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SOIL MOISTURE AND TEMPERATURE EFFECTS ON NITROGEN AVAILABILITY FROM ORGANIC NITROGEN SOURCES

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SOIL MOISTURE AND TEMPERATURE EFFECTS ON NITROGEN AVAILABILITY FROM ORGANIC NITROGEN SOURCES

By

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ABSTRACT

SOIL MOISTURE AND TEMPERATURE EFFECTS ON NITROGEN AVAILABILITY FROM ORGANIC NITROGEN SOURCES

By

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Soil incubation and growth chamber studies were conducted to evaluate the effects of soil moisture (50, 70, and 90% of water holding capacity) and temperature (15/10, 20/15, and 25/20°C day/night) on nitrogen (N) availability from four organic N sources. In the soil incubation study, differential N release kinetics of the N sources was determined by measuring ammonium- and nitrate-N contents periodically over 12 weeks. Net N released, as a percentage of organic N, was greatest in the order: urea (91-96%) > blood meal (BM) (56-61%) > alfalfa pellets (AP) (41-52%) > partially composted chicken manure (CM) (37-45%). Increasing soil moisture increased net N released from AP and CM by 12 and 21%, respectively, but did not affect net N released from urea and BM. Increasing temperature increased net N released from AP, BM, and CM by 25, 10, and 13%, respectively, but did not affect net N released from urea. In the growth chamber study, kale (*Brassica oleracea* L.) was grown as an indicator of N availability. Regardless of soil moisture and temperature, N use efficiency by kale was greatest in the order: urea > BM > AP > CM. Soil moisture influenced N availability differently in the two studies, whereas temperature influenced N availability similarly in the two studies. Our results indicate that soil incubation data will be useful for evaluating variations in N availability among N sources and temperatures on a field scale. Increasing temperature improves N availability from natural organic materials, thereby contributing to better crop production.

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KEY TO SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of variance
ANUE	Apparent nitrogen use efficiency
AP	Alfalfa pellets
BM	Blood meal
CEC	Cation exchange capacity
CM	Partially composted chicken manure
DAT	Days after transplanting
OM	Organic matter
meq	Milliequivalent
R^2	Coefficient of determination
SAS	Statistical Analysis System
SE	Standard error
TKN	Total Kjeldahl-nitrogen
WHC	Water holding capacity

CHAPTER 1

SOIL MOISTURE AND TEMPERATURE EFFECTS ON NITROGEN RELEASE FROM ORGANIC NITROGEN SOURCES

INTRODUCTION

Nitrogen (N) release pattern and N supplying capacity of applied N sources must be known for efficient management of N inputs. Because N release process of organic N sources involves a biological decomposition, the N availability is controlled by chemical composition (Fox et al., 1990; Ajwa et al., 1998; Rowell et al., 2001; Kumar and Goh, 2003) and soil environment (Sims, 1986; Vigil and Kissel, 1995; Seneviratne et al., 1998; Whalen et al., 2001; Cookson et al., 2002). Thus, it is difficult to predict the pattern and amount of available N released from organic N sources during the growing season. Sufficient N is necessary for optimum crop production, whereas excessive fertilization will increase the risk of nitrate leaching. From a management standpoint, it is important to understand how chemical composition and soil environment influence availability of N from organic N sources.

Availability of N from organic N sources varies considerably. For example, urea [$CO(NH_2)_2$], a synthetic organic N fertilizer, is rapidly hydrolyzed to ammonia (NH_4^+) by the enzyme urease after applied to soil. MacLean and McRae (1987) found rapid hydrolysis rates of over 90% within 5 days at temperatures between 9 and 18°C in an acid podzolic soil. Tomar and Soper (1981) reported that the rate of urea hydrolysis after 4 weeks of incubation averaged 83% in 11 different soils. Unlike urea, natural organic materials, such as animal manures and plant residues, are mineralized slowly to NH_4^+. Chae and Tabatabai (1986) reported that net N mineralization rate of cow, hog, and chicken manure averaged 35, 39, and 53%, respectively, in five different soils over 26 weeks of incubation. Li and Mahler (1995) reported that when ground alfalfa, spring pea, and winter wheat were incorporated with soil at the rate of 2% (w/w), their net

