vol. 67, no. 3, pp. 817–825, 2003.

[21] H. Trindade, J. Coutinho, S. Jarvis, and N. Moreira, "Effects of different rates and timing of application of nitrogen as slurry and mineral fertilizer on yield of herbage and nitrate-leaching potential of a maize/Italian ryegrass cropping system in north-west Portugal," *Grass and Forage Science*, vol. 64, no. 1, pp. 2–11, 2009.

[22] J. M. Jemison and R. H. Fox, "Nitrate leaching from nitrogen-fertilized and manured corn measured with zero-tension pan lysimeters," *Journal of Environmental Quality*, vol. 23, no. 2, pp. 337–343, 1994.

[23] A. Bakhsh, R. S. Kanwar, C. Pederson, and T. B. Bailey, "N-source effects on temporal distribution of NO_3-N leaching losses to subsurface drainage water," *Water, Air, and Soil Pollution*, vol. 181, no. 1–4, pp. 35–50, 2007.

[24] T. G. Sommerfeldt, C. Chang, and T. Entz, "Long-term annual manure applications increase soil organic matter and nitrogen, and decrease carbon to nitrogen ratio," *Soil Science Society of America*, vol. 52, no. 6, pp. 1668–1672, 1988.

[25] W. E. Jokela, "Nitrogen fertilizer and dairy manure effects on corn yield and soil nitrate," *Soil Science Society of America*, vol. 56, no. 1, pp. 148–154, 1992.

[26] R. B. Ferguson, J. A. Nienaber, R. A. Eigenberg, and B. L. Woodbury, "Long-term effects of sustained beef feedlot manure application on soil nutrients, corn silage yield, and nutrient uptake," *Journal of Environmental Quality*, vol. 34, no. 5, pp. 1672–1681, 2005.

[27] J. Nyiraneza and S. Snapp, "Integrated management of inorganic and organic nitrogen and efficiency in potato systems," *Soil Science Society of America*, vol. 71, no. 5, pp. 1508–1515, 2007.

[28] G. F. Ge, Z. J. Li, F. L. Fan, G. X. Chu, Z. A. Hou, and Y. C. Liang, "Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers," *Plant and Soil*, vol. 326, no. 1, pp. 31–44, 2009.

[29] H. A. Ajwa and M. A. Tabatabai, "Decomposition of different organic materials in soils," *Biology and Fertility of Soils*, vol. 18, no. 3, pp. 175–182, 1994.

[30] H. Chu, X. J. Lin, T. Fujii et al., "Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management," *Soil Biology and Biochemistry*, vol. 39, no. 11, pp. 2971–2976, 2007.

[31] W. H. Zhong, T. Gu, W. Wang et al., "The effects of mineral fertilizer and organic manure on soil microbial community and diversity," *Plant and Soil*, vol. 326, no. 1-2, pp. 511–522, 2010.

[32] A. Debose and M. W. Klungland, *Soil Survey of Snohomish County Area*, USDA Soil Conservation Service, Washigton, DC, USA, 1983.

[33] R. G. Gavlak, D. A. Horneck, and R. O. Miller, *Soil, Plant, and Water Reference Methods for the Western Region*, vol. 125, Region Extension Publications, 3rd edition, 2005.

[34] D. M. Sullivan and C. G. Cogger, *Post-Harvest Soil Nitrate Testing for Manured Cropping Systems*, Oregon State University Extension Services, 2003.

[35] G. C. Topp and P. A. Ferré, "The soil solution phase," in *Methods of Soil Analysis. Part 4-Physical Methods*, J. H. Dane and G. C. Topp, Eds., vol. 5 of *Soil Science Society of America Book Series, Vol. 5*, pp. 422–423, Soil Science Society of America, 2nd edition, 2002.

[36] E. G. Bligh and W. J. Dyer, "A rapid method of total lipid extraction and purification," *Canadian Journal of Biochemistry and Physiology*, vol. 37, no. 8, pp. 911–917, 1959.

[37] S. O. Petersen and M. J. Klug, "Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community," *Applied and Environmental Microbiology*, vol. 60, no. 7, pp. 2421–2430, 1994.

[38] A. M. Ibekwe and A. C. Kennedy, "Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions," *FEMS Microbiology Ecology*, vol. 26, no. 2, pp. 151–163, 1998.

[39] K. A. Pritchett, A. C. Kennedy, and C. G. Cogger, "Management effects on soil quality in organic vegetable systems in western Washington," *Soil Science Society of America*, vol. 75, no. 2, pp. 605–615, 2011.

[40] SAS Institute Incorporation, *SAS User's Guide: Statistics. Version 9.2.*, SAS Institute Incorporation, Cary, NC, USA, 2008.

[41] C. G. Cogger, D. M. Sullivan, A. I. Bary, and S. C. Fransen, "Nitrogen recovery from heat-dried and dewatered biosolids applied to forage grasses," *Journal of Environmental Quality*, vol. 28, no. 3, pp. 754–759, 1999.

[42] F. V. Jørgensen and S. F. Ledgard, "Contribution from stolons and roots to estimates of the total amount of N_2 fixed by white clover (*Trifolium repens* L.)," *Annals of Botany*, vol. 80, no. 5, pp. 641–648, 1997.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 817343, 5 pages
doi:10.1155/2012/817343

Research Article

Nitrogen and Carbon Cycling in a Grassland Community Ecosystem as Affected by Elevated Atmospheric CO_2

H. A. Torbert,[1] H. W. Polley,[2] and H. B. Johnson[2]

[1] *National Soil Dynamics Laboratory, USDA Agricultural Research Service, 411 S. Donahue, Auburn, AL 36832, USA*
[2] *Grassland Soil and Water Research Laboratory, USDA Agricultural Research Service, 808 E Blackland Road, Temple, TX 76502, USA*

Correspondence should be addressed to H. A. Torbert, allen.torbert@ars.usda.gov

Received 4 January 2012; Accepted 7 February 2012

Academic Editor: Dexter B. Watts

Increasing global atmospheric carbon dioxide (CO_2) concentration has led to concerns regarding its potential effects on terrestrial ecosystems and the long-term storage of carbon (C) and nitrogen (N) in soil. This study examined responses to elevated CO_2 in a grass ecosystem invaded with a leguminous shrub *Acacia farnesiana* (L.) Willd (Huisache). Seedlings of *Acacia* along with grass species were grown for 13 months at CO_2 concentrations of 385 (ambient), 690, and 980 μmol mol^{-1}. Elevated CO_2 increased both C and N inputs from plant growth which would result in higher soil C from litter fall, root turnover, and excretions. Results from the incubation indicated an initial (20 days) decrease in N mineralization which resulted in no change in C mineralization. However, after 40 and 60 days, an increase in both C and N mineralization was observed. These increases would indicate that increases in soil C storage may not occur in grass ecosystems that are invaded with *Acacia* over the long term.

1. Introduction

The rise of CO_2 in the atmosphere is well documented [1]; what has not been documented are the sinks for this C, with an estimated unknown sink of 1.4×10^{15} g C yr^{-1} arising from the global C balance [2]. Carbon dioxide is a prime chemical input to the metabolism of higher plants and has a major role in governing plant-water relations and water use efficiency. The increased growth of most plants under higher levels of CO_2 [3–6] has prompted recent speculation on the ability of terrestrial ecosystems to sequester C [7]. However, the fate of C within ecosystems is affected by a biological chain of events which includes competition between plants. The ability of terrestrial ecosystems to sequester C will depend on the cycling of C among the various biomass and soil C pools and on the residence time of C in these pools.

The rate of C mineralization during decomposition of residue derived from plants grown under elevated CO_2 has not been resolved. It has been theorized that the commonly observed increase in plant C : N ratio under elevated CO_2 could lead to slower residue decomposition resulting in increased soil C storage and reduction in available N for plant production [8]. However, slower decomposition of leaf litter due to elevated CO_2 is not supported by the literature on litter quality [9]. Others have suggested that increased biomass might enhance microbial activity, resulting in a "priming effect" thereby leading to no increase in C storage [10]. Alternatively, microbial preference for easily decomposable plant material produced under CO_2-enriched conditions could reduce the turnover of more resistant organic material, thereby increasing soil C [11, 12]. Observations from field and laboratory studies indicate that with elevated atmospheric CO_2, N may limit the rate of plant residue decomposition and slow the release of N from decomposing plant material [13]. This indicates that understanding N cycling as affected by elevated CO_2 is fundamental to understanding the potential for soil C storage on a global scale.

It has also been speculated that changes in elevated CO_2 could impact the competitiveness between plant species, especially between native and invasive plant species. A study by Runion et al. [14] indicated that elevated atmospheric CO_2 increased biomass production in a longleaf ecosystem compared to ambient CO_2 conditions. However, close examination of biomass data for each species in this plant

community indicated that only longleaf pine showed a significant positive response to elevated atmospheric CO_2 treatment. In grasslands, invasion by woody legumes such as *Acacia* can alter hydrology, nutrient accumulation and cycling, and C sequestration. The rate and magnitude of these changes are likely to be sensitive to the effects of atmospheric CO_2 enrichment on growth and water and N dynamics of leguminous shrubs. Polley et al. [15] indicated that, at the highest CO_2 concentration studied, biomass production of grass-tree communities increased more than 2.5-fold as a result of increased leaf photosynthetic rates, leaf area, and N_2 fixation.

Previous research, considering the effect of elevated CO_2 on the decomposition of individual plant parts and monoculture plant systems has indicated that increased soil C storage could occur [16, 17]. However, these past studies did not consider the impact of increased biomass input and the changes in soil brought about by differing responses of plant species. The objective of this study was to determine the impact of atmospheric CO_2 enrichment on potential soil C and N mineralization in a grassland community.

2. Materials and Methods

A study on the effects of elevated CO_2 concentrations on the productivity, water use, and N dynamics of the grassland invader *Acacia farnesiana* (L.) Willd (Huisache) was conducted by Polley et al. [15]. To study the impact of atmospheric CO_2 enrichment on potential soil C and N mineralization within a grassland community, an incubation study was conducted on soil collected from this study. Descriptions of the study site and the model ecosystem have been previously reported [15]. Briefly, seeds of *Acacia* were planted in fine sandy loam soil (Udic Paleustalfs [18], in wheeled, 380-liter containers (0.9 m deep and 0.65 m on each side)). Three *Acacia* plants were established in each container.

Three individuals each of the C_3 grass *Stipa leucotricha* Trin. and Rupr. (Texas winter grass) and the C_4 grass *Schizachyrium scoparium* (Michx.) Nash. (little bluestem) were also established from tillers in each container to provide a competitive environment for *Acacia* growth. Three 380-liter containers of the species mix were maintained for 13 months at average CO_2 concentrations of 385 (ambient), 690, or 980 μmol mol^{-1} in air-conditioned greenhouse bays.

Carbon dioxide gas was injected into the greenhouse bays as necessary to maintain the desired concentrations. The CO_2 concentration and dewpoint temperature of air in each bay were measured at 4 min intervals with an infrared gas analyzer (Model 6262, Li-Cor, Inc., Lincoln, NE). The CO_2 readings were corrected for atmospheric pressure using a pressure indicator (Model DPI 260, Druck Inc., New Fairfield, CT). The CO_2 concentration of air averaged 385, 690, and 980 μmol mol^{-1} for the three CO_2 treatments. Air temperature was measured with 25 mm diameter thermocouples, and bay temperature was changed seasonally to approximate outdoor temperature by manually adjusting thermostatic controls.

At the conclusion of the study (13 months), soil samples were collected from 0–20, 20–50, and 50–80 cm depth increments for use in the incubation study. Soil subsamples were dried (55°C) and ground to pass a 0.15 mm sieve. Soil inorganic N (NO_2-N + NO_3-N and NH_4-N) was extracted with 2 M KCl and measured by standard colorimetric procedures using a Technicon Autoanalyzer 3 (Bran + Luebbe, Buffalo Grove, IL). Soil subsamples were weighed (25 g dry weight basis) and placed in plastic containers. Deionized water was added to adjust soil water content to an equivalent of -20 kPa at a bulk density of 1.3 mg m^{-3}. The containers were placed in sealed glass mason jars with 10 mL of water for humidity control; each jar also had a vial containing 10 mL of 1 M NaOH which functioned as a CO_2 trap. The jars were incubated in the dark at 25°C and removed after 20 and 60 days for inorganic N determination. Carbon dioxide in the NaOH traps was determined by titrating the excess base with 1 M HCl in the presence of $BaCl_2$ after 20, 40, and 60 days. Potential C mineralization was the difference between CO_2-C captured in sample traps and in blanks. Potential N mineralization was the difference between final and initial inorganic N contents. The C mineralization divided by total C (initial soil C concentration) was used to calculate C turnover. Statistical analyses were performed using the mixed procedure of SAS [19], and means were separated using least significant difference at an *a priori* 0.10 probability level.

3. Results and Discussion

Increasing atmospheric CO_2 concentration to 980 μmol mol^{-1} increased biomass production of the woody legume *Acacia* more than 2.5-fold during the initial year of growth due to increased leaf photosynthetic rates, leaf area, and N_2 fixation [15]. The increase in biomass at elevated CO_2 required no more water than was consumed by the shrubs grown near ambient CO_2 concentration. As a result, apparent water use efficiency and biomass production increased by similar relative amounts. Elevated CO_2 greatly increased N_2 fixation by *Acacia*, with the total N fixed per plant at the highest CO_2 concentration being 1.5 times greater compared to ambient CO_2 conditions. This positive effect of CO_2 on N_2 fixation by *Acacia* during its initial year of growth is generally consistent with that reported for woody legumes [20, 21]. Nitrogen fixation promoted by elevated CO_2 would be expected to provide additional N to a grassland ecosystem due to the N addition to the soil following litter fall, root turnover, and exudation. These are important factors by which increasing atmospheric CO_2 concentration can modify the impact of woody legumes on grasslands. Studies have shown that plant matter grown under elevated CO_2 can greatly impact N mineralization [16, 17]. This incubation study was conducted to determine the impact of the elevated CO_2 concentration on soil N mineralization in a grassland system.

After 20 days of incubation of the 0–20 cm depth soil, N mineralization was significantly reduced by elevated CO_2, with N mineralization being 95% higher at ambient CO_2 compared to the 980 μmol mol^{-1} treatment level (Table 1). During this same measuring period, soil C mineralization and C turnover were not significantly impacted. This indicated that changes under elevated CO_2 initially reduced N

Table 1: Effect of atmospheric CO_2 concentration on soil N mineralization during a 60-day soil incubation at three soil depth increments[†].

CO_2 level	0–20 days			0–60 days		
	Depth					
	0–20 cm	20–50 cm	50–80 cm	0–20 cm	20–50 cm	50–80 cm
(μmol mol^{-1})	($mg kg^{-1})					
385	6.86 a	1.91 a	1.37 a	7.51 a	4.01 a	4.54 a
690	2.91 b	0.59 a	0.69 a	7.85 b	3.86 a	4.89 a
980	3.52 c	2.91 a	0.46 a	10.04 c	3.30 a	3.89 a

[†] Values represent means of three replications. Means within a column for each sampling date followed by the same letter do not differ significantly ($\alpha = 0.1$).

mineralization rates. While greater plant inputs were entering the system, with greater total N from the N_2 fixation, the quality of the plant residues were such that N mineralization was being limited. This reduction in inorganic N in soil solution in turn limited C mineralization, resulting in no difference in C respiration or C turnover at the 20-day sampling period (Tables 2 and 3). This is consistent with studies by Torbert et al. [17] which indicated that the impact of elevated atmospheric CO_2 concentration on plant residue quality may be more important than the impact on plant residue quantity in determining C cycling in soil. They observed that the potential effect of elevated atmospheric CO_2 concentration on C storage in agroecosystems will be dependent on the crop species grown, where N cycling within the plant/soil system would likely be the controlling factor for C storage in these systems. Likewise, these results agree with work from a free-air CO_2 enrichment (FACE) study utilizing cotton (*Gossypium hirsutum* L.) [22] and wheat (*Triticum aestivum* L.) [23], which provided evidence for increased soil C storage even though differences between the two species (cotton and wheat) were noted.

However, unlike the agroecosystem studies, limitations to N mineralization did not persist in this grass ecosystem with *Acacia*. At the 40-day sampling period, a significant increase was observed with C mineralization as atmospheric CO_2 was increased (Table 2). Likewise, an increase in the level of C turnover was observed with increasing atmospheric CO_2 levels. At the 60-day measurement period, C mineralization was also significantly increased at the 980 μmol mol^{-1} compared to the ambient level as was C turnover (Tables 2 and 3). The C mineralization for the 980 μmol mol^{-1} treatment was 98% higher than for the ambient treatment. At this sampling period, unlike the 20-day period, a significant increase in N mineralization was also observed (Table 1). This indicated that after 60 days, N was no longer limiting microbial decomposition of plant material. Since increased biomass inputs into the soil system were observed in this study [15] and the limitations due to N were no longer impacting the decomposition processes, C mineralization also increased.

No significant differences were observed for N mineralization or C mineralization at the 20–50 cm depth or the 50–80 cm depth (Tables 1 and 3). This indicated that most changes in the soil system can be expected to occur in the top 20 cm of the soil profile. This is the portion of the soil that would be most impacted from litter fall and could result in movement of N to grass species from N_2 fixation by the *Acacia*. While this study included a deep rooted species in the grass ecosystem, the greatest changes in C and N cycling occurred in the top of the soil profile where most plant rooting occurs.

It has been theorized that a reduction in N availability in ecosystems could reduce plant response to elevated CO_2 conditions [24–26]. The results from this incubation study indicate that N mineralization was increased and therefore the potential for N limitations to reduce plant response to elevated CO_2 in this grass ecosystem might not occur. This would indicate that over the long term in a grass ecosystem invaded with woody legumes such as *Acacia*, N resources should not be limiting for either microbial decomposition of residues or plant growth in future elevated atmospheric CO_2 conditions compared to today's conditions.

In a study to examine responses to elevated CO_2 in a typical regenerating longleaf pine-wiregrass community, Torbert et al. [27] reported, as in this study with mixed plant species, an increase in N mineralization with elevated CO_2. At least over the short term in a regenerating longleaf pine system, N resources should not be limiting for either microbial decomposition of residues or plant growth in future elevated atmospheric CO_2 conditions compared to today's conditions. However, elevated CO_2 decreased soil C respiration and C turnover in the regenerating longleaf pine-wiregrass community, indicating long-term C sequestration would be likely. In the current study with grass species, an actual increase in soil C mineralization was observed with elevated CO_2. This was likely due to the large input of N due to increase in N_2 fixation by the *Acacia* into the plant/soil system. Polley et al. [15] reported total N accretion by *Acacia* increased with each increase in CO_2 concentration: 55% from 385 to 690 μmol mol^{-1} CO_2, and another 29% from 690 to 980 μmol mol^{-1} CO_2. The increased C mineralization indicated that soil C levels in the elevated CO_2 are being reduced compared to the ambient treatments.

These findings are in contrast to other research which reported that C sequestration is likely with increasing atmospheric CO_2. Low-N availability of soil frequently limits production on grasslands [23, 24] and may also limit the response of net primary production to changes in CO_2 concentration. However, with the *Acacia* included in the grass ecosystem, CO_2 enrichment caused large N inputs into the soil system and large increases in biomass (a 2.5-fold increase during the initial year of growth, [15]). Although C inputs to soil increased, C mineralization also increased.

Table 2: Effect of atmospheric CO_2 concentration on soil C mineralization during a 60-day soil incubation at three soil depth increments†.

CO_2 level	0–20 days			20–40 days			40–60 days		
	Depth								
	0–20 cm	20–50 cm	50–80 cm	0–20 cm	20–50 cm	50–80 cm	0–20 cm	20–50 cm	50–80 cm
(μmol mol^{-1})	($mg kg^{-1}$)								
385	57.52 a	63.94 a	36.84 a	59.15 a	67.15 a	59.44 a	210.76 a	263.46 a	229.40 a
690	92.86 a	41.98 a	66.94 a	100.55 b	55.80 a	72.29 a	199.20 b	217.40 a	246.32 a
980	87.93 a	70.15 a	34.26 a	124.98 c	42.52 a	69.40 a	417.46 c	169.43 a	256.82 a

† Values represent means of three replications. Means within a column for each sampling date followed by the same letter do not differ significantly ($\alpha = 0.1$).

Table 3: Effect of atmospheric CO_2 concentration on soil C turnover during a 60-day soil incubation at three soil depth increments†.

CO_2 level	0–20 days			20–40 days			40–60 days		
	Depth								
	0–20 cm	20–50 cm	50–80 cm	0–20 cm	20–50 cm	50–80 cm	0–20 cm	20–50 cm	50–80 cm
(μmol mol^{-1})	($mg kg^{-1}$)								
385	1.01 a	1.12 a	0.65 a	0.85 a	1.18 a	1.04 a	3.70 a	4.62 a	4.02 a
690	1.63 a	0.74 a	1.17 a	1.75 b	0.98 a	1.27 a	3.49 b	3.81 a	4.32 a
980	1.54 a	1.23 a	5.34 a	2.19 c	0.75 a	1.22 a	2.19 c	2.97 a	4.51 a

† Values represent means of three replications. Means within a column for each sampling date followed by the same letter do not differ significantly ($\alpha = 0.1$).

The results from this short-term study indicate that CO_2 may not increase soil C storage in this grassland ecosystem. Thus, long-term studies with elevated CO_2 will be necessary to clearly determine the impact of *Acacia* invasion in this grassland ecosystems on soil C storage.

References

[1] C. D. Keeling, R. B. Bacastow, A. F. Carter et al., "A three dimensional model of atmospheric CO_2 transport based on observed winds: observational data and preliminary analysis," in *Aspects of Climate Variability in the Pacific and the Western America*, D. H. Peterson, Ed., vol. 55, pp. 165–235, Geophysical Monograph, 1989.

[2] D. Schimel, I. G. Enting, M. Heimann et al., "CO_2 and the carbon cycle," in *Climate Change 1994*, J. T. Houghton, Ed., pp. 35–71, Cambridge University Press, Cambridge, UK, 1995.

[3] B. R. Strain and D. J. Cure, Eds., "Direct effects of increasing carbon dioxide on vegetation," DOE/ER–0238, Office of Energy Research, U.S. Department of Energy, Washington, DC, USA, 1985.

[4] S. A. Prior, H. H. Rogers, G. B. Runion, and J. R. Mauney, "Effects of free-air CO_2 enrichment on cotton root growth," *Agricultural and Forest Meteorology*, vol. 70, no. 1–4, pp. 69–86, 1994.

[5] S. H. Wittwer, *Food, Climate, and Carbon Dioxide: The Global Environment and World Food Production*, CRC Press, Boca Raton, Fla, USA, 1995.

[6] S. G. Pritchard, H. H. Rogers, S. A. Prior, and C. M. Peterson, "Elevated CO_2 and plant structure: a review," *Global Change Biology*, vol. 5, no. 7, pp. 807–837, 1999.

[7] R. M. Gifford, J. L. Lutze, and D. J. Barrett, "Global atmospheric change effects on terrestrial carbon sequestration: exploration with a global C- and N-cycle model (CQUESTN)," *Plant and Soil*, vol. 187, no. 2, pp. 369–387, 1996.

[8] Y. Luo, B. Su, W. S. Currie et al., "Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide," *Bioscience*, vol. 54, no. 8, pp. 731–739, 2004.

[9] J. Hom, "Global change and forest soils," in *The Potential of US Forest Soils to Sequester Carbon and Mitigate the Greenhouse Effect*, J. M. Kimble, Ed., pp. 127–134, CRC Press, Boca Raton, Fla, USA, 2002.

[10] M. R. Lamborg, W. F. Hardy, and E. A. Paul, "Microbial effects," in *CO_2 and Plants: The Response of Plants to Rising Levels of Atmospheric CO_2*, E. R. Lemon, Ed., pp. 131–176, American Association for the Advancement of Science Selected Symposium, Washington, DC, USA, 1984.

[11] J. Goudriaan and H. E. de Ruiter, "Plant growth in response to CO_2 enrichment at two levels of nitrogen and phosphorus supply. 1. Dry matter, leaf area, and development," *Netherlands Journal Agriculture Science*, vol. 31, pp. 157–169, 1983.

[12] L. J. A. Lekkerkerk, S. C. van de Geijn, and J. A. van Veen, "Effects of elevated atmospheric CO_2 levels on the carbon economy of a soil planted with wheat," in *Soils and the Greenhouse Effect*, A. F. Bouwman, Ed., pp. 423–429, John Wiley & Sons, New York, NY, USA, 1990.

[13] R. A. Gill, H. W. Polley, H. B. Johnson, L. J. Anderson, H. Maherali, and R. B. Jackson, "Nonlinear grassland responses to past and future atmospheric CO_2," *Nature*, vol. 417, no. 6886, pp. 279–282, 2002.

[14] G. B. Runion, M. A. Davis, S. G. Pritchard et al., "Effects of elevated atmospheric carbon dioxide on biomass and carbon accumulation in a model regenerating longleaf pine community," *Journal of Environmental Quality*, vol. 35, no. 4, pp. 1478–1486, 2006.

[15] H. W. Polley, H. B. Johnson, and H. S. Mayeux, "Leaf physiology, production, water use, and nitrogen dynamics of the grassland invader *Acacia smallii* at elevated CO_2 concentrations," *Tree Physiology*, vol. 17, no. 2, pp. 89–96, 1997.

[16] H. A. Torbert, S. A. Prior, and H. H. Rogers, "Elevated atmospheric carbon dioxide effects on cotton plant residue decomposition," *Soil Science Society of America Journal*, vol. 59, no. 5, pp. 1321–1328, 1995.

[17] H. A. Torbert, S. A. Prior, H. H. Rogers, and G. B. Runion, "Crop residue decomposition as affected by growth under elevated atmospheric CO_2," *Soil Science*, vol. 163, no. 5, pp. 412–419, 1998.

[18] J. W. Huckabee Jr., D. R. Thompson, J. C. Wyrick, and E. G. Paulat, *Soil Survey of Bell County, Texas*, USDA, Soil Conservation Service, Washington, DC, USA, 1977.

[19] SAS, *SAS System for Personal Computers. Release 9.03*, SAS, Cary, NC, USA, 2003.

[20] R. B. Thomas, D. D. Richter, H. Ye, P. R. Heine, and B. R. Strain, "Nitrogen dynamics and growth of seedlings of an N-fixing tree (*Gliricidia sepium* (Jacq.) Walp.) exposed to elevated atmospheric carbon dioxide," *Oecologia*, vol. 88, no. 3, pp. 415–421, 1991.

[21] C. S. Vogel and P. S. Curtis, "Leaf gas exchange and nitrogen dynamics of N_2-fixing, field-grown *Alnus glutinosa* under elevated atmospheric CO_2," *Global Change Biology*, vol. 1, pp. 55–61, 1995.

[22] C. W. Wood, H. A. Torbert, H. H. Rogers, G. B. Runion, and S. A. Prior, "Free-air CO_2 enrichment effects on soil carbon and nitrogen," *Agricultural and Forest Meteorology*, vol. 70, no. 1–4, pp. 103–116, 1994.

[23] S. A. Prior, H. A. Torbert, G. B. Runion et al., "Free-air CO_2 enrichment of wheat: soil carbon and nitrogen dynamics," *Journal of Environmental Quality*, vol. 26, no. 4, pp. 1161–1166, 1997.

[24] D. S. Schimel, T. G. F. Kittel, A. K. Knapp, T. R. Seastedt, W. J. Parton, and V. B. Brown, "Physiological interactions along resource gradients in a tallgrass prairie," *Ecology*, vol. 72, no. 2, pp. 672–684, 1991.

[25] T. R. Seastedt, J. M. Briggs, and D. J. Gibson, "Controls of nitrogen limitation in tallgrass prairie," *Oecologia*, vol. 87, no. 1, pp. 72–79, 1991.

[26] B. R. Strain and F. A. Bazzaz, "Terrestrial plant communities," in *CO_2 and Plants*, E. R. Lemon, Ed., pp. 177–222, Westview Press, Boulder, Colo, USA, 1983.

[27] H. A. Torbert, S. A. Prior, G. B. Runion, M. A. Davis, S. G. Pritchard, and H. H. Rogers, "Nitrogen and carbon cycling in a model longleaf pine community as affected by elevated atmospheric CO_2," *Environmental Management*, vol. 33, supplement 1, pp. S132–S138, 2004.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 549745, 9 pages
doi:10.1155/2012/549745

Research Article

Effects of Atmospheric CO_2 Enrichment on Soil CO_2 Efflux in a Young Longleaf Pine System

G. Brett Runion,[1] J. R. Butnor,[2] S. A. Prior,[1] R. J. Mitchell,[3] and H. H. Rogers[1]

[1] *National Soil Dynamics Laboratory, USDA-ARS, 411 S. Donahue Drive, Auburn, AL 36832, USA*
[2] *Southern Research Station, USDA Forest Service, 3041 Cornwallis Road, Research Triangle Park, NC 27709, USA*
[3] *Joseph W. Jones Ecological Research Center at Ichuaway, 3988 Jones Center Drive, Newton, GA 39870, USA*

Correspondence should be addressed to G. Brett Runion, brett.runion@ars.usda.gov

Received 15 December 2011; Accepted 4 January 2012

Academic Editor: H. Allen Torbert

The southeastern landscape is composed of agricultural and forest systems that can store carbon (C) in standing biomass and soil. Research is needed to quantify the effects of elevated atmospheric carbon dioxide (CO_2) on terrestrial C dynamics including CO_2 release back to the atmosphere and soil sequestration. Longleaf pine savannahs are an ecologically and economically important, yet understudied, component of the southeastern landscape. We investigated the effects of ambient and elevated CO_2 on soil CO_2 efflux in a young longleaf pine system using a continuous monitoring system. A significant increase (26.5%) in soil CO_2 efflux across 90 days was observed under elevated CO_2; this occurred for all weekly and daily averages except for two days when soil temperature was the lowest. Soil CO_2 efflux was positively correlated with soil temperature with a trend towards increased efflux response to temperature under elevated CO_2. Efflux was negatively correlated with soil moisture and was best represented using a quadratic relationship. Soil CO_2 efflux was not correlated with root biomass. Our data indicate that, while elevated CO_2 will increase feedback of CO_2 to the atmosphere via soil efflux, terrestrial ecosystems will remain potential sinks for atmospheric CO_2 due to greater biomass production and increased soil C sequestration.

1. Introduction

The rural southeastern landscape is dominated by three vegetation types (crops, forests, and pastures), all of which have the ability to store atmospheric carbon (C) as standing biomass (including plant roots) or in soil. One particularly important ecosystem is longleaf pine savannahs. Prior to European settlement, the coastal plains of the southeastern United States were dominated by nearly pure stands of longleaf pine (*Pinus palustris* Mill.) with a diverse understory plant community; some longleaf ecosystems have the highest reported values for species richness, including many threatened and endangered species, in the temperate Western Hemisphere [1]. This system now occupies only 2% of its former range [2], a loss comparable to or exceeding that of most endangered communities throughout the world including the North American tallgrass prairie, the moist tropical coastal forest of Brazil, and the dry forests along the Pacific coast of Central America [3]. Longleaf pine forests in the southeast currently occupy sites at the more xeric end of the moisture continuum and are often found on soils with low N availability. In fact, it is not unusual to find disjunct longleaf pine communities in the rural farm landscape. Thus, landowner interest in this species has increased dramatically over the last decade, not only due to its ecological significance but also because of superior lumber quality, fire tolerance, and resistance to some of the more devastating southern forest insects (e.g., bark beetles) and diseases (e.g., fusiform rust). Given that longleaf pine systems may become a more important component of the rural farm landscape, it is important to determine how the rising level of carbon dioxide (CO_2) in the atmosphere [4] will impact these systems.

Carbon dioxide is the first molecular link from atmosphere to biosphere. Most plant species increase biomass

production when exposed to above-ambient levels of atmospheric CO_2 (e.g., [5–9]). Positive plant responses to higher CO_2 can be attributed to increased photosynthetic capacity [10], water use efficiency [8, 11], and nutrient uptake and utilization efficiency [6].

The rising level of atmospheric CO_2 has prompted speculation on the ability of terrestrial ecosystems to sequester C as a means of mitigating this rise and its potential impacts on climate. However, as the ability of terrestrial ecosystems to store C (in biomass and/or in soil) is not based solely on net primary productivity [12], elevated atmospheric CO_2 may also impact terrestrial ecosystem C storage through alterations in plant tissue quality, which will impact soil microbes, decomposition processes, and subsequent soil C storage. Plant tissue produced under high CO_2 often has higher C : N ratios [13, 14] and may be structurally different, with alterations in leaf anatomy [15] and epicuticular waxes [16, 17]. Plants grown under elevated CO_2 may also exhibit altered tissue chemistry, including lower N concentrations [18, 19], higher concentrations of carbohydrates [19, 20], and increased levels of defense compounds such as phenolics [21, 22].

The fate of C within plant systems is affected by a chain of biological events starting with transfer of C from air to leaf, transformation within the plant, translocation within the plant/soil system, return of plant residue to the soil, and decomposition and is impacted by the effects of other environmental factors (e.g., temperature, nutrients, and water) on these processes. Therefore, the ability of terrestrial ecosystems to sequester C will depend on C cycling among the various biomass and soil pools and on the residence time of the C within these pools [23].

At many stages in the cycling of C within terrestrial ecosystems, CO_2 is transferred back to the atmosphere by both autotrophic and heterotrophic respiration. Soil respiration is a significant source of CO_2 flux from terrestrial ecosystems to the atmosphere [24], with global estimates ranging from 68 to 100 Pg C yr^{-1} [25, 26]. Therefore, even small shifts in soil CO_2 efflux could have serious implications for increasing or decreasing atmospheric CO_2 concentration and the resulting impacts on climate change [27]. Through its impact on the quantity and quality of C within the plant/soil system, elevated CO_2 can affect this feedback of C to the atmosphere. For example, increased root growth under elevated CO_2 could increase root respiration [28], while changes in root exudation and/or quality might enhance [23, 29] or suppress [30] microbial respiration. The combined effects on total soil CO_2 respired back to the atmosphere, and the potential for C sequestration, are difficult to predict.

One review of soil and microbial respiration demonstrates that elevated atmospheric CO_2 generally increases belowground respiration, with overall estimates ranging from 40 to 50% for soil respiration and from 20 to 35% for microbial respiration [31]; these estimates agree with another review that reported an overall increase of 37% for forest species [32]. Other elevated CO_2 studies report stimulation of root or total soil respiration in the range of 15–50% [33–35], with even greater stimulation reported in some cases [36, 37]. Enhanced root or soil respiration under high CO_2 is often related to increased root biomass, that is, autotrophic respiration [31, 34, 36, 37] and/or increases in the size or activity of the microbial community, that is, heterotrophic respiration [31, 34, 37, 38]. However, some cases [30, 33] showed elevated CO_2 to suppress soil respiration or to have no effect [39, 40]. Soil CO_2 efflux can be highly variable on temporal and spatial scales within a single field experiment [34, 41] and among experiments; therefore, even relatively large increases in soil efflux under elevated CO_2 may not be statistically significant [31]. Some of the variation among individual studies may be due to differences in plant species, experimental conditions, or methods used for determination of CO_2 efflux.