mineralization rates were 31, 23, and 16%, respectively, after 20 weeks of incubation. Ciavatta et al. (1997) found a relatively high mineralization rate for blood meal of about 75% after 120 days incubation. The variation in N availability among different types of animal manures and plant residues has been attributed to the chemical composition, such as N content (Fox et al., 1990; Constantinides and Fownes, 1994; Aulakh et al., 2000), C/N ratio (Aulakh et al., 2000; Trinsoutrot et al., 2000; Rowell et al., 2001), lignin/N ratio (Melillo et al., 1982; Constantinides and Fownes, 1994; Kumar and Goh, 2003), and polyphenol content (Fox et al., 1990; Palm and Sanchez, 1991; Constantinides and Fownes, 1994).

Soil moisture and temperature are the major environmental factors affecting N availability from organic N sources. Because urea is readily soluble in water, urea hydrolysis is largely dependent on diffusion of dissolved urea in soil (Sadeghi, 1989). Urease activity is generally highest near field capacity and declines as soil moisture decreases (Vlek and Carter, 1983; Saharawat, 1984). Urea hydrolysis is also accelerated with increasing temperature as urea diffusion rate in soil is positively correlated with temperature (Pang et al., 1977; Sadeghi et al., 1988). Urease activity increases in relation to temperature with maximum urea hydrolysis occurring between 60 and 70°C (Overrein and Moe, 1967; Saharawat, 1984; Moyo et al., 1989; Lai and Tabatabai, 1992). MacLean and MacRae (1987) studied the rate of urea hydrolysis in soil incubated at different temperatures, and reported that 52, 67, 80, and 93% of urea was hydrolyzed at 4, 9, 13, and 18°C, respectively, after three days of incubation.

Mineralization of natural organic materials is mediated by heterotrophic bacteria and fungi, and their microbial activity is also influenced by soil moisture and temperature.

Soil moisture regulates oxygen diffusion in soil with maximum aerobic microbial activity occurring between 50 and 70% of water holding capacity (WHC) (Linn and Doran, 1984; Franzluebbers, 1999). On the other hand, low soil moisture inhibits microbial activity by reducing diffusion of soluble substrates (Griffin, 1981; Schjønning et al., 2003), microbial mobility (Killham et al., 1993), and intracellular water potential (Csonka, 1989; Stark and Firestone, 1995). Doel et al. (1990) conducted a 198-day soil incubation study with white lupin at -0.30, -0.03, and -0.01 MPa. Although immobilization was not overcome at -0.30 MPa throughout incubation, net mineralization occurred at -0.03, and -0.01 MPa after 168 and 187 days of incubation, respectively. De Neve and Hofman (2002) incubated fresh carrot leaves in soil at different soil moistures ranging from 18 to 60% WHC for 98 days. Net mineralized N increased with increasing soil moisture from 18 to 45% WHC, and was constant with further increases to 60% WHC. Similarly, increases in temperature enhance mineralization by stimulating microbial activity and accelerating diffusion of soluble substrates in soil (Nicolardot et al., 1994; MacDonald et al., 1995; Zak et al., 1999). Increases in temperature also induce a shift in the composition of microbial communities (Richards et al., 1985; Carreiro and Koske, 1992; Zogg et al., 1997), which parallels an increase in microbial activity (Zogg et al., 1997). Griffin and Honeycutt (2000) incubated soil amended with dairy, poultry, and swine manures for 112 days, and found that increasing temperature from 10 to 24°C significantly accelerated the mineralization rate. Cookson et al. (2002) used clover residues in a soil incubation study, and reported that 22, 33, 41, and 60% of N was mineralized at 2, 5, 10, and 15°C, respectively after 160 days of incubation.