A major drawback of most methods for determining soil CO_2 efflux concerns the timescale of measurements (i.e., cumulative totals across hours to days with NaOH traps or discreet points in time with soil collars and gas exchange devices); efflux between measurement periods is then generally assumed to be linearly integrative across the intervening time periods [34, 37]. Given the varying responses of soil CO_2 efflux to elevated atmospheric CO_2 and the limitations of current measurement technology, more research is needed before we can confidently predict the impacts of elevated atmospheric CO_2 on the ability of terrestrial ecosystems to sequester C. The objective of this experiment was to assess the response of soil CO_2 efflux (root plus microbial respiration) to three years of atmospheric CO_2 enrichment in a model regenerating longleaf pine community using a novel, continuous CO_2 efflux monitoring system; correlations of efflux with changes in root biomass, populations of microbes and micro- and mesofauna, and soil C were also investigated.

2. Materials and Methods

2.1. Study Site. A model regenerating longleaf pine-wiregrass ecosystem was constructed in Spring 1998 at the National Soil Dynamics Laboratory in Auburn, AL; descriptions of the study site and model ecosystem have been previously reported [42]. Briefly, an assemblage of five early successional forest species representing major functional guilds within a typical longleaf pine-wiregrass community was chosen for study: longleaf pine (*Pinus palustris*, a C_3 evergreen conifer), wiregrass (*Aristida stricta*, a C_4 bunch grass), sand post oak (*Quercus margaretta*, a C_3 broadleaf tree), rattlebox (*Crotalaria rotundifolia*, a C_3 perennial herbaceous legume), and butterfly weed (*Asclepias tuberose*, a C_3, nonleguminous, herbaceous perennial). These species are common associates throughout the southeastern USA. The model forest community was assembled in April 1998 on an outdoor soil bin (2 m deep, 6 m wide, and 76 m long) containing a Blanton loamy sand (loamy, siliceous, and thermic Grossarenic Paleudults). The planting regime used [42] reflected densities found in naturally regenerating longleaf pine-wiregrass ecosystems [43, 44].

Open top chambers [45], encompassing 7.3 m^2 of ground surface area, were used to deliver target CO_2 concentrations of 365 μmol mol^{-1} (ambient) or 720 μmol mol^{-1} (elevated)

beginning June 1998 using a delivery system described by Mitchell et al. [46]. The study area was divided into six blocks, and each CO_2 treatment was randomly assigned to one open top chamber within each block; therefore, the experimental design was a randomized complete block design, with blocks occurring along the length of the soil bin.

2.2. Soil Respiration Measurements. Soil CO_2 efflux was measured using the Automated Carbon Efflux System (ACES) (US Patent 6,692,970), developed at USDA Forest Service, Southern Research Station Laboratory in Research Triangle Park, NC; a description of the ACES has been previously reported [47]. Briefly, ACES is a chamber-based, multiport respiration measurement system, which uses open system, dynamic soil respiration chambers measuring 25 cm diameter (491 cm^2) equipped with air and soil thermocouples (soil thermocouples were inserted to depth of 5 cm). The soil chambers are designed with pressure equilibration ports to ensure that differences in chamber pressure do not compromise the quality of the respiration measurement [48]. Each ACES has 15 sample chambers and one null calibration chamber, which are measured sequentially for 10 minutes each, allowing a complete run every 2 hours and 40 minutes or nine complete runs per day. When not being actively sampled, all chambers are refreshed with reference air to prevent buildup of CO_2. The ACES units constructed for our study were modified to allow use of reference air from two sources, owing to the differential atmospheric CO_2 concentrations employed; soil chambers in ambient CO_2 open top chambers were refreshed with ambient CO_2 air, while those in elevated open top chambers were refreshed with elevated CO_2 air. Ambient CO_2 reference air was obtained by placing an air compressor in an additional, empty, ambient open top chamber located on an adjacent soil bin and using the same CO_2 delivery system as the main study; elevated CO_2 reference air was similarly obtained by placing a second air compressor in an additional, empty, elevated open top chamber. The air compressors replace the ballast tanks commonly used with the ACES, which provide reference air for the ACES that is buffered against fluctuations in atmospheric CO_2 concentration [47].

Constraints on distance between soil respiration chambers and the main ACES unit (housing the infrared gas analyzer and datalogger) necessitated use of two ACES units in this study; one was used for blocks 1–3 and a second for blocks 4–6. Two soil chambers were placed into each of the 12 open top chambers; the three additional soil chambers for each system were placed outside of open top chambers. Calibration chambers were placed into the ambient open top chamber nearest to each main ACES unit. A soil moisture probe was placed adjacent to each calibration chamber and inserted to a depth of 20 cm.

To minimize the effect of precipitation exclusion on the soil substrate within the soil chambers, soil chambers were moved every 3-4 days between two sample points (A and B) within each open top chamber. Litter on the soil surface was not removed from each sample point, but all points were kept free of live vegetation. The ACES units were installed on March 6, 2001, at which time the study had been continuously exposed to CO_2 treatments for 33 months. The ACES units were run continuously until June 4, 2001 (day of year (DOY) 65 through 155), with the exception of brief periods for maintenance or due to system/power failures; at this time they were removed to allow for a complete destructive harvest of the study. Details of the harvest, along with associated biomass and plant and soil C data, have been previously reported [49].

2.3. Soil Biology Assessments. Root-zone soil, from the 0–15 cm depth increment, was collected using large soil cores (24.5 cm diameter × 60 cm deep) and an extraction method of our own design [50]. The soil was then passed through a 2 mm mesh stainless steel sieve until 10–20 g of sieved soil was collected. Dehydrogenase activity, a reliable index of microbial activity in soil [51], was determined from modified procedures described by Tabatabai [52]. Sieved soil (≈1 g) for triplicate subsamples from each plot was placed in test tubes (15 × 100 mm), covered with 1 mL of 3% aqueous (w/v) 2,3,5-triphenyltetrazolium chloride and stirred with a glass rod. After 96 hr incubation (27°C), 10 mL of methanol was added to each test tube, and the suspension was vortexed for 30 sec. Tubes were then incubated for 1 hr to allow suspended soil to settle. The resulting supernatant (≈5 mL) was carefully transferred to clean test tubes using Pasteur pipets. Absorbance was read spectrophotometrically at 485 nm, and formazan concentration was calculated using a standard curve produced from known concentrations of triphenyl formazan. One subsample of sieved soil (≈1 g) from each soil sample was used for determination of soil moisture so that formazan concentrations could be expressed per gram soil dry weight.

Soil taken from the previously described cores was extracted for relative populations of Collembola and Acari by a modified version of the Tullgren system as described by Wiggins et al. [53]. Soil samples in large funnels, with stems positioned over water in a collecting tube, were arranged in series under 40 W light bulbs. The animals, migrating in advance of the slowly drying soil (5–7 days), were collected live. Populations, counted under a dissecting microscope, were expressed as numbers per kg of air-dried soil. A subsample of the soil collected for soil animals was sent to the Department of Entomology and Plant Pathology, Auburn University for assessment of nematode populations.

2.4. Data Analysis. All soil respiration data were analyzed for system and power failures; obvious "systematic" errors were parsed from the data set. A total of 18,813 soil CO_2 efflux observations were taken over the 90-day measurement period; of these, 94.4% were deemed acceptable for analysis. Data analysis was conducted using the mixed model procedures (Proc Mixed) of the Statistical Analysis System [54]. Data were initially analyzed to determine if differences existed between the two ACES units employed in the study or between soil chamber positions (A versus B); as no significant unit or positional effects were noted, data were not segregated prior to analysis. Effects of CO_2

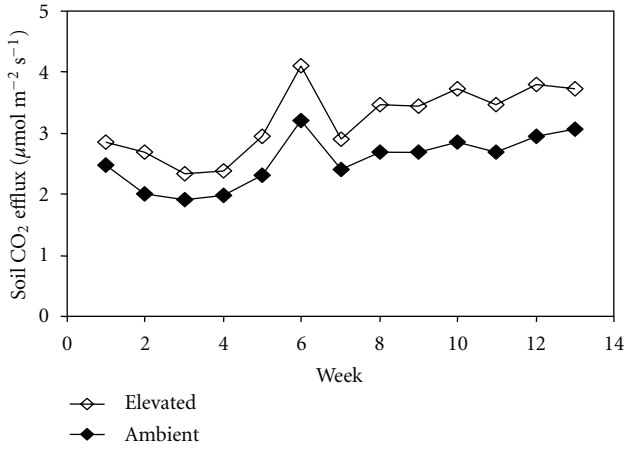


FIGURE 1: Weekly average soil CO_2 efflux (μmol CO_2 m^{-2} s^{-1}) for ambient (365 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2 plots in a model regenerating longleaf pine-wiregrass ecosystem.

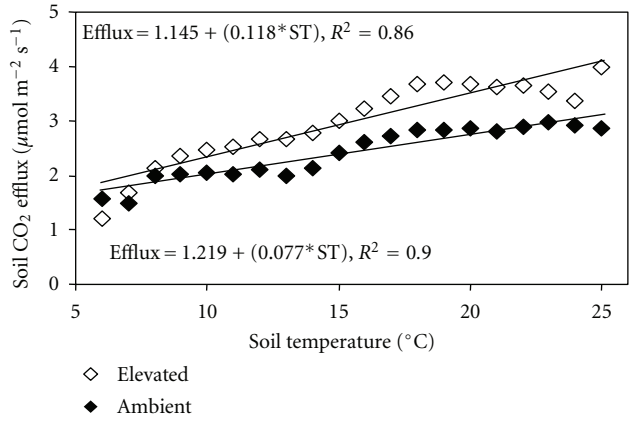


FIGURE 2: Response of soil CO_2 efflux (μmol CO_2 m^{-2} s^{-1}) to soil temperature for ambient (365 μmol CO_2 mol^{-1}) and elevated (720 μmol CO_2 mol^{-1}) CO_2 plots in a model regenerating longleaf pine-wiregrass ecosystem. Equations describing each line, with fit statistics, are provided above (elevated) and below (ambient) the lines.

concentration were determined using the analysis of variance statistics derived from the mixed procedure of SAS; effects were determined by day, by week, and across the entire measurement period. All data from each ACES chamber (total = 24) were then averaged for 1.0°C intervals of soil temperature measured at a depth of 5 cm at each ACES chamber, regardless of DOY; averaging served to reduce the influence of outliers on the response of soil CO_2 efflux to temperature throughout the experiment. Linear regression [55] was then used on the averaged data to determine the relationship between soil CO_2 efflux and soil temperature; a similar procedure was used to investigate the relationship between soil CO_2 efflux and soil moisture. The relationship of soil CO_2 efflux with components of root biomass and with soil C and N (previously reported by Runion et al. [49]) was also investigated using linear regression. Specific respiration rates were calculated by dividing cumulative soil CO_2 efflux (g C m^{-2}) over the entire measurement period by total root biomass, total root C and N, and total soil C and N (g m^{-2}) to give g C respired per g root dry weight, root C or N, or per g soil C or N.

3. Results

Soil CO_2 efflux, averaged across the entire 90 day measurement period, from ambient plots was 2.54 (±0.008; n = 8884) μmol CO_2 m^{-2} s^{-1}, while elevated plots averaged 3.22 (±0.011; n = 8878) μmol CO_2 m^{-2} s^{-1}; this represented a significant increase ($P < 0.0001$) of 26.8% or a total increase of ≈60 g C m^{-2}. When averaged on a weekly basis (Figure 1), elevated CO_2 plots consistently had significantly higher ($P < 0.0001$) soil respiration rates than did ambient plots, with the increase ranging from 15 to 33%. Further, when analyzed on a daily average basis (data not shown), elevated CO_2 significantly increased ($P < 0.05$) soil respiration on all but two days (DOY 79, P = 0.08, and DOY 80, P = 0.99); these two days had the lowest daily average soil temperatures recorded during the entire duration of the study (7.3 and 8.0, resp.).

Regression of averaged soil CO_2 efflux on soil temperature (Figure 2) showed strong positive linear relationships for both ambient and elevated CO_2 plots (r^2 = 0.90 and 0.86 for ambient and elevated, resp.). Assessment of nonlinear models did not improve the fit of these data over the linear models. The slope of the line for elevated CO_2 plots was significantly steeper ($P < 0.01$) than for ambient plots; Y-intercepts for these two regression lines did not differ (P = 0.15). Using these regressions, we calculated the change in soil CO_2 efflux for a 10°C change in soil temperature for each set of plots; these values were 0.77 and 1.18 μg m^{-2} s^{-1} for ambient and elevated CO_2 plots, respectively, indicating a 53% increase in the response of efflux to increasing temperature under elevated CO_2. Soil moisture, collected only in two ambient CO_2 plots (Figure 3), also showed a strong linear correlation with soil CO_2 efflux, albeit a negative relationship (r^2 = 0.76). However, these data showed a better fit when a quadratic function was employed (r^2 = 0.96).

Soil CO_2 efflux was not correlated with fine, coarse, or total root biomass (r^2 range = 0.01 to 0.35), whether analyzed for each plant species or for total across all species. Similar trends were observed when correlating soil CO_2 efflux with either root N or C (data not shown).

No effects of CO_2 treatment on dehydrogenase were observed (P = 0.40). Soil CO_2 efflux was not correlated with dehydrogenase (r^2 = 0.40 and 0.05 for ambient and elevated CO_2 treatments, resp.). While numbers of both nematodes and soil animals were higher under elevated CO_2, these effects were not significant (P = 0.40 and 0.15 for nematodes and soil animals, resp.). Soil CO_2 efflux was, again, not correlated with numbers of nematodes or soil animals (r^2 range = 0.01 to 0.35).

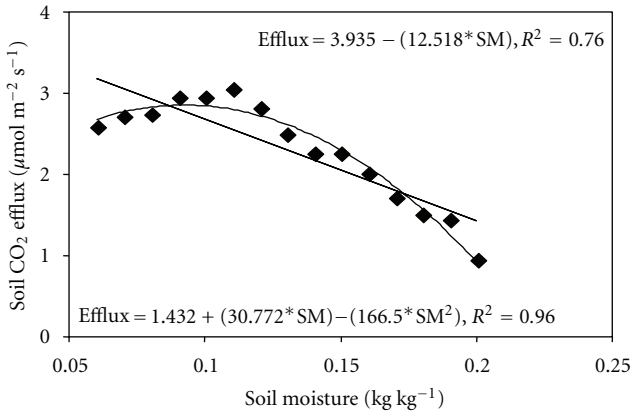


FIGURE 3: Response of soil CO_2 efflux (μmol CO_2 m^{-2} s^{-1}) to soil moisture for ambient (365 μmol CO_2 mol^{-1}) CO_2 plots only in a model regenerating longleaf pine-wiregrass ecosystem. Equations describing this line, with fit statistics, are provided above (linear) and below (quadratic) the lines.

Soil C and N content did not differ among plots at initiation of the study (1852 and 197 g m^{-2} for soil C and N, resp.). These variables also varied little at study termination (3042 and 2975 g C m^{-2} and 165 and 163 g N m^{-2} for ambient and elevated plots, resp.). Soil CO_2 efflux was positively correlated with both soil C and N content measured at the end of the study; the significance of these correlations varied by soil profile depth (Table 1). Correlations of soil CO_2 efflux with soil C tended to be stronger than correlations with soil N.

Specific soil CO_2 efflux rates per g root dry weight or per g root C were significantly lower in elevated than in ambient CO_2 plots; specific respiration rate per g root N was not different between CO_2 treatments (Table 2). However, specific respiration rates per g soil N and C were significantly higher in elevated than in ambient CO_2 plots.

4. Discussion

The observed increase in soil CO_2 efflux under elevated CO_2 in this study is consistent with other reports in the literature (e.g., [31]). The ≈60 g m^{-2} increase observed across the 90-day measurement period is also comparable with the ≈178 g C m^{-2} increase reported by Butnor et al. [47] who used ACES over a 220-day period in a 17-year old loblolly pine (*P. taeda*) stand. The consistency of the increase we observed, on a weekly or daily basis, further demonstrates that growth under high CO_2 had a sustained impact on soil CO_2 efflux in the model longleaf pine community following 33–36 months of constant exposure to a twice ambient concentration of atmospheric CO_2.

Temperature is known to strongly influence soil respiration [56, 57], with efflux increasing as temperature increases, as observed in this study. Soil CO_2 efflux has generally been shown to increase as an exponential function of temperature [57]; however, in the present study this relationship was more than adequately described using a linear function for both ambient and elevated CO_2 treatments. Under elevated CO_2, the increased responsiveness (i.e., steeper slope) of soil CO_2 efflux to temperature might suggest increased feedback of C to the atmosphere under global warming. However, when we attempted to fit quadratic relationships of soil respiration to soil temperature (data not shown), we observed differences in the inflection points of the curves (30 and 24°C for ambient and elevated plots, resp.), as well as a slight increase in fit statistics (r^2 = 0.92 and 0.94 for ambient and elevated plots, resp.). This analysis suggested that, at soil temperatures above 24°C, the increase in soil CO_2 efflux under elevated CO_2 was reduced; extrapolation of these curves indicated that efflux for both CO_2 treatments would be nearly equal at ≈33°C. Additional research is needed to verify these extrapolations.