Although many studies have evaluated the relationships between N availability

and chemical composition, soil moisture, or temperature for various organic N sources, few have been focused on an interaction between source of N and soil moisture or temperature regarding N availability. Soil moisture and temperature may influence availability of N from organic N sources differently depending on chemical composition. Such information will be valuable to making decisions for the most efficient use of organic N sources especially in greenhouse production systems, where irrigation and temperature control can be easily managed.

Four organic N sources in this study, including urea, alfalfa pellets, blood meal, and partially composted chicken manure, were chosen because of wide variation in their chemical compositions. The objectives of this study were to: (1) examine the effects of soil moisture and temperature on N availability from the organic N sources, and (2) evaluate the interaction between the sources of N and soil moisture or temperature regarding N availability.

MATERIALS AND METHODS

Soil

The soil used in this study was a Granby sandy clay loam (sandy, mixed, mesic Typic Haplaquolls). Approximately 20 kg of surface soil (15 cm) was collected from the Michigan State University Horticulture Teaching and Research Center in East Lansing MI, in May 2002 and August 2003. The soil was passed through a 5 mm sieve, thoroughly mixed to ensure uniformity, and stored in a covered plastic container under field moisture condition (10% w/w) at room temperature (20-23°C) until the incubation was initiated to minimize disturbance of the microbial population (Pramer and Bartha, 1972; Honeycutt, 1999).

The chemical and physical properties of the soil are listed in Table 1.1. Soil samples for analysis were, unless otherwise noted, immediately dried at 38°C for 48 hr, ground, and passed through a 2 mm sieve. Soil pH was measured with a combination reference glass pH electrode using a soil:water ratio of 1:1 (w/v) (Watson and Brown, 1998). Total Kjeldahl-N (TKN) content was determined by the micro-Kjeldahl digestion procedure (Bremmer and Mulvany, 1982) followed by colorimetric determination using a Lachat rapid flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). Ammonium (NH_4^+) and nitrate (NO_3^-) N were determined by the ammonia-salicylate method and cadmium reduction method, respectively, using a Lachat rapid flow injection autoanalyzer following extraction with 1N KCl (Keeney and Nelson, 1982). No attempt was made to measure nitrite (NO_2^-) N separately in the extracts, but NO_2^--N was measured with the NO_3^--N. Since TKN does not account for NO_3^--N, total N was calculated as the sum of TKN and NO_3^--N. Available P was determined colorimetrically

Table 1.1. Chemical and physical properties of soils used in this study.

Property	Soil (2002)†	Soil (2003)‡
pH	5.7	5.8
Sand (%)	54.7	54.7
Silt (%)	17.4	15.4
Clay (%)	27.8	29.8
CEC (cmol kg^{-1})	52.2	51.3
OM content (%)	3.6	3.5
Total C (g kg^{-1})	20.9	20.0
Total N (g kg^{-1})	4.4	4.1
NH_4^+-N (mg kg^{-1})	5.4	5.1
NO_3^--N (mg kg^{-1})	22.0	17.8
P (mg kg^{-1})	206	175
K (mg kg^{-1})	133	198
Ca (mg kg^{-1})	546	627
Mg (mg kg^{-1})	176	99

† Soil (2002) was used in the soil moisture and temperature studies performed in 2002.