Soil moisture is known to affect soil CO_2 efflux through both physical (displacing soil gases) and biological (impacts on root and microbial activity) means [56]. Although the relationship is generally positive, negative relationships (as observed in the present study) have been reported [56, 58]. The improved fit of our data to a quadratic relationship suggests the existence of a soil moisture content at which soil CO_2 efflux is maximized; this would, obviously, be dependent on soil type. Therefore, as most prior soil CO_2 efflux data have been collected using a series of spot measurements or measurements integrated across relatively short time scales, it is possible that the varying responses (i.e., positive versus negative) of soil CO_2 efflux to soil moisture in previous studies [56, 58] might be explained by knowing where data fell on the quadratic response curve (Figure 3).

Increased rates of soil CO_2 efflux under high CO_2 have often been shown to be related to increases in root biomass [31, 34, 36, 37]. Course, fine, and total root biomass were all increased by elevated CO_2 in this experiment [49]. Therefore, the lack of strong correlations between soil CO_2 efflux and root biomass, root C, or root N was unexpected. Most likely, this lack of correlation was due to high variability within the data, particularly variability among species [49]. The lower specific respiration rates for root dry weight and root C under elevated CO_2 are primarily due to the fact that root biomass increased more than soil CO_2 efflux. In contrast, the higher specific respiration rates per g soil C or N are primarily due to the fact that elevated CO_2 increased soil CO_2 efflux to a greater degree than soil C or N.

Assessments of soil microbial activity and populations of soil micro- and mesofauna at study termination showed no differences between CO_2 exposure treatments, again suggesting that root respiration was primarily responsible for the observed differences in soil CO_2 efflux. However, since microbial parameters are highly variable even on short temporal scales, it is likely that assessment of these parameters solely at the end of the study does not accurately reflect their overall contribution to soil CO_2 efflux across the 90-day measurement period. Further, the strong correlations of CO_2 efflux with soil N and, especially, C might also indicate a greater contribution of heterotrophic respiration to total soil efflux than the microbial assessments suggested. Separation of heterotrophic and autotrophic respiration would have aided explanation of these trends.

Table 1: Regression parameters and statistics for relationships of total soil CO_2 efflux ($g\,C\,m^{-2}$) over the 90-day measurement period with soil N and C content.

CO_2[a]	Soil profile depth	Soil elemental content ($g\,m^{-2}$)[b]	Intercept	Variable	Significance of variable ($pr > |t|$)	r^2
Ambient	0–15 cm	N	163.970	1.550	0.17	0.42
	15–30 cm	N	172.578	1.516	0.17	0.41
	30–45 cm	N	136.918	2.381	0.12	0.49
	45–60 cm	N	185.687	1.101	0.30	0.26
	0–60 cm	N	165.534	0.403	0.18	0.39
Elevated	0–15 cm	N	169.059	2.805	0.07	0.60
	15–30 cm	N	179.336	2.908	0.26	0.30
	30–45 cm	N	207.544	2.197	0.22	0.35
	45–60 cm	N	220.245	1.795	0.30	0.26
	0–60 cm	N	190.030	0.633	0.18	0.39
Ambient	0–15 cm	C	57.834	0.215	0.21	0.36
	15–30 cm	C	−34.372	0.379	0.05	0.67
	30–45 cm	C	−130.076	0.499	0.04	0.69
	45–60 cm	C	−20.394	0.315	0.12	0.50
	0–60 cm	C	−142.144	0.123	0.02	0.79
Elevated	0–15 cm	C	−61.083	0.443	0.01	0.88
	15–30 cm	C	−79.153	0.528	0.08	0.57
	30–45 cm	C	75.940	0.309	0.11	0.51
	45–60 cm	C	19.100	0.356	0.14	0.46
	0–60 cm	C	−120.255	0.139	0.01	0.83

[a] Ambient $CO_2 \approx 365\,\mu mol\,mol^{-1}$; elevated $CO_2 \approx 720\,\mu mol\,mol^{-1}$. [b] N: soil nitrogen; C: soil carbon.

Table 2: Specific respiration rates (g C respired per g) for root biomass, root C and N and soil C and N.

CO_2[a]	Root dry weight	Root N	Root C	Soil N	Soil C
Ambient	0.172	34.493	0.375	1.437	0.076
Elevated	0.146	32.262	0.315	1.848	0.098
ANOVA	$P = 0.03$	$P = 0.32$	$P = 0.02$	$P < 0.01$	$P < 0.01$

[a] Ambient $CO_2 \approx 365\,\mu mol\,mol^{-1}$; elevated $CO_2 \approx 720\,\mu mol\,mol^{-1}$.

Previous research with container-grown longleaf pine seedling showed that N was the controlling factor; under low N conditions, longleaf growth response to elevated CO_2 was negligible [14]. In the current study, Torbert et al. [59] found increased soil N mineralization under elevated CO_2, indicating that N resources should not be limiting for either microbial decomposition of residues or plant growth in future regenerating longleaf pine systems. Therefore, despite this study receiving no N additions throughout the three years, a positive growth response to elevated CO_2 was observed for longleaf pine [49].

We assessed the overall impact of CO_2 enrichment on this model longleaf pine community through its first three years of growth by extrapolating biomass (above- and belowground, as well as litter), soil C and N [49], and soil respiration data from this study. Elevated CO_2 resulted in a significant increase of $4.07\,Mg\,C\,ha^{-1}\,yr^{-1}$ sequestered in standing biomass (ambient $CO_2 = 6.29\,Mg\,C\,ha^{-1}\,yr^{-1}$; elevated $CO_2 = 10.36\,Mg\,C\,ha^{-1}\,yr^{-1}$) with an additional significant increase of $0.54\,Mg\,C\,ha^{-1}\,yr^{-1}$ in litter (ambient $CO_2 = 0.72\,Mg\,C\,ha^{-1}\,yr^{-1}$; elevated $CO_2 = 1.26\,Mg\,C\,ha^{-1}\,yr^{-1}$). The change in soil C was not significantly different between CO_2 treatments at termination of the study (3.97 and $3.74\,Mg\,C\,ha^{-1}\,yr^{-1}$ for ambient and elevated CO_2, resp.). Therefore, the entire system showed a gain of $4.38\,Mg\,C\,ha^{-1}\,yr^{-1}$ due to exposure to elevated atmospheric CO_2 (ambient $CO_2 = 10.98\,Mg\,C\,ha^{-1}\,yr^{-1}$; elevated $CO_2 = 15.36\,Mg\,C\,ha^{-1}\,yr^{-1}$). It should be noted that soil respiration rates from the final three months of exposure were used to estimate soil CO_2 efflux over the three-year study period. Also, we did not assess nighttime plant respiration; it is unlikely this would have significantly impacted the analysis since plant respiration has been shown to be relatively unresponsive to elevated CO_2 [60]. Despite

increased soil respiration of 2.54 Mg CO_2-C ha^{-1} yr^{-1} under elevated CO_2, our estimates suggest a net increased storage of 1.84 Mg C ha^{-1} yr^{-1} with the majority of the added C residing in plant biomass. Torbert et al. [59] found decreased soil C turnover under elevated CO_2, suggesting that increased C sequestration in soil is possible in these longleaf systems.

In general, elevated CO_2 increases soil CO_2 efflux due to increases in autotrophic respiration from increased root growth and/or increased heterotrophic respiration associated with microbial use of increased C inputs [31, 34]. However, despite increased soil CO_2 efflux under elevated atmospheric CO_2, terrestrial ecosystems can still be potential sinks for atmospheric CO_2 due to greater biomass production and increased soil C sequestration. This may be particularly true for forest systems. For example, our research indicates that regenerating longleaf pine systems have the potential to be sinks for atmospheric CO_2 in a future elevated CO_2 environment. These findings are especially important given that longleaf pines currently occupy less productive, low N sites and given the increasing landowner interest in this species due to its superior economic and ecological attributes.

Acknowledgments

This work was supported by the Experimental Program to Stimulate Competitive Research, US Environmental Protection Agency, Contract no. R826259-01, by the Alabama Agricultural Experiment Station, Project no. ALA-60-008, and by Interagency Agreement no. DE-AI05-95ER62088 from the US Department of Energy, Environmental Sciences Division. Any opinions, findings, and conclusions, or recommendations expressed in this paper are those of the authors and do not necessarily reflect the views of the US Environmental Protection Agency, the Alabama Agricultural Experiment Station, or the US Department of Energy. The authors thank Renee Paddock and Barry Dorman for assistance in constructing the ACES units and Barry Dorman, Jerry Carrington, and Milam Saxon for assistance with system maintenance.

References

[1] R. K. Peet and D. J. Allard, "Longleaf pine vegetation of the southern Atlantic and eastern gulf coast regions: a preliminary classification," in *Proceedings of the 18th Tall Timbers Fire Ecology Conference, The Longleaf Pine Ecosystem: Ecology, Restoration and Management*, S. M. Hermann, Ed., pp. 45–81, Tall Timbers Research Station, 1993.

[2] J. L. Landers, D. H. van Lear, and W. D. Boyer, "Longleaf pine forests of the southeast: requiem or renaissance?" *Journal of Forestry*, vol. 93, pp. 39–44, 1995.

[3] R. F. Noss, "Longleaf pine and wiregrass: keystone components of an endangered ecosystem," *Natural Areas Journal*, vol. 9, pp. 211–213, 1989.

[4] C. D. Keeling and T. P. Whorf, "Atmospheric CO_2 records from the sites in the SIO air sampling network," in *Trends '93: A Compendium of Data on Global Change*, T. A. Boden et al., Ed., ORNL/CDIAC-65, pp. 16–26, The Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, Tenn, USA, 1994.

[5] B. A. Kimball, "Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations," *Agronomy Journal*, vol. 75, pp. 779–788, 1983.

[6] H. H. Rogers, G. Brett Runion, and S. V. Krupa, "Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere," *Environmental Pollution*, vol. 83, no. 1-2, pp. 155–189, 1994.

[7] B. R. Strain and J. D. Cure, *Direct Effects of Atmospheric CO_2 Enrichment on Plants and Ecosystems: An Updated Bibliographic Database*, ORNL/CDIAC-70, The Carbon Dioxide Information Analysis Center, Oak Ridge, Tenn, USA, 1994.

[8] J. S. Amthor, "Terrestrial higher-plant response to increasing atmospheric (CO_2) in relation to the global carbon cycle," *Global Change Biology*, vol. 1, no. 4, pp. 243–274, 1995.

[9] M. H. Jones and P. S. Curtis, *Bibliography on CO_2 Effects on Vegetation and Ecosystems: 1990–1999 Literature*, ORNL/CDIAC-129, The Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, Tenn, USA, 2000.

[10] S. P. Long and B. G. Drake, "Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations," in *Crop Photosynthesis: Spatial and Temporal Determinants*, N. R. Baker and H. Thomas, Eds., pp. 69–107, Elsevier, New York, NY, USA, 1992.

[11] H. H. Rogers and R. C. Dahlman, "Crop responses to CO_2 enrichment," *Vegetatio*, vol. 104-105, no. 1, pp. 117–131, 1993.

[12] Z. G. Cardon, "Influence of rhizodeposition under elevated CO_2 on plant nutrition and soil organic matter," *Plant and Soil*, vol. 187, no. 2, pp. 277–288, 1996.

[13] J. M. Mellilo, "Will increases in atmospheric CO_2 concentrations affect decay processes?" in *Ecosystems Center Annual Report*, pp. 10–11, Marine Biology Laboratory, Woods Hole, Mass, USA, 1983.

[14] S. A. Prior, G. B. Runion, R. J. Mitchell, H. H. Rogers, and J. S. Amthor, "Effects of atmospheric CO_2 on longleaf pine: productivity and allocation as influenced by nitrogen and water," *Tree Physiology*, vol. 17, no. 6, pp. 397–405, 1997.

[15] J. F. Thomas and C. N. Harvey, "Leaf anatomy of four species grown under continuous CO_2 enrichment," *Botanical Gazette*, vol. 144, no. 3, pp. 303–309, 1983.

[16] E. A. Graham and P. S. Nobel, "Long-term effects of a doubled atmospheric CO_2 concentration on the CAM species *Agave deserti*," *Journal of Experimental Botany*, vol. 47, no. 294, pp. 61–69, 1996.

[17] S. A. Prior, S. G. Pritchard, G. B. Runion, H. H. Rogers, and R. J. Mitchell, "Influence of atmospheric CO_2 enrichment, soil N, and water stress on needle surface wax formation in *Pinus palustris* (Pinaceae)," *American Journal of Botany*, vol. 84, no. 8, pp. 1070–1077, 1997.

[18] R. J. Norby, E. G. O'Neill, and R. J. Luxmoore, "Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil," *Plant Physiology*, vol. 82, pp. 83–89, 1986.

[19] G. B. Runion, J. A. Entry, S. A. Prior, R. J. Mitchell, and H. H. Rogers, "Tissue chemistry and carbon allocation in seedlings of *Pinus palustris* subjected to elevated atmospheric CO_2 and water stress," *Tree Physiology*, vol. 19, no. 4-5, pp. 329–335, 1999.

[20] S. Yelle, R. C. Beeson Jr., M. J. Trudel, and A. Gosselin, "Acclimation of two tomato species to high atmospheric CO_2. I. Starch and sugar concentrations," *Plant Physiology*, vol. 90, pp. 1465–1472, 1989.

[21] R. L. Lindroth, K. K. Kinney, and C. L. Platz, "Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry, and insect performance," *Ecology*, vol. 74, no. 3, pp. 763–777, 1993.

[22] S. Pritchard, C. Peterson, G. B. Runion, S. Prior, and H. Rogers, "Atmospheric CO_2 concentration, N availability, and water status affect patterns of ergastic substance deposition in longleaf pine (*Pinus palustris* mill.) foliage," *Trees*, vol. 11, no. 8, pp. 494–503, 1997.

[23] B. A. Hungate, E. A. Holland, R. B. Jackson, F. S. Chapin, H. A. Mooney, and C. B. Field, "The fate of carbon in grasslands under carbon dioxide enrichment," *Nature*, vol. 388, no. 6642, pp. 576–579, 1997.

[24] W. H. Schlesinger and J. A. Andrews, "Soil respiration and the global carbon cycle," *Biogeochemistry*, vol. 48, no. 1, pp. 7–20, 2000.

[25] J. W. Raich and W. H. Schlesinger, "The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate," *Tellus*, vol. 44, no. 2, pp. 81–99, 1992.

[26] R. C. Musselman and D. G. Fox, "A review of the role of temperate forests in the global CO_2 balance," *Journal of the Air and Waste Management Association*, vol. 41, no. 6, pp. 798–807, 1991.

[27] L. E. Rustad, T. G. Huntington, and R. D. Boone, "Controls on soil respiration: implications for climate change," *Biogeochemistry*, vol. 48, no. 1, pp. 1–6, 2000.

[28] A. S. Ball and B. G. Drake, "Stimulation of soil respiration by carbon dioxide enrichment of marsh vegetation," *Soil Biology and Biochemistry*, vol. 30, no. 8-9, pp. 1203–1205, 1998.

[29] Y. Luo, R. B. Jackson, C. B. Field, and H. A. Mooney, "Elevated CO_2 increases belowground respiration in California grasslands," *Oecologia*, vol. 108, no. 1, pp. 130–137, 1996.

[30] P. Ineson, P. A. Coward, and U. A. Hartwig, "Soil gas fluxes of N_2O, CH_4 and CO_2 beneath *Lolium perenne* under elevated CO_2: the Swiss free air carbon dioxide enrichment experiment," *Plant and Soil*, vol. 198, no. 1, pp. 89–95, 1998.

[31] D. R. Zak, K. S. Pregitzer, J. S. King, and W. E. Holmes, "Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis," *New Phytologist*, vol. 147, no. 1, pp. 201–222, 2000.

[32] I. A. Janssens and R. Ceulemans, "The response of soil CO_2 efflux under trees grown in elevated atmospheric CO_2: a literature review," *Phyton*, vol. 40, no. 4, pp. 97–101, 2000.

[33] R. M. Gifford, H. Lambers, and J. I. L. Morison, "Respiration of crop species under CO_2 enrichment," *Physiologia Plantarum*, vol. 63, pp. 351–356, 1985.

[34] F. S. Nakayama, G. Huluka, B. A. Kimball, K. F. Lewin, J. Nagy, and G. R. Hendrey, "Soil carbon dioxide fluxes in natural and CO_2-enriched systems," *Agricultural and Forest Meteorology*, vol. 70, no. 1–4, pp. 131–140, 1994.

[35] P. S. J. Verburg, A. Gorissen, and W. J. Arp, "Carbon allocation and decomposition of root-derived organic matter in a plant-soil system of *Calluna vulgaris* as affected by elevated CO_2," *Soil Biology and Biochemistry*, vol. 30, no. 10-11, pp. 1251–1258, 1998.

[36] K. L. Griffin, M. A. Bashkin, R. B. Thomas, and B. R. Strain, "Interactive effects of soil nitrogen and atmospheric carbon dioxide on root/rhizosphere carbon dioxide efflux from loblolly and ponderosa pine seedlings," *Plant and Soil*, vol. 190, no. 1, pp. 11–18, 1997.

[37] J. M. Vose, K. J. Elliott, D. W. Johnson, D. T. Tingey, and M. G. Johnson, "Soil respiration response to three years of elevated CO_2 and N fertilization in ponderosa pine (*Pinus ponderosa* Doug. ex Laws.)," *Plant and Soil*, vol. 190, no. 1, pp. 19–28, 1997.

[38] C. W. Rice, F. O. Garcia, C. O. Hampton, and C. E. Owensby, "Soil microbial response in tallgrass prairie to elevated CO_2," *Plant and Soil*, vol. 165, no. 1, pp. 67–74, 1994.

[39] S. F. Oberbauer, W. C. Oechel, and G. H. Riechers, "Soil respiration of Alaskan tundra at elevated atmospheric carbon dioxide concentrations," *Plant and Soil*, vol. 96, no. 1, pp. 145–148, 1986.

[40] D. W. Johnson, B. A. Hungate, P. Dijkstra, G. Hymus, and B. Drake, "Effects of elevated carbon dioxide on soils in a Florida scrub oak ecosystem," *Journal of Environmental Quality*, vol. 30, no. 2, pp. 501–507, 2001.

[41] M. Xu and Y. Qi, "Soil-surface CO_2 efflux and its spatial and temporal variations in a young ponderosa pine plantation in northern California," *Global Change Biology*, vol. 7, no. 6, pp. 667–677, 2001.

[42] S. G. Pritchard, M. A. Davis, R. J. Mitchell et al., "Root dynamics in an artificially constructed regenerating longleaf pine ecosystem are affected by atmospheric CO_2 enrichment," *Environmental and Experimental Botany*, vol. 46, no. 1, pp. 55–69, 2001.

[43] M. J. Hainds, *Legume population dynamics in a frequently burned longleaf pine-wiregrass ecosystem*, M.S. thesis, Auburn University, Auburn, Ala, USA, 1995.

[44] E. I. Jacqmain, *Controls on Oak composition and population size structure in frequently burned longleaf pine-wiregrass savannahs*, M.S. thesis, Auburn University, Auburn, Ala, USA, 1996.

[45] H. H. Rogers, W. W. Heck, and A. S. Heagle, "A field technique for the study of plant responses to elevated carbon dioxide concentrations," *Journal of the Air Pollution Control Association*, vol. 33, no. 1, pp. 42–44, 1983.

[46] R. J. Mitchell, G. B. Runion, S. A. Prior, H. H. Rogers, J. S. Amthor, and F. P. Henning, "Effects of nitrogen on *Pinus palustris* foliar respiratory responses to elevated atmospheric CO_2 concentration," *Journal of Experimental Botany*, vol. 46, no. 291, pp. 1561–1567, 1995.

[47] J. R. Butnor, K. H. Johnsen, R. Oren, and G. G. Katul, "Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stands," *Global Change Biology*, vol. 9, no. 6, pp. 849–861, 2003.

[48] C. Fang and J. B. Moncrieff, "An improved dynamic chamber technique for measuring CO_2 efflux from the surface of soil," *Functional Ecology*, vol. 10, no. 2, pp. 297–305, 1996.

[49] G. B. Runion, M. A. Davis, S. G. Pritchard et al., "Effects of elevated atmospheric carbon dioxide on biomass and carbon accumulation in a model regenerating longleaf pine community," *Journal of Environmental Quality*, vol. 35, no. 4, pp. 1478–1486, 2006.

[50] S. A. Prior, G. B. Runion, H. A. Torbert, and D. C. Erbach, "A hydraulic coring system for soil-root studies," *Agronomy Journal*, vol. 96, no. 4, pp. 1202–1205, 2004.

[51] I. L. Stevenson, "Dehydrogenase activity in soils," *Canadian Journal of Microbiology*, vol. 5, no. 2, pp. 229–235, 1959.

[52] M. A. Tabatabai, "Soil enzymes: dehydrogenases," in *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, A. L. Page, Ed., Agronomy Monograph 9, pp. 937–940, American Society of Agronomy and Soil Science Society of America, Madison, Wis, USA, 2nd edition, 1982.

[53] E. A. Wiggins, E. A. Curl, and J. D. Harper, "Effects of soil fertility and cotton rhizosphere on populations of Collembola," *Pedobiologia*, vol. 19, pp. 75–82, 1979.

[54] R. C. Littell, G. A. Milliken, W. W. Stroup, and R. D. Wolfinger, *SAS System for Mixed Models*, SAS Institute, Cary, NC, USA, 1996.

[55] SAS Institute, *SAS User's Guide: Statistics—Version 5 Edition*, Statistical Analysis System (SAS) Institute, Cary, NC, USA, 1985.

[56] J. S. Singh and S. R. Gupta, "Plant decomposition and soil respiration in terrestrial ecosystems," *The Botanical Review*, vol. 43, no. 4, pp. 449–528, 1977.

[57] C. Fang and J. B. Moncrieff, "The dependence of soil CO_2 efflux on temperature," *Soil Biology and Biochemistry*, vol. 33, no. 2, pp. 155–165, 2001.

[58] T. J. Bouma and D. R. Bryla, "On the assessment of root and soil respiration for soils of different textures: interactions with soil moisture contents and soil CO_2 concentrations," *Plant and Soil*, vol. 227, no. 1-2, pp. 215–221, 2000.

[59] H. A. Torbert, S. A. Prior, G. B. Runion, M. A. Davis, S. G. Pritchard, and H. H. Rogers, "Nitrogen and carbon cycling in a model longleaf pine community as affected by elevated atmospheric CO_2," *Environmental Management*, vol. 33, supplement 1, pp. S132–S138, 2004.

[60] J. S. Amthor, G. W. Koch, J. R. Willms, and D. B. Layzell, "Leaf O_2 uptake in the dark is independent of coincident CO_2 partial pressure," *Journal of Experimental Botany*, vol. 52, no. 364, pp. 2235–2238, 2001.

Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 163054, 13 pages
doi:10.1155/2012/163054

Research Article

Changes in Soluble-N in Forest and Pasture Soils after Repeated Applications of Tannins and Related Phenolic Compounds

Jonathan J. Halvorson,[1] Javier M. Gonzalez,[1] and Ann E. Hagerman[2]

[1] *USDA-ARS Appalachian Farming Systems Research Center, 1224 Airport Road, Beaver, WV 25813-9423, USA*
[2] *Department of Chemistry and Biochemistry, Miami University, 160 Hughes Laboratories, 701 East High Street, Oxford, OH 45056, USA*

Correspondence should be addressed to Jonathan J. Halvorson, jonathan.halvorson@ars.usda.gov

Received 15 September 2011; Accepted 7 December 2011

Academic Editor: Dexter B. Watts

Tannins (produced by plants) can reduce the solubility of soil-N. However, comparisons of tannins to related non-tannins on different land uses are limited. We extracted soluble-N from forest and pasture soils (0–5 cm) with repeated applications of water (Control) or solutions containing procyanidin from sorghum, catechin, tannic acid, β-1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG), gallic acid, or methyl gallate (10 mg g^{-1} soil). After eight treatments, samples were rinsed with cool water (23°C) and incubated in hot water (16 hrs, 80°C). After each step, the quantity of soluble-N and extraction efficiency compared to the Control was determined. Tannins produced the greatest reductions of soluble-N with stronger effects on pasture soil. Little soluble-N was extracted with cool water but hot water released large amounts in patterns influenced by the previous treatments. The results of this study indicate hydrolyzable tannins like PGG reduce the solubility of labile soil-N more than condensed tannins like sorghum procyanidin (SOR) and suggest tannin effects will vary with land management. Because they rapidly reduce solubility of soil-N and can also affect soil microorganisms, tannins may have a role in managing nitrogen availability and retention in agricultural soils.

1. Introduction

Tannins are reactive secondary metabolites produced by plants that affect important biological, chemical, and physical processes in soil and couple primary productivity to biogeochemical cycles [1–4]. Tannin effects on decomposition and nitrogen availability in soil have been a subject of research for more than fifty years [5, 6]. However, development of strategies for use of tannins as soil management tools has lagged, in part because few studies have specifically related them to improving plant productivity or soil fertility. Early tannin research was conducted on temperate agricultural soils [7–9], while recent work has concentrated more on their role in forest ecosystems [10–12] and tropical soils [13–15]. These studies, however, have tended to emphasize the impacts of tannins on microbially mediated processes rather than on the more immediate abiotic interactions between tannins and soil and have made little attempt to frame their findings into the context of landscape effects.

Tannins are believed to affect the nitrogen cycle through several direct and indirect mechanisms that reduce rates of net mineralization or nitrification. Some tannins are directly toxic to plants or microorganisms [16, 17] but their effects vary with particular tannin chemistry or among taxonomic groups [18]. Some tannins or related phenolic compounds are used by soil microorganisms as substrates increasing microbial demand for nitrogen and immobilization in microbial biomass [2, 12, 19]. Tannins can also reduce rates of mineralization or decomposition by affecting the activity of enzymes [20, 21] or by forming complexes with other proteins or organic nitrogen compounds via reversible noncovalent processes such as hydrogen bonding and hydrophobic interactions (cf. [2, 22, 23]). The availability of the nitrogen sequestered in tannin-protein complexes varies among species of plants, taxa of microorganisms, or even among strains of mycorrhizae [11, 24–28]. Tannins and related phenolics may also affect soil-N through interactions with inorganic soil fractions [3, 23, 29]. For example, tannin-related

phenolic compounds can interact with nitrite, produced during nitrification, to form more recalcitrant organic forms in a process termed nitrosation [30–32].

Our earlier studies revealed some tannins were rapidly sorbed by soil and reduced the solubility of labile soil-N [33, 34]. Significant amounts of retained tannin-C remained in soil even after repeated rinses with hot water [33]. A single application of a gallotannin (β-1,2,3,4,6-penta-*O*-galloyl-D-glucose) produced a persistent reduction in the solubility of organic-N not observed with gallic acid, its simple monomeric constituent, suggesting the rapid formation of stable complexes with soil [33]. Tannic acid also influenced the recovery and composition of Bradford-reactive soil protein, associated with glomalin, produced by arbuscular mycorrhizae [35]. These observations suggested plant tannins are capable of affecting critical soil ecosystem processes such as formation of soil organic matter and rates of nutrient cycles and thus may have a role in managing nitrogen availability and retention in soil.

This report is a portion of a two-part study designed to expand the body of basic information about the effects of tannins and related non-tannin phenolics on soil organic matter and nutrient cycling. In part 1, we reported patterns of sorption of phenolic-C and showed Appalachian forest and pasture soils had a high affinity and a fixed capacity for tannins while related phenolic compounds were retained less [34]. This work summarizes the effects of repeated applications of chemically well-defined hydrolyzable and condensed tannins (polymers) and related non-tannin phenolic substances (monomers) on the solubility of soil-N. We compared surface soil from pasture to soil from the surrounding woodlands to gain insight into the magnitude of change associated with conversion from woodlands to silvopasture and assessed their potential significance on a landscape basis. Our ultimate goal is to gather and develop information needed to devise new management strategies that use the phenolic compounds added by plant residues, leachates, livestock manure or, from intentional amendments, to achieve desired agronomic or environmental goals.

2. Materials and Methods

2.1. Sample Collection and Preparation. Surface soil (0–5 cm) was collected from four farm units in Southern West Virginia, each with areas in forest (mixed deciduous or pine) and pasture use as described in greater detail by Halvorson and Gonzalez [36] and Halvorson et al. [34]. Each sample consisted of a composite of 10 soil cores (6.35 cm in diameter) collected along transects. In the lab, composite samples were sieved (2 mm), dried at 55°C, and stored until further analysis.

2.2. Soil Properties. Bulk density (BD) was determined gravimetrically from intact soil cores [36]. Soil chemical properties were determined for each composite sample (Table 1). Soil pH and electrical conductance (EC) were measured by electrode (1 : 1 soil : water). Total inorganic-N (TIN) was estimated as the sum of water extractable inorganic-N plus KCl extractable-N remaining in soil after the first extraction reported in previous work [33]. Total soil-C content was determined by dry combustion [37] with a FlashEA 1112 NC Analyzer (CE Elantech, Lakewood, NJ). The texture of the soil samples was determined by hydrometer (Midwest labs, Omaha, NE, http://www.midwestlabs.com/). Cation exchange capacity (CEC) was measured at the inherent soil pH by exchange with cobalt hexamine trichloride [38–40].

2.3. Effects of Test Compounds on Extraction of Soil-N. As part of a larger study [34], soluble-N extracted from soil was determined by difference after each of eight repeated applications of aqueous treatment solutions followed by a sequential extraction with cool (23°C) and hot (80°C) water [41, 42].

2.3.1. Test Compounds. Soil samples were treated with deionized water (Control) or with solutions containing model tannins or non-tannin phenolic compounds (organic acids and flavonoids), selected to represent a range of phenolic compounds of varying complexity present in the plant-soil continuum [43]. Our representative condensed tannin was a polymeric flavonoid-based procyanidin isolated from sorghum grain [*Sorghum bicolor* (L.) Moench] (SOR) [44]. We also evaluated tannic acid (TA), a common but imprecisely defined mixture of galloyl esters, and β-1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG), a well-defined gallotannin purified from the tannic acid. Non-tannin phenolics included the flavonoid catechin (CAT), the phenolic acid gallic acid (GA), and its ester, methyl gallate, (MG) (Figure 1, Table 2).

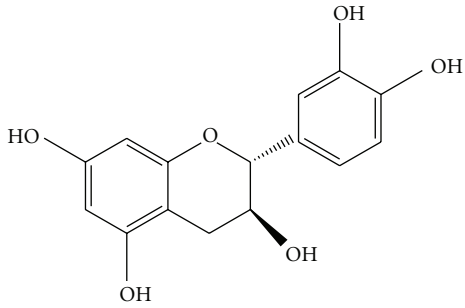
2.3.2. Procedure. Samples of soil (2.5 g) were weighed into *Oak Ridge* centrifuge tubes and treated with 25 mL of deionized water (Control) or with 25 mL of test solution to yield a final amendment of 10 mg test compound g^{-1} soil. After reciprocal shaking at 200 rpm for 1 hour at room temperature, samples were centrifuged for 3 min at 11,952 g and decanted. Supernatants were analyzed for soluble-N with a Shimadzu TOC-VCPN analyzer equipped with a TNM-1 module (Shimadzu Scientific Instruments, Columbia, MD). The treatment application step was repeated seven more times by adding an additional 25 mL of Control or treatment solution to the soil pellet, shaking, and extracting as above. After the final treatment, all samples were extracted with 25 mL of cool (23°C) water and assayed again. Finally, more water (25 mL) was added to soil samples, which were then vortexed, incubated overnight in a hot water bath (16 hrs, 80°C), and assayed for soluble-N as before.

Data were corrected to account for any nitrogen added from the treatments, or carryover from the previous treatment step. Treatment effects were determined for absolute values (mg kg^{-1} soil) but the amount of net soluble-N extracted by treatment solutions was also expressed relative to the Control and used to determine if treatments decreased (net Treatment $<$ net Control) or increased (net Treatment $>$ net Control) extraction of soluble-N

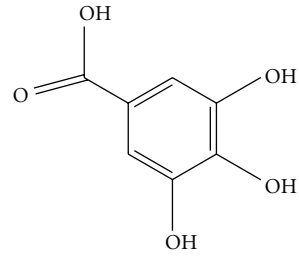
Table 1: Selected soil properties adapted from Halvorson et al. [34]. Mean (SEM), $n = 4$.

Land use	BD†	pH(1:1)	EC (μS cm soil^{-1})	TIN‡ (mg N kg soil^{-1})	Total C (mg C g soil^{-1})	Total N (mg N g soil^{-1})	CEC (cmolc kg soil^{-1})	Sand %	Silt %	Clay %
Forest	0.95 (0.03)	4.47 (0.26)	103 (13)	17.3 (3.4)	56.0 (3.5)	4.0 (0.2)	9.8 (1.9)	73.0 (3.1)	20.5 (2.2)	6.5 (1.0)
Pasture	1.14 (0.09)	5.27 (0.08)	151 (14)	26.5 (4.4)	42.9 (2.4)	4.5 (0.4)	10.1 (1.0)	69.0 (6.0)	25.0 (5.3)	6.0 (0.8)

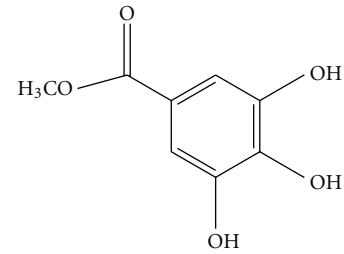
† Bulk density (BD) for the study was determined gravimetrically.
‡ Total inorganic-N (TIN) was estimated as the sum of water extractable inorganic-N plus KCl extractable-N remaining in soil [33].