‡ Soil (2003) was used in the temperature study performed in 2003.

by the Bray and Kurtz P-1 method (Frank et al., 1992). Exchangeable K, Ca, and Mg were extracted with 1N NH_4OAc and measured by an atomic absorption spectrometer (Warncke and Brown, 1998). Cation exchange capacity (CEC) was estimated by summing the milliequivalent (meq) exchangeable acidity, which was obtained from SMP buffer pH measurement (Watson and Brown, 1998), and the meq exchangeable bases (K, Ca, and Mg) (Warncke and Brown, 1998). Soil gravimetric water content was determined by oven drying samples at 105°C for 24 hr. Water holding capacity (WHC) was calculated from the difference in the weights between the moist soil allowed to drain for 48 hr after fully saturating with water and the oven dry soil. The maximum gravimetric

water content was described as 100% WHC. Soil samples dried at 105°C for 48 hr and ground to pass through a 0.15 mm sieve were used for carbon (C) analysis. Total C content was determined by dry combustion using a Leco carbon analyzer (Leco Corp., St. Joseph, MI). Organic matter (OM) content was estimated based on weight loss on ignition after determination of C (Combs and Nathan, 1998). The unground soil samples dried at 38°C for 48 hr were used for particle size analysis. Soil particle size distribution was estimated by the hydrometer method (Gee and Bauder, 1986). All soil analyses were performed in duplicate or triplicate.

Nitrogen sources

Urea [$CO(NH_2)_2$] was used as a synthetic organic N fertilizer (Figure 1.1). Three natural organic materials were used (Figure 1.1). Alfalfa pellets (AP), which were obtained from Bradfield Industries, Inc. (Springfield, MI), are alfalfa-based fertilizers blended with animal protein, natural sulfate of potash, and molasses. Blood meal (BM) was obtained from Glorious Gardens Blood Meal Growing Markets, Inc. (West Des Moines, IA). Partially composted chicken manure (CM) was obtained from Herbruck's Poultry Ranch, Inc. (Saranac, MI).

Chemical properties of these N sources are listed in Table 1.2. Urea was ground to be in powder form, and AP and CM were ground and passed through a 2 mm sieve prior to use. Since BM was originally powdered, it was used without grinding. The pH, total C, total N, NH_4^+-N, and NO_3^--N were determined by the same procedures used for soil. Total P, K, Ca, and Mg contents were measured by a direct current plasma atomic emission spectrophotometer following dry ashing at 500°C and digestion with 3 N HNO_3

containing 1000 ppm of LiCl. All analyses were performed in duplicate. Dry matter weight was determined by oven drying samples at 105°C for 24 hr.

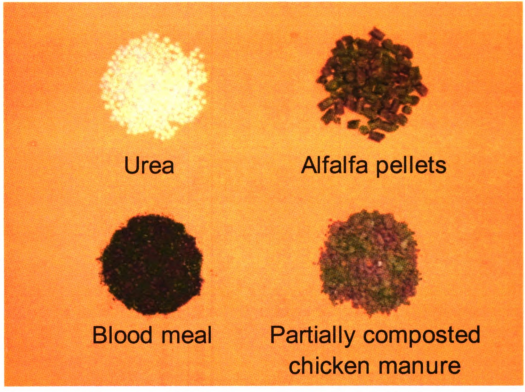

Figure 1.1. Four organic N sources used in this study.

Table 1.2. Chemical characteristics of four organic N sources used in this study.[†]

N source	Moisture	pH	Total C	Total N	C/N ratio	NH_4^+	NO_3^-	P	K	Ca	Mg	Fe
	%		----%	----		--g N kg^{-1} --		----------%				
Urea	--	--	--	46.00	--	--	--	--	--	--	--	--
AP	4.46	5.73	39.3	3.60	10.93	0.49	0.15	0.58	4.55	1.69	0.16	0.06
BM	5.01	7.30	41.3	12.65	3.26	0.15	0.02	0.51	0.64	1.12	0.11	0.11
CM	7.92	8.69	26.6	3.80	6.99	1.02	0.15	2.71	3.30	14.42	0.80	0.07

[†] All values are expressed on a dry basis (105°C).

Experimental design

The N release kinetics of the organic N sources was determined at different soil moistures and temperatures. The experimental design for the soil moisture study was a completely randomized design with three replications. Treatments consisted of a factorial combination of three soil moisture levels (50, 70, and 90% WHC), five N sources (control, urea, AP, BM, and CM), and six incubation times (0, 1, 2, 4, 8, and 12 weeks).

The experimental design for the temperature study was a split plot design in two randomized blocks with three subsamples. Treatments consisted of a factorial combination of three temperature levels [15/10, 20/15, and 25/20°C day/night (14/10 hr)], five N sources (control, urea, AP, BM, and CM), and six incubation times (0, 1, 2, 4, 8, and 12 weeks). Temperature was designated as a main plot, and N source and incubation time were designated as subplots.

The soil moisture study was conducted during June to August 2002 using the soil collected in May 2002. Due to limited availability of growth chambers, the temperature study was conducted twice to be replicated. The study was repeated during August to October 2003 with the soil collected in August 2003.

Soil incubation

Twenty grams dry weight equivalent of moist soil was placed in 125 ml Erlenmeyer flasks. Urea, AP, BM, and CM were mixed with the soil at the rate of 63, 100, 92, and 150 mg N kg^{-1} soil (oven dry basis), respectively. The application rates were calculated to provide approximately equal amounts of 60 mg available N kg^{-1} soil (134 kg available N ha^{-1}) using Equation [1].

$$\text{Estimated available N} = N_i + fN_o \quad [1]$$

where N_i is the inorganic N (NH_4^+-N + NO_3^--N) content, N_o is the organic N (total N – inorganic N) content, and f is the proportion of organic N fraction expected to be released during incubation (Griffin and Honeycutt, 2000). Coefficient f of 0.95, 0.59, 0.65, and 0.39 was applied for urea, AP, BM, and CM, respectively, based on a previous 2-week soil incubation experiment (data not shown).

In the soil moisture study, the samples were treated with distilled water to provide 50, 70, and 90% WHC, and were randomly placed in a growth chamber set at 20/15°C day/night (14/10 hr) (Figure 1.2). In the temperature study, the samples were treated with distilled water to provide 70% WHC, and were randomly placed in the growth chambers set at 15/10, 20/15, and 25/20°C day/night (14/10 hr).

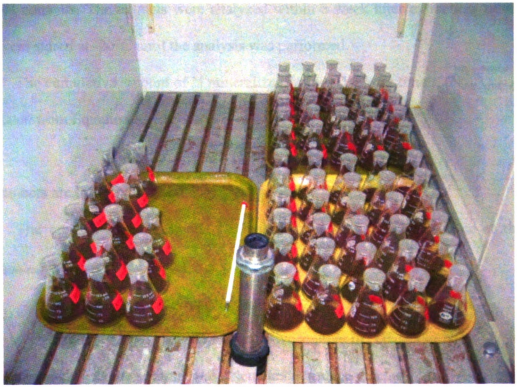

Figure 1.2. Soil samples incubated in a growth chamber.

Incubation was carried out in dark condition. The flasks were covered with parafilm (Figure 1.2), which allows air exchange and retards moisture loss from the samples. Soil moisture was adjusted every week by weighing the samples and adding the required amount of distilled water. Soil samples with no N source were incubated as a control to estimate the soil N mineralization and subtract from the treatments to be able to estimate the rate of N release from the organic N sources.

Inorganic N analysis and calculations for N release

Initial NH_4^+-N and NO_3^--N contents were determined in samples extracted with 50 ml of 1N KCl added directly to the flask immediately after incorporation of each N source. After 1, 2, 4, 8, and 12 weeks of incubation, samples were removed from the growth chambers and extracted with 50 ml of 1N KCl for determination of NH_4^+-N and NO_3^--N contents. The extracts were analyzed within a week after extraction, otherwise they were stored at -20°C until the analysis was performed.

The cumulative amount of N mineralized from soil OM at time *t*, $(N_{min})_{control}$, was calculated from Equation [2].

$$(N_{min})_{control} = N_i\ (control)_t - N_i\ (control)_{t=0} \qquad [2]$$

All numbers are in mg N kg^{-1} soil.