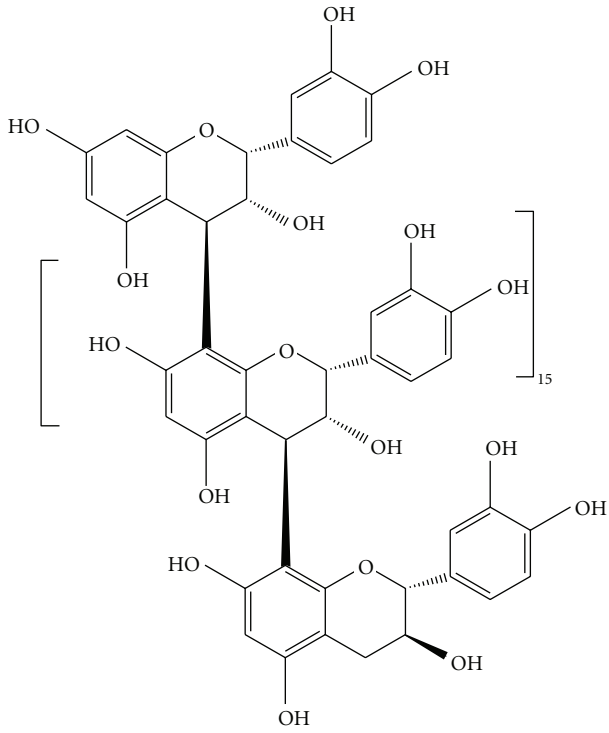
(a) (+)-Catechin



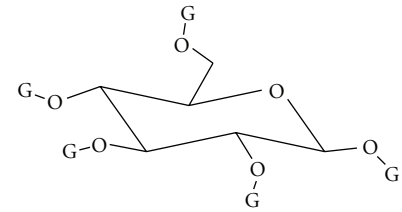
(b) Gallic acid



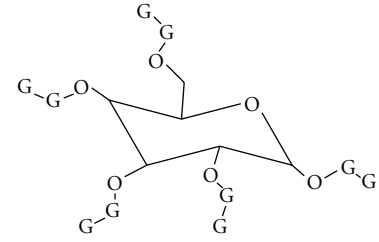
(c) Methyl gallate



(d) Sorghum procyanidin



(e) β-1, 2, 3, 4 , 6-penta- *O*-galloyl-D-glucose



(f) Tannic acid
G = gallic acid ester

Figure 1: Chemical structures for (a) (+)-catechin (CAT), (b) gallic acid (GA), (c) methyl gallate (MG), (d) sorghum procyanidin (SOR), (e) β-1,2,3,4,6-penta-*o*-galloyl-d-glucose (PGG), and (f) tannic acid (TA). The structure shown for tannic acid is a representative molecule for tannic acid, an imprecisely defined mixture of galloyl esters [44]. Figure redrawn from [34].

Table 2: Details about treatment compounds [34].

Treatment			Compound characteristics					Solution characteristics[¶]			
	Class	Source	MW[†]	C[‡] (%)	N[‡] (%)	C (g mol^{-1})	$K_{ow}^{§}$	Soluble-C (mg kg^{-1} soil)	Soluble-N (mg kg^{-1} soil)	Phenolics[#] (mmol GA equiv kg^{-1} soil)	pH (1mg/mL)
Methyl 3,4,5-trihydroxybenzoate, 98% (methyl gallate)(MG)	Phenolic organic ester	Indofine Chemical Co., Hillsborough, NJ	184	51.7	0.084	96	6.3	5141	0.1	37.2	4.4
Gallic acid, certified (GA)	Phenolic organic acid	Fisher Scientific,Pittsburgh, PA	170	47.7	0.106	85	0.3	4739	0	52.6	3.3
Tannic acid, certified (TA)	Mixture of gallotannins	Fisher Scientific, Pittsburgh, PA	902	49.4	0.142	474	ND	5042	2.5	30.7	3.5
β-1,2,3,4,6 penta-*O*-galloyl-D-glucose (PGG)	Gallotannin	Purified fromTannic Acid (Fisher)	941	49.7	0.099	492	129	5037	0.7	26.1	5.1
(+)-Catechin hydrate, >98% (CAT)	Flavonoid	Sigma, St Louis, MO	290	61.6	0	180	2.4	6067	1.0	32.1	5.6
[(4β- > 8)-epicatechin$_{15}$-(4β- > 8)-catechin] (Sorghum procyanidin) (SOR)	Polymeric flavonoid	[*Sorghum bicolor* (L.) Moench]	4624	48.6	0.094	2880	0.002	5189	8.2	13.7	6.0

[†] Number average molecular weight for TA estimated by RP-HPLC by Hagerman (Personal Communication) and used to calculate weighed average g C mol^{-1}.

[‡] Total C and N were determined in triplicate with a FlashEA 1112 NC Analyzer (CE Elantech, Lakewood, NJ).

[§] Octanol-water partition coefficients (K_{ow}) for PGG CAT and SOR from [45] GA and MG from Lu et al. [46]. Low values correspond to hydrophilic compounds while higher values are indicative of hydrophobic ones.

[¶] Supplied g^{-1} soil application^{-1}.

[#] Determined by the Modified Prussian Blue assay.

from soil. We determined percentage change in soluble-N attributable to treatments, ΔSol-N, as

$$\Delta\text{Sol-N} = 100 * \frac{(\text{Sol-N}_{\text{trt}} - \text{Sol-N}_{\text{control}})}{\text{Sol-N}_{\text{control}}}, \quad (1)$$

where $\text{Sol-N}_{\text{trt}}$ and $\text{Sol-N}_{\text{control}}$ indicate the amount of net soluble-N extracted from soil samples treated with phenolic compounds or water alone, respectively.

2.4. Statistical Analysis. Significant effects of test compounds and land use were identified by analysis of variance (ANOVA) with SAS 9.1 and PROC MIXED using a model that accounted for both fixed (land use, treatment) and random (sample location) effects [47, 48]. We used the KR (Kenward-Roger) option to calculate degrees of freedom and selected covariance structures to minimize Akaike's Information Criterion. Assumptions of data normality were evaluated and appropriate data transformations identified with SAS/ASSIST. We assumed a value of 5% as the minimum criterion for significance. Significant main effects were separated by pairwise comparisons among means, adjusted by the Tukey-Kramer method. The SLICE option in PROC MIXED was used to test significant Treatment × Use interaction. Significant deviation of ΔSol-N from zero, indicative of a meaningful change in soluble-N due to the treatment, was determined by the LSMEANS statement in PROC MIXED. Values indicated in text and graphs are the arithmetic mean, ± the standard error of the mean, expressed on air-dry soil basis.

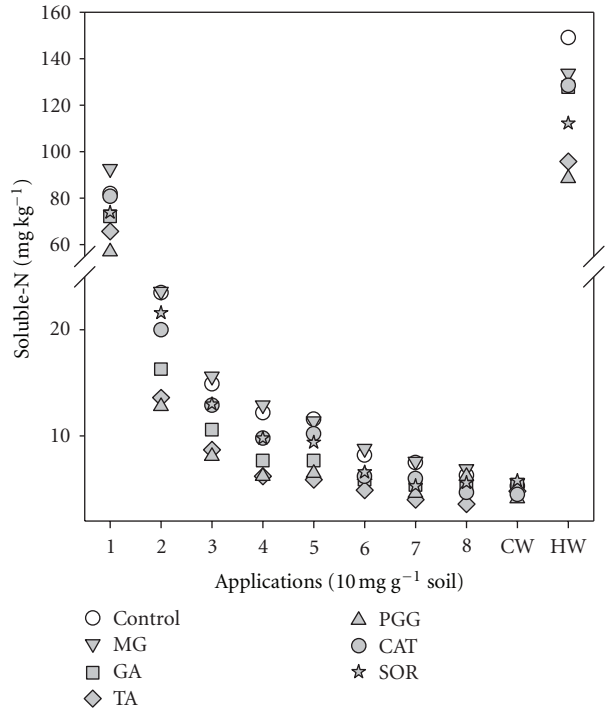


Figure 2: Soluble-N extracted from 0–5 cm soil with eight treatment cycles of water (Control) or treatment solutions (10 mg g^{-1} soil) followed by extractions with cool (CW) and hot (HW) water. Values are the mean of 4 farms × 2 uses (forest and pasture), $n = 8$. Treatment abbreviations are defined in Figure 1.

3. Results

3.1. General Patterns. Multiple applications of phenolic solutions produced overall extraction pattern for soluble-N shown in Figure 2. The first of the eight cycles had the greatest effect, extracting 50–60% of the cumulative total amount, while each of the seven subsequent applications removed incrementally less N. The rinse with cool water after the treatment cycles extracted little additional soluble-N but the final incubation in hot water released a relatively large pulse of soluble-N from all treatments and the control. The distinct patterns of ΔSol-N, established with the first treatment application, generally varied little with subsequent phenolic treatments or cool water rinse but showed some differences between forest and pasture soils (Figures 3(a) and 3(b)).

3.2. Treatment with Phenolic Compounds. The amount of soluble-N extracted by the first treatment cycle varied with simple main effects of Treatment and Use. Soluble-N was comparable for most treatments with amounts from samples treated with MG slightly greater than the Control (Table 3). In contrast, both TA and PGG reduced amounts of soluble-N. Pasture soil produced more soluble-N than forest soil. Extraction efficiency of treatments compared to the water Control, ΔSol-N, varied as a Treatment × Use interaction. Treatment with MG significantly increased the amount of soluble-N from forest soil by about 19% compared to 9% for pasture soil (Figure 4). Treatment with CAT did not affect soluble-N from pasture or forest soil. Gallic acid produced no effect on forest soil, but reduced soluble-N from pasture soil by 19%. Soluble-N was reduced from both soil types by SOR, TA, and PGG, but decreases were stronger in pasture soil. Greatest reductions resulted from the PGG treatment, which lowered soluble-N from forest and pasture by 24 and 34%, respectively.

Patterns established with the first treatment cycle persisted throughout the seven subsequent applications of phenolics and thus cumulative extractions, after all eight treatment cycles, varied with main effects of Treatment and Use (Table 3). While MG slightly increased soluble-N, amounts were significantly reduced by GA, TA, and PGG treatments. Pasture soil yielded more soluble-N than forest soil. Cumulative treatment ΔSol-N varied as a Treatment × Use interaction. Eight treatment cycles with MG extracted 13% more soluble-N from forest soil than water alone but had little effect on pasture soil. By contrast, treatment with CAT reduced soluble-N by about 9% for both soils. Soluble-N was also reduced by the other phenolic compounds but treatment effects were discernibly stronger for pasture soil. Greatest reductions, with PGG, resulted in 28 and 40% less soluble-N, than the water control, from forest and pasture, respectively.

3.3. Cool and Hot Water Extractions. Only small amounts, 5.1 ± 0.2 mg kg^{-1}, of soluble-N, were extracted by cool water, after the final treatment cycle, limiting the interpretive value

Table 3: Soluble-N (mg kg^{-1} soil) extracted with treatment solutions[†].

	Single treatment		Cumulative after treatment 8			Cool water			Hot water			Final total		
Trt	Forest	Pasture	Avg. ($n = 8$)	Forest	Pasture	Avg. ($n = 8$)	Forest	Pasture	Avg. ($n = 8$)	Forest	Pasture	Avg. ($n = 8$)	Forest	Pasture	Avg. ($n = 8$)
H_2O	70 (1)	94 (10)	82 (7)AB	147 (4)	186 (19)	166 (11)AB	4.9 (0.3)	5.8 (0.8)	5.3 (0.4)AB	107 (4)	191 (27)	149 (20)A	259 (6)	383 (45)	321 (31)A
MG	83 (3)	102 (10)	93 (6)A	166 (6)	193 (19)	179 (11)A	5.5 (0.5)	5.7 (0.5)	5.6 (0.3)A	98 (5)	170 (26)	134 (18)AB	269 (10)	368 (44)	319 (28)A
CAT	70 (1)	91 (8)	81 (6)AB	137 (3)	165 (14)	151 (9)BC	4.2 (0.4)	4.8 (1.0)	4.5 (0.5)BC	91 (3)	166 (24)	129 (18)AB	231 (6)	336 (37)	284 (26)A
SOR	65 (1)	82 (8)	74 (5)BC	136 (3)	154 (13)	145 (7)BC	6.2 (0.3)	5.4 (0.5)	5.8 (0.3)A	95 94)	129 (8)	112 (8)BC	237 (6)	289 (20)	263 (14)A
GA	69 (1)	75 (7)	72 (3)BC	131 (3)	131 (12)	131 (6)CD	5.5 (0.4)	5.0 (0.4)	5.3 (0.3)AB	107 (3)	148 (19)	128 (12)AB	244 (5)	284 (31)	264 (16)A
TA	61 (1)	70 (5)	66 (3)C	110 (2)	116 (9)	113 (5)DE	5.0 (0.7)	4.5 (0.7)	4.8 (0.5)ABC	86 (3)	106 (9)	96 (6)CD	201 (4)	226 (17)	213 (10)B
PGG	53 (2)	61 (6)	57 (3)D	105 (3)	110 (9)	108 (4)E	4.2 (0.2)	4.0 (0.5)	4.1 (0.2)C	78 (4)	99 (8)	89 (6)D	188 (4)	213 (17)	200 (9)B
Avg. ($n = 28$)	67 (2)Y	82 (4)X		133 (4)Y	151 (8)X		5.1 (0.2)	5.0 (0.2)		95 (2)Y	144 (9)X		233 (6)Y	300 (16)X	

[†] Treatment solutions consisted of a water control or supplied 10 mg of methyl gallate (MG), gallic acid (GA), catechin (CAT) condensed tannin from sorghum (SOR), tannic acid (TA), or β-1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG) per g soil. Data are arithmetic average (standard error) ($n = 4$). Main effects of land use (column averages, $n = 28$) or treatments (row averages, $n = 8$) are denoted by capital letters (Tukey's HSD, $P < 0.05$).

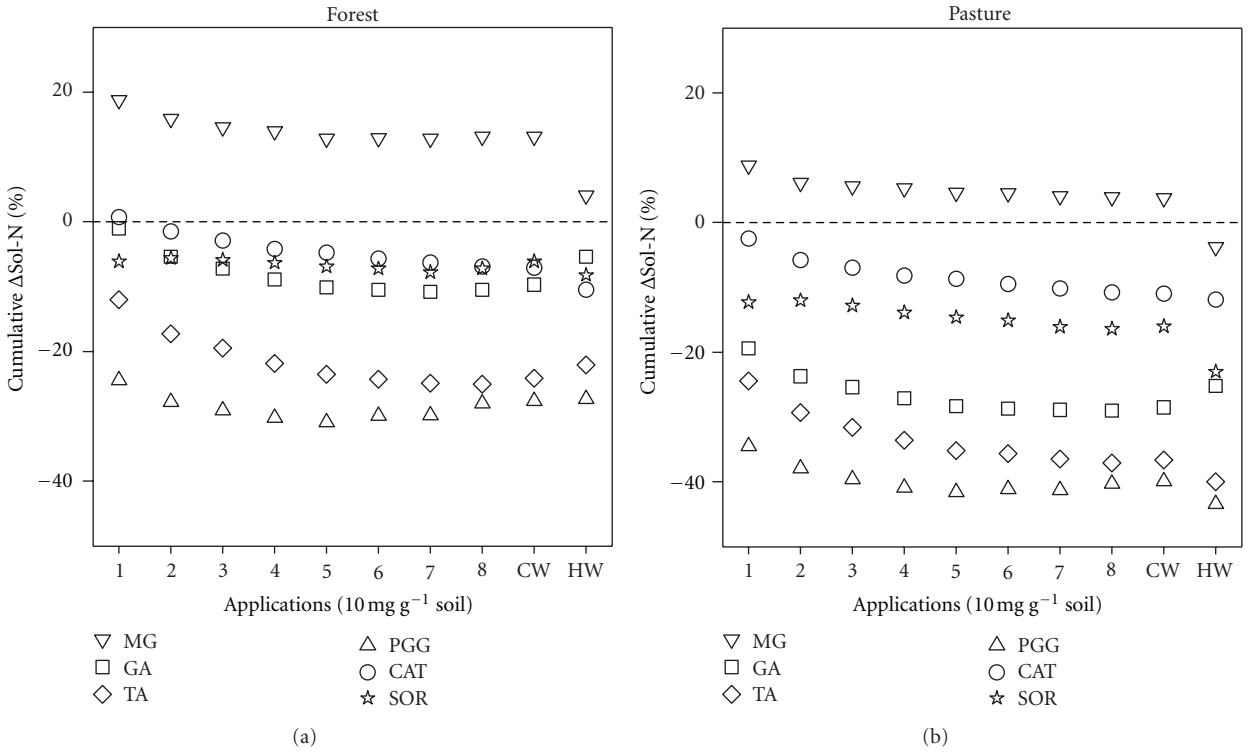


Figure 3: Percent change in soluble-N extractions attributable to treatments, ΔSol-N for (a) forest and (b) pasture soils calculated with (1) ($n = 4$). Treatment abbreviations are defined in Figure 1.

of intertreatment ΔSol-N comparisons (Table 3). Variation among treatment effects was less than 2 mg kg^{-1} soil but samples treated with PGG yielded less cool water soluble-N than others.

In contrast, hot water extracted large amounts of soluble-N that varied with simple main effects of Treatment and Use (Table 3). The greatest amount of soluble-N, extracted from the Control treatment, was comparable to the amount extracted by the preceding eight treatment cycles (Table 3). Significantly less soluble-N was extracted with hot water from TA- and PGG-treated samples than other treatments. Pasture produced about 50% more soluble-N than forest soil.

Hot water ΔSol-N varied as an interaction between Treatment and Use (Figure 5). Gallic acid had no effect on hot water extractions from forest soil, compared to the Control, but reduced soluble-N from pasture samples by 22%. Previous treatments with MG and CAT reduced soluble-N similarly from forest and pasture soils, by 10 and 14%, respectively. Reductions in soluble-N, observed for samples treated with SOR, TA, or PGG, were greater in pasture than forest soil. Hot water soluble-N was reduced from samples previously treated with PGG, by 27% for forest soil and by 47% for pasture.

3.4. Cumulative Extraction of Soluble-N. Final cumulative soluble-N extracted by eight treatment cycles and subsequent cool and hot rinses differed by Treatment and Use (Table 3). Treatment effects segregated into two groups with less total soluble-N extracted from soils treated with TA or PGG. Pasture soil yielded an average of 300 mg kg^{-1} soluble-N or about 29% more than forest soil.

Final cumulative ΔSol-N varied as an interaction between Treatment and Use (Figure 6). In both forest and pasture soils, ΔSol-N was highest from samples treated with MG and lowest from samples treated with TA or PGG. The repeated treatments with MG did not significantly influence cumulative extraction of soluble-N from forest or pasture soil. Forest soil was also unaffected by GA but soluble-N was reduced from pasture soil by 25% (nearly 100 mg kg^{-1}). Treatments with CAT reduced soluble-N similarly from both land uses by an average of 11% (about 37 mg kg^{-1}). Tannins produced the greatest reductions of soluble-N with significantly stronger effects on pasture soil. The condensed tannin, SOR, reduced cumulative extractions of soluble-N from forest and pasture soils by 8 and 23%, respectively, (21 and 93 mg kg^{-1}). Tannic acid, reduced soluble-N from forest soil by 22% (58 mg kg^{-1}) and by 40% (156 mg kg^{-1}) from pasture soil. The hydrolyzable tannin, PGG, produced greatest reductions, 27 and 43% from forest and pasture soils, respectively, (71 and 176 mg kg^{-1}).

4. Discussion

When expressed on a landscape basis, the size of the pool of soil N affected by tannins and other phenolic compounds appears significant. Water alone (Control) extracted the equivalent of 33 and 54 kg of soluble-N ha^{-1} from 0–5 cm of forest and pasture soils with the first treatment cycle

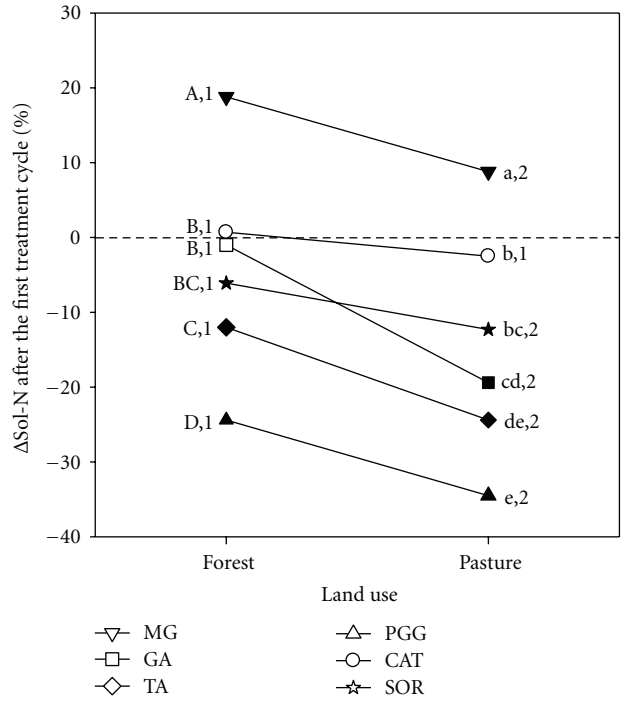


Figure 4: Treatment × Use interactions for ΔSol-N after the first application of treatment solutions (10 mg g^{-1} soil). Within each land use, letters denote differences among treatments. Differences between uses are denoted by numbers. Filled symbols denote significant deviations from the control. Treatment abbreviations are defined in Figure 1.

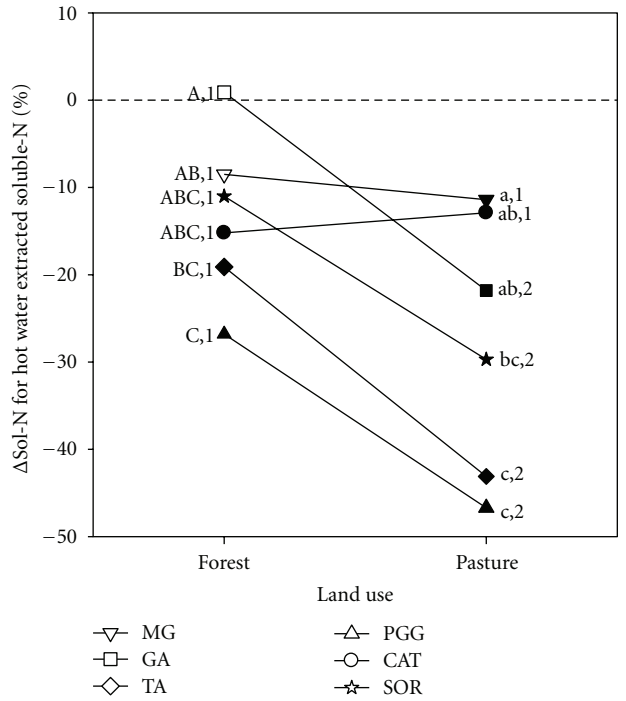


Figure 5: Treatment × Use interactions for ΔSol-N calculated for hot water extractions (not cumulative). Within each land use, letters denote differences among treatments. Differences between uses are denoted by numbers. Filled symbols denote significant deviations from the control. Treatment abbreviations are defined in Figure 1.

(Tables 1 and 3). Compared against these baseline values, the first treatment with MG increased losses of soluble-N from forest and pasture soils by 5-6 kg N ha^{-1} while PGG conserved 8 and 19 kg N ha^{-1} in forest and pasture samples, respectively. These reductions of soluble-N were strongly correlated with the amount of phenolic treatment-C sorbed by the soil [34] (Figure 7).

Incremental extractions of nitrogen after eight successive treatment applications were small suggesting a majority of the most labile pool of soil-N had been removed (Figure 2). The difference between Control extractions, equivalent to 70 and 106 kg ha^{-1} from forest and pasture soils, and treatment extractions infer the magnitude of the soil-N pool most responsive to the phenolic inputs. These suggest the eight treatments with MG mobilized an additional 19 and 7 mg N kg^{-1} forest or pasture soil, compared to water, or 9 and 4 kg N ha^{-1}. Conversely, PGG reduced the solubility of labile soil-N by 41 and 76 mg kg^{-1} in forest and pasture soils, respectively, equivalent to retention of 21 and 43 kg N ha^{-1}.

Negligible extractions of soluble-N with cool water, exhibiting only small differences among treatments, were unexpected (Table 3). We had, instead, anticipated relatively large releases of soluble-N from samples that had previously retained N once the treatments were omitted.

In contrast to cool water, hot water released large amounts of soluble-N, accounting for between 40 and 50% of the cumulative extraction, suggesting it originated from a

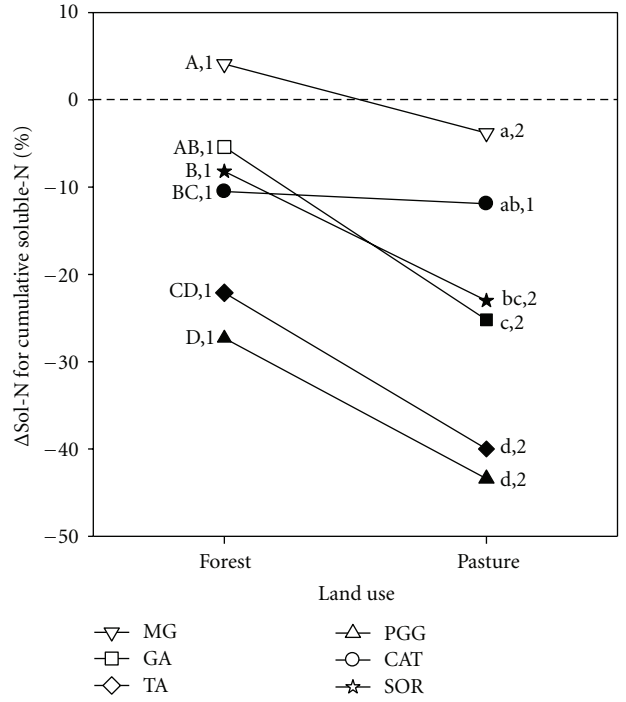


Figure 6: Treatment × Use interactions for ΔSol-N calculated for cumulative extractions. Within each land use, letters denote differences among treatments. Differences between uses are denoted by numbers. Filled symbols denote significant deviations from the control. Treatment abbreviations are defined in Figure 1.

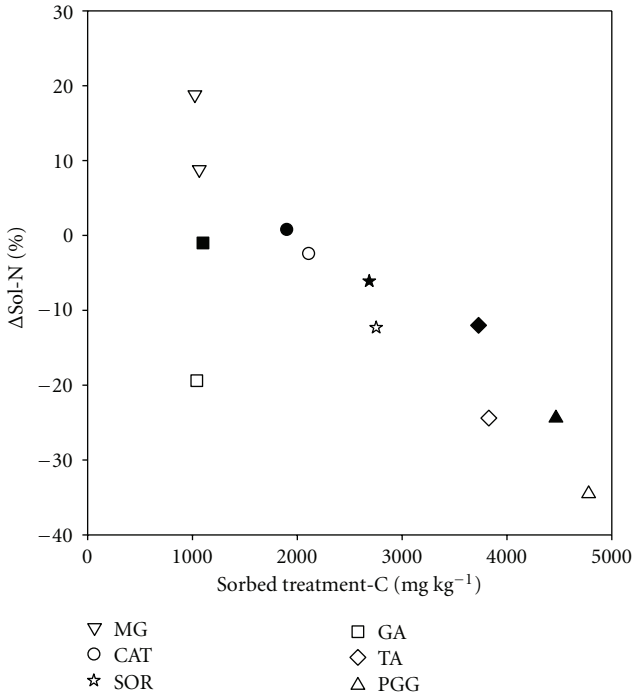


Figure 7: Relationship between ΔSol-N and sorbed treatment-C after the first application of treatments. Data from forest and pasture soils are denoted by filled and open symbols, respectively. Values for treatment-C are from [34]. Treatment abbreviations are defined in Figure 1.

different pool of soil organic matter. The hot water-soluble pool from forest soil, 51 kg N ha^{-1}, was less than half that from pasture soil, 110 kg N ha^{-1}. Hot water-extractable soil-N is thought to be primarily composed of unspecified forms of organic-N, particularly amino-N and amides, associated with soil microbial biomass and organic matter, with the remainder consisting of NH_4-N, generated by hydrolysis of heat-labile organic N [41, 42, 49]. In a previous experiment, Halvorson et al. [33] found organic-N comprised more than 80% of soluble-N extracted by hot water after a single treatment with GA, TA or PGG.

The patterns of treatment effects observed for hot water soluble-N, along with those observed for the preceding extractions, suggest phenolic compounds, like MG, may simply affect the efficacy of the extraction process while others including the tannins and CAT somehow increase the ability for organic-N to resist hydrolysis or physically restrain it in the soil matrix. While eight treatments with MG increased soluble-N in supernatants by an average 7 kg N ha^{-1} (Table 3), hot water soluble-N from samples treated with MG was reduced by 8 kg N ha^{-1} (Figure 5). Thus, final cumulative extractions from MG-treated forest and pasture soils, though distinct from each other (129 compared to 211 kg N ha^{-1}), were not appreciably different from cumulative extractions with water (Figure 6). In contrast, the PGG treatment reduced soluble-N in treatment supernatants, the cool water rinse, and the final hot water incubation with reductions of hot water soluble-N equivalent to 14 and 53 kg N ha^{-1} from forest and pasture (Figures 4 and 5, Table 3). All together, PGG reduced soluble-N from the forest soil by 71 mg kg^{-1} soil or 35 kg ha^{-1} and from pasture soil by 170 mg kg^{-1} soil or 97 kg ha^{-1}.

Reductions in N availability by condensed tannins are attributed to formation of protein-tannin complexes with soil protein that are more recalcitrant than those formed with hydrolyzable tannins in part because they are less available to microorganisms as substrate [10, 50–52]. However, the strong reductions in solubility of labile soil-N, observed for TA and PGG, suggest hydrolyzable tannins may play a more prominent role in abiotic immobilization of organic soil-N than previously thought. A study by Hagerman et al. [45] concluded PGG, a nonpolar tannin (Table 2), precipitated with bovine serum albumin (BSA) by forming a hydrophobic coat around the protein while the more polar, SOR, formed hydrogen-bond cross-links between BSA molecules.

In addition to precipitation, polymerization resulting from condensation reactions between phenolic and amino-containing compounds in solution can occur. Oxidation of phenolics, forming semiquinones and quinones, can be carried out biotically by the polyphenol oxidase enzymes and/or abiotically by redox reactions with manganese and iron oxides [53, 54]. When PGG is oxidized, it forms covalently linked complexes with protein [55].

Additions of some tannins/phenolics to the soil resulted in the dissolution of Mn oxides, evinced by increased Mn content in the supernatant [56]. Mobilization of other soil metals such as Fe, Al and Si by tannins has also been observed [29, 57]. During the dissolution of Mn oxides, the Mn may be reduced from insoluble Mn(III and IV) to soluble Mn(II), which is available for plants. Phenolic compounds could be oxidized, forming quinones and semiquinones, the latter a highly reactive radical that readily self-polymerizes or copolymerizes with other compounds [58]. Tannic acid, gallic acid, and other polyhydroxy phenols with OH–OH in the orthopositions are known to be highly effective in the dissolution of Mn in soils with high Mn oxides content [59] or in synthetic Mn oxide [60]. Thus, the lower soluble-N extracted with PGG, GA, or TA solutions compared to other compounds might be due in part to redox reactions with soil Mn together with oxidation of these organic compounds into quinones or semiquinones and formation of "humic-like" polymers with amino-containing compounds that were retained in the soil matrix.

The significant interactions between treatment and land use, observed for ΔSol-N, indicated the effects of phenolic plant inputs were of greater consequence in land managed as pasture than the surrounding woodlands (Figures 4–6). Variations in soil microbial community composition, related to land use, have been suggested to explain variations in the mineralization of tannin-protein complexes [13]; however this study and our related work [33] also suggest land use can affect the initial reactions between tannins and soil.

The effects of tannins on soil organic matter and nutrient cycling have important implications for livestock production in mixed systems such as silvopastures that include a mixture of forages together with browse and overstory tree species [61–64]. Appalachian silvopasture soil typically differs from

the surrounding unmanaged woodland because it is limed to increase soil pH, receives additional N-inputs from fertilizers and manure, and can develop greater bulk density due to compaction by livestock (Table 1). In addition, transitioning from either forest or pasture to a silvopastoral mixed stand, containing both forages and overstory, may affect soil nitrogen pools by redistributing the patterns of nutrients in soils and biomass. Decreases in soil C and N have been associated with afforestation, especially in the case of pines [65]. Two years after conversion from a mixed hardwood woodland, Staley et al. [66] reported losses of organic-C and -N from West Virginia silvopasture soil of 17 and 9%, respectively, which they attributed to litter decomposition.

Along with their effects on soil organic matter and the availability of nitrogen, tannins and related phenolic compounds can interact with important metals in soil, such as Ca, Mn, Al, and Fe, probably through chelation and oxidation/reduction reactions [67, 68]. Interactions between metals and phenolic compounds may inhibit or promote plant growth in forest soil. For example, tannic acid has been reported to reduce the rate of root growth by itself but has also been shown to mitigate the toxic effects of Al on roots [69] and in soil [10, 26]. In addition tannins affect nutrient value of forages (e.g., [70, 71]) and animal health [72, 73]. Thus, tannins can link plant productivity, ruminant physiology, pathogen survival, and environmental quality in agroecosystems.

The results of this study indicate hydrolyzable tannins like PGG can quickly reduce the solubility of labile soil-N more than condensed tannins like SOR and suggest tannin effects will vary with land management. However information about short-term reactions that incorporate tannin-C onto the soil matrix and immobilize soil-N must be considered together with their potential for chemical and biological degradation [33]. Further work is required to determine the persistence of tannin effects as they are degraded by soil microorganisms or other soil biota [74], physically lost by leaching, or chemically oxidized after interacting with soil metals [67, 68].

Tannins and other phenolic compounds affect a number of important biological, chemical, and physical processes in plants animals and soil. Studies such as this improve our understanding of the effects of natural phenolic inputs on soil organic matter and nutrient cycling and will ultimately lead to new management strategies. Future research on tannins and other plant polyphenolics in soil ecosystems should be focused towards understanding their effects on plant productivity and soil function. Functional definitions linking specific tannin chemistry to soil processes are required that can also serve as a rationale for comparing tannins. Research remains hampered by a lack of standardized methods that simplify the extraction, identification, and quantification of tannins from plants and soil and that can be adapted to field measurements. Experimental field work will remain difficult until suitable model tannins can be identified that are available in reasonable quantities and expense.

Because they are chemically and biologically active, tannins appear to have the potential to be used to improve nitrogen retention by soils but additional work is needed to determine how long their effects can last and whether the retained N is readily available to crop or forage plants. The effects of tannins on nutrient cycling will likely be influenced by specific tannin chemistry [13, 75, 76], vary with tannin concentrations, and the quantity and quality of soil N [77].

Disclaimer

USDA is an equal opportunity provider and employer. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

Acknowledgments

The authors thank J. Harrah and T. Robertson for excellent analytical assistance. This work is dedicated to P. J. A. Halvorson.

References

[1] S. Hättenschwiler and P. M. Vitousek, "The role of polyphenols in terrestrial ecosystem nutrient cycling," *Trends in Ecology and Evolution*, vol. 15, no. 6, pp. 238–242, 2000.

[2] T. E. C. Kraus, R. A. Dahlgren, and R. J. Zasoski, "Tannins in nutrient dynamics of forest ecosystems—a review," *Plant and Soil*, vol. 256, no. 1, pp. 41–66, 2003.

[3] J. D. Horner, J. R. Gosz, and R. G. Cates, "The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems," *American Naturalist*, vol. 132, no. 6, pp. 869–883, 1988.

[4] M. von Lützow, I. Kögel-Knabner, K. Ekschmitt et al., "Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions—a review," *European Journal of Soil Science*, vol. 57, no. 4, pp. 426–445, 2006.

[5] C. B. Coulson, R. I. Davies, and D. A. Lewis, "Polyphenols in plant, humus and soil. 1. Polyphenols of leaves, litter and superficial humus from mull and mor sites," *Journal of Soil Science*, vol. 11, no. 1, pp. 20–29, 1960.

[6] W. R. C. Handley, "Further evidence for the importance of residual leaf protein complexes in litter decomposition and the supply of nitrogen for plant growth," *Plant and Soil*, vol. 15, no. 1, pp. 37–73, 1961.

[7] J. Lewis and R. Starkey, "Vegetable tannins, their decomposition and effects of decomposition of some organic compounds," *Soil Science*, vol. 106, pp. 241–247, 1968.

[8] J. Basaraba, "Influence of vegetable tannins on nitrification in soil," *Plant and Soil*, vol. 21, no. 1, pp. 8–16, 1964.

[9] J. Basaraba and R. L. Starkey, "Effect of plant tannins on decomposition of organic substances," *Soil Science*, vol. 101, no. 1, pp. 17–23, 1966.

[10] P. Kraal, K. G. J. Nierop, J. Kaal, and A. Tietema, "Carbon respiration and nitrogen dynamics in Corsican pine litter amended with aluminium and tannins," *Soil Biology and Biochemistry*, vol. 41, no. 11, pp. 2318–2327, 2009.

[11] G. D. Joanisse, R. L. Bradley, C. M. Preston, and G. D. Bending, "Sequestration of soil nitrogen as tannin-protein complexes may improve the competitive ability of sheep laurel (*Kalmia angustifolia*) relative to black spruce (*Picea mariana*)," *New Phytologist*, vol. 181, no. 1, pp. 187–198, 2009.

[12] T. E. C. Kraus, R. J. Zasoski, R. A. Dahlgren, W. R. Horwath, and C. M. Preston, "Carbon and nitrogen dynamics in a forest soil amended with purified tannins from different plant species," *Soil Biology and Biochemistry*, vol. 36, no. 2, pp. 309–321, 2004.

[13] R. Mutabaruka, K. Hairiah, and G. Cadisch, "Microbial degradation of hydrolysable and condensed tannin polyphenol-protein complexes in soils from different land-use histories," *Soil Biology and Biochemistry*, vol. 39, no. 7, pp. 1479–1492, 2007.

[14] K. Sivapalan, V. Fernando, and M. W. Thenabadu, "Humified phenol-rich plant residues and soil urease activity," *Plant and Soil*, vol. 70, no. 1, pp. 143–146, 1983.

[15] K. Sivapalan, V. Fernando, and M. W. Thenabadu, "N-mineralization in polyphenol-rich plant residues and their effect on nitrification of applied ammonium sulphate," *Soil Biology and Biochemistry*, vol. 17, no. 4, pp. 547–551, 1985.

[16] A. Scalbert, "Antimicrobial properties of tannins," *Phytochemistry*, vol. 30, no. 12, pp. 3875–3883, 1991.

[17] F. A. Einhellig, "Mode of allelochemical action of phenolic compounds," in *Allelopathy: Chemistry and Mode of Action*, F. A. Macías et al., Ed., pp. 217–238, CRC Press, Boca Raton, Fla, USA, 2004.

[18] T. K. Bhat, B. Singh, and O. P. Sharma, "Microbial degradation of tannins—a current perspective," *Biodegradation*, vol. 9, no. 5, pp. 343–357, 1998.

[19] J. P. Schimel, R. G. Cates, and R. Ruess, "The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga," *Biogeochemistry*, vol. 42, no. 1-2, pp. 221–234, 1998.

[20] R. E. Benoit and R. L. Starkey, "Enzyme inactivation as a factor in the inhibition of decomposition of organic matter by tannins," *Soil Science*, vol. 105, no. 4, pp. 203–208, 1968.

[21] G. D. Joanisse, R. L. Bradley, C. M. Preston, and A. D. Munson, "Soil enzyme inhibition by condensed litter tannins may drive ecosystem structure and processes: the case of *Kalmia angustifolia*," *New Phytologist*, vol. 175, no. 3, pp. 535–546, 2007.

[22] M. C. Rillig, B. A. Caldwell, H. A. B. Wösten, and P. Sollins, "Role of proteins in soil carbon and nitrogen storage: controls on persistence," *Biogeochemistry*, vol. 85, no. 1, pp. 25–44, 2007.

[23] B. Adamczyk, S. Adamczyk, A. Smolander, and V. Kitunen, "Tannic acid and Norway spruce condensed tannins can precipitate various organic nitrogen compounds," *Soil Biology and Biochemistry*, vol. 43, no. 3, pp. 628–637, 2011.

[24] E. Castells, "Indirect effects of phenolics on plant performance by altering nitrogen cycling: another mechanism of plant–plant negative interactions," in *Allelopathy in Sustainable Agriculture and Forestry*, R. S. Zeng, A. U. Mallik, and S. M. Luo, Eds., pp. 137–156, Springer, New York., NY, USA, 2008.

[25] N. Wurzburger and R. L. Hendrick, "Plant litter chemistry and mycorrhizal roots promote a nitrogen feedback in a temperate forest," *Journal of Ecology*, vol. 97, no. 3, pp. 528–536, 2009.

[26] R. R. Northup, R. A. Dahlgren, and J. G. McColl, "Polyphenols as regulators of plant-litter-soil interactions in northern California's pygmy forest: a positive feedback?" *Biogeochemistry*, vol. 42, no. 1-2, pp. 189–220, 1998.

[27] G. D. Bending and D. J. Read, "Effects of the soluble polyphenol tannic acid on the activities of ericoid and ectomycorrhizal fungi," *Soil Biology and Biochemistry*, vol. 28, no. 12, pp. 1595–1602, 1996.

[28] G. D. Bending and D. J. Read, "Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi," *Soil Biology and Biochemistry*, vol. 28, no. 12, pp. 1603–1612, 1996.

[29] J. Kaal, K. G. J. Nierop, and J. M. Verstraten, "Retention of tannic acid and condensed tannin by Fe-oxide-coated quartz sand," *Journal of Colloid and Interface Science*, vol. 287, no. 1, pp. 72–79, 2005.

[30] S. El Azhar, R. Verhe, M. Proot, P. Sandra, and W. Verstraete, "Binding of nitrite-N on polyphenols during nitrification," *Plant and Soil*, vol. 94, no. 3, pp. 369–382, 1986.

[31] S. El Azhar, J. Vandenabeele, and W. Verstraete, "Nitrification and organic nitrogen formation in soils," *Plant and Soil*, vol. 94, no. 3, pp. 383–399, 1986.

[32] R. D. Fitzhugh, G. M. Lovett, and R. T. Venterea, "Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA," *Global Change Biology*, vol. 9, no. 11, pp. 1591–1601, 2003.

[33] J. J. Halvorson, J. M. Gonzalez, A. E. Hagerman, and J. L. Smith, "Sorption of tannin and related phenolic compounds and effects on soluble-N in soil," *Soil Biology and Biochemistry*, vol. 41, no. 9, pp. 2002–2010, 2009.

[34] J. J. Halvorson, J. M. Gonzalez, and A. E. Hagerman, "Repeated applications of tannins and related phenolic compounds are retained by soil and affect cation exchange capacity," *Soil Biology and Biochemistry*, vol. 43, no. 6, pp. 1139–1147, 2011.

[35] J. J. Halvorson and J. M. Gonzalez, "Tannic acid reduces recovery of water-soluble carbon and nitrogen from soil and affects the composition of Bradford-reactive soil protein," *Soil Biology and Biochemistry*, vol. 40, no. 1, pp. 186–197, 2008.

[36] J. J. Halvorson and J. M. Gonzalez, "Bradford reactive soil protein in Appalachian soils: distribution and response to incubation, extraction reagent and tannins," *Plant and Soil*, vol. 286, no. 1-2, pp. 339–356, 2006.

[37] D. W. Nelson and L. E. Sommers, "Total carbon, organic carbon and organic matter," in *Methods of Soil Analysis Part 3: Chemical Methods*, D. L. Sparks et al., Ed., Soil Science Society of America Books Series No. 5, pp. 961–1010, Soil Science Society of America, Inc., Madison, Wis, USA, 1996.

[38] H. Ciesielski and T. Sterckeman, "Determination of cation exchange capacity and exchangeable cations in soils by means of cobalt hexamine trichloride. Effects of experimental conditions," *Agronomie*, vol. 17, no. 1, pp. 1–7, 1997.

[39] H. Ciesielski and T. Sterckeman, "A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils," *Agronomie*, vol. 17, no. 1, pp. 9–16, 1997.

[40] ISO 23470:2007, "Soil quality—determination of effective cation exchange capacity (CEC) and exchangeable cations using a hexamminecobalt trichloride solution," ISO/TC 190, Soil quality Subcommitte SC3, Chemical methods and soil characteristics, 2007.

[41] A. Ghani, M. Dexter, and K. W. Perrott, "Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation," *Soil Biology and Biochemistry*, vol. 35, no. 9, pp. 1231–1243, 2003.

[42] D. Curtin, C. E. Wright, M. H. Beare, and F. M. McCallum, "Hot water-extractable nitrogen as an indicator of soil nitrogen availability," *Soil Science Society of America Journal*, vol. 70, no. 5, pp. 1512–1521, 2006.

[43] C. Gallet and P. Lebreton, "Evolution of phenolic patterns in plants and associated litters and humus of a mountain forest ecosystem," *Soil Biology and Biochemistry*, vol. 27, no. 2, pp. 157–165, 1995.

[44] A. E. Hagerman, *The Tannin Handbook*, Miami University, Oxford, Ohio, USA, 2002.

[45] A. E. Hagerman, M. E. Rice, and N. T. Ritchard, "Mechanisms of Protein Precipitation for Two Tannins, Pentagalloyl Glucose and Epicatechin16 (4 → 8) Catechin (Procyanidin)," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 7, pp. 2590–2595, 1998.

[46] Z. Lu, G. Nie, P. S. Belton, H. Tang, and B. Zhao, "Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives," *Neurochemistry International*, vol. 48, no. 4, pp. 263–274, 2006.

[47] R. C. Littell, G. A. Milliken, W. W. Stroup, and R. D. Wolfinger, *SAS System for Mixed Models*, SAS Institute Inc., Cary, NC, USA, 1996.

[48] SAS, "SAS OnlineDoc, Version 8," SAS Institute, Inc., Cary, NC, USA, 1999.

[49] P. Leinweber, H. R. Schulten, and M. Korschens, "Hot water extracted organic matter: chemical composition and temporal variations in a long-term field experiment," *Biology and Fertility of Soils*, vol. 20, no. 1, pp. 17–23, 1995.

[50] R. L. Bradley, B. D. Titus, and C. P. Preston, "Changes to mineral N cycling and microbial communities in black spruce humus after additions of $(NH_4)_2SO_4$ and condensed tannins extracted from *Kalmia angustifolia* and balsam fir," *Soil Biology and Biochemistry*, vol. 32, no. 8-9, pp. 1227–1240, 2000.

[51] K. G. J. Nierop and J. M. Verstraten, "Fate of tannins in Corsican pine litter," *Journal of Chemical Ecology*, vol. 32, no. 12, pp. 2709–2719, 2006.

[52] B. Adamczyk, V. Kitunen, and A. Smolander, "Polyphenol oxidase, tannase and proteolytic activity in relation to tannin concentration in the soil organic horizon under silver birch and Norway spruce," *Soil Biology and Biochemistry*, vol. 41, no. 10, pp. 2085–2093, 2009.

[53] F. J. Stevenson, *Humus Chemistry: Genesis, Composition, Reactions*, John Wiley & Sons, 2nd edition, 1994.

[54] P. J. Hernes, R. Benner, G. L. Cowie, M. A. Goi, B. A. Bergamaschi, and J. I. Hedges, "Tannin diagenesis in mangrove leaves from a tropical estuary: a novel molecular approach," *Geochimica et Cosmochimica Acta*, vol. 65, no. 18, pp. 3109–3122, 2001.

[55] Y. Chen and A. E. Hagerman, "Reaction pH and protein affect the oxidation products of β-pentagalloyl glucose," *Free Radical Research*, vol. 39, no. 2, pp. 117–124, 2005.

[56] J. J. Halvorson, J. M. Gonzalez, and A. E. Hagerman, "Effects of tannins on soil carbon, cation exchange capacity, and metal solubility," in *Proceedings of the 94th Ecological Society of America Annual Meeting*, Albuquerque, NM, USA, August 2009.

[57] J. Kaal, K. G. J. Nierop, and J. M. Verstraten, "Interactions between tannins and goethite- or ferrihydrite-coated quartz sand: influence of pH and evaporation," *Geoderma*, vol. 139, no. 3-4, pp. 379–387, 2007.

[58] R. Liu, "Comment on "surface complexation of catechol to metal oxides: an ATR-FTIR, adsorption, and dissolution study"," *Environmental Science and Technology*, vol. 44, no. 16, pp. 6517–6518, 2010.

[59] N. V. Hue, S. Vega, and J. A. Silva, "Manganese toxicity in a Hawaiian Oxisol affected by soil pH and organic amendments," *Soil Science Society of America Journal*, vol. 65, no. 1, pp. 153–160, 2001.

[60] A. T. Stone and J. J. Morgan, "Reduction and dissolution of manganese(III) and manganese(IV) oxides by organics: 2. Survey of the reactivity of organics," *Environmental Science and Technology*, vol. 18, no. 8, pp. 617–624, 1984.

[61] R. P. Udawatta and L. D. Godsey, "Agroforestry comes of age: putting science into practice," *Agroforestry Systems*, vol. 79, no. 1, pp. 1–4, 2010.

[62] C. M. Feldhake, J. P. S. Neel, and D. P. Belesky, "Establishment and production from thinned mature deciduous-forest silvopastures in Appalachia," *Agroforestry Systems*, vol. 79, no. 1, pp. 31–37, 2010.

[63] J. P. S. Neel, C. M. Feldhake, and D. P. Belesky, "Influence of solar radiation on the productivity and nutritive value of herbage of cool-season species of an understorey sward in a mature conifer woodland," *Grass and Forage Science*, vol. 63, no. 1, pp. 38–47, 2008.

[64] V. D. Nair, S. G. Haile, G. A. Michel, and P. K. R. Nair, "Environmental quality improvement of agricultural lands through silvopasture in Southeastern United States," *Scientia Agricola*, vol. 64, no. 5, pp. 513–519, 2007.

[65] S. T. Berthrong, E. G. Jobbágy, and R. B. Jackson, "A global meta-analysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation," *Ecological Applications*, vol. 19, no. 8, pp. 2228–2241, 2009.

[66] T. E. Staley, J. M. Gonzalez, and J. P. S. Neel, "Conversion of deciduous forest to silvopasture produces soil properties indicative of rapid transition to improved pasture," *Agroforestry Systems*, vol. 74, no. 3, pp. 267–277, 2008.

[67] E. H. Majcher, J. Chorover, J. M. Bollag, and P. M. Huang, "Evolution of CO_2 during birnessite-induced oxidation of ^{14}C-labeled catechol," *Soil Science Society of America Journal*, vol. 64, no. 1, pp. 157–163, 2000.

[68] H. M. Appel, "Phenolics in ecological interactions: the importance of oxidation," *Journal of Chemical Ecology*, vol. 19, no. 7, pp. 1521–1552, 1993.

[69] T. B. Kinraide and A. E. Hagermann, "Interactive intoxicating and ameliorating effects of tannic acid, aluminum (Al^{3+}), copper (Cu^{2+}), and selenate (SeO_4^{2-}) in wheat roots: a descriptive and mathematical assessment," *Physiologia plantarum*, vol. 139, no. 1, pp. 68–79, 2010.

[70] T. N. Barry and W. C. McNabb, "The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants," *British Journal of Nutrition*, vol. 81, no. 4, pp. 263–272, 1999.

[71] B. R. Min, T. N. Barry, G. T. Attwood, and W. C. McNabb, "The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review," *Animal Feed Science and Technology*, vol. 106, no. 1–4, pp. 3–19, 2003.

[72] H. Hoste, F. Jackson, S. Athanasiadou, S. M. Thamsborg, and S. O. Hoskin, "The effects of tannin-rich plants on parasitic nematodes in ruminants," *Trends in Parasitology*, vol. 22, no. 6, pp. 253–261, 2006.

[73] I. Mueller-Harvey, "Unravelling the conundrum of tannins in animal nutrition and health," *Journal of the Science of Food and Agriculture*, vol. 86, no. 13, pp. 2010–2037, 2006.

[74] M. Coulis, S. Hättenschwiler, S. Rapior, and S. Coq, "The fate of condensed tannins during litter consumption by soil animals," *Soil Biology and Biochemistry*, vol. 41, no. 12, pp. 2573–2578, 2009.

[75] T. E. C. Kraus, Z. Yu, C. M. Preston, R. A. Dahlgren, and R. J. Zasoski, "Linking chemical reactivity and protein precipitation to structural characteristics of foliar tannins," *Journal of Chemical Ecology*, vol. 29, no. 3, pp. 703–730, 2003.

[76] S. Coq, J. M. Souquet, E. Meudec, V. Cheynier, and S. Hättenschwiler, "Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana," *Ecology*, vol. 91, no. 7, pp. 2080–2091, 2010.

[77] J. M. Talbot and A. C. Finzi, "Differential effects of sugar maple, red oak, and hemlock tannins on carbon and nitrogen cycling in temperate forest soils," *Oecologia*, vol. 155, no. 3, pp. 583–592, 2008.