The cumulative amount of N released from an applied N source at time *t*, $(N_{rel})_{N\ source}$, was calculated from Equation [3].

$$(N_{rel})_{N\ source} = N_i\ (N\text{-treated soil})_t - N_i\ (control)_t - N_i\ (N\ source)_{t=0} \qquad [3]$$

All numbers are in mg N kg^{-1} soil. The cumulative amount of NH_4^+-N or NO_3^--N released from an applied N source at time *t* was calculated in a same manner.

The percentage of N that was released from organic fraction of applied N at time *t*, ($\%\ N_{rel})_{N\ source}$, was calculated from Equation [4].

$$(\%N_{rel})_{N\ source} = [(N_{rel})_{N\ source} / N_o\ (N\ source)] \times 100 \quad [4]$$

Statistical analysis and N release models

A three-way analysis of variance (ANOVA) was conducted to test significant differences in treatment effects and interactions using the MIXED procedure of Statistical Analysis System (SAS) (SAS Institute, 1990). When statistically significant differences existed, treatment means were separated using LSMEANS procedure, and then tested using paired *t* test at $\alpha = 0.05$.

Soil N mineralization was fit to a zero-order model using the linear regression procedure REG (SAS Institute, 1990) as follows:

$$N_{min} = kt \quad [5]$$

where N_{min} (mg N kg^{-1}) is the cumulative N mineralized from soil OM at time *t*, and *k* (mg N kg^{-1} $week^{-1}$) is the zero-order rate constant. Significant differences among slopes, *k*, at different soil moistures or temperatures were tested with orthogonal contrasts.

To describe the N release kinetics of the organic N sources, the NLIN (SAS Institute, 1990), a nonlinear curve-fitting procedure, was used to fit a first-order model (Stanford and Smith, 1972) to the cumulative N release as follows:

$$N_{rel} = N_0(1 - \exp^{-k_0 t}) \quad [6]$$

where N_{rel} (mg N kg^{-1}) is the cumulative N released from an applied N source at time *t*, N_0 (mg N kg^{-1}) is the size of potentially mineralizable N, exp is the exponential constant with numerical value $\cong$ 2.718, and k_0 ($week^{-1}$) is the first-order rate constant. The

parameters among N release models were deemed significantly different ($\alpha = 0.05$) if the 95% confidence intervals around the estimated values did not overlap.

All equations were fit using all data points, although only mean values are shown in the figures below.

RESULTS AND DISCUSSION

Chemical composition of N sources

Chemical composition varied among N sources (Table 1.2). Urea [$CO(NH_2)_2$], a synthetic organic N fertilizer, contains 46% N. Among the natural organic materials used in this study, BM contained the highest total N content (12.65%), whereas AP (3.60%) and CM (3.80%) had similar total N contents. The C/N ratio of BM was relatively narrow (3.26) compared to that of AP (10.93) and CM (6.99).

The inorganic N contents in AP, BM, and CM were 0.64, 0.17, and 1.17 g kg^{-1}, accounting for 1.78, 0.13, and 3.07% of their total N contents, respectively. The proportions of NH_4^+-N to inorganic N were 76, 87, and 88% for AP, BM, and CM, respectively, suggesting that initial inorganic N existed mainly as NH_4^+.

Patterns of N release

The cumulative amount of N released was significantly greater in the N-treated soils than in the control throughout incubation, suggesting that net immobilization did not occur or was completed before the first measurement was made. Three different patterns of N release were shown in both soil moisture and temperature studies. First pattern, in the control a slow linear N release occurred (Figure 1.3). Second pattern, in the urea treatment a rapid N release occurred with most of the urea (> 75%) hydrolyzed in the first week (Figure 1.4 and 1.5). Third pattern, the AP, BM, and CM treatments showed a rapid N release in the first 2 weeks followed by a slow N release (Figure 1.4 and 1.5). These N release patterns will be described and discussed here before discussing the effects of soil moisture and temperature.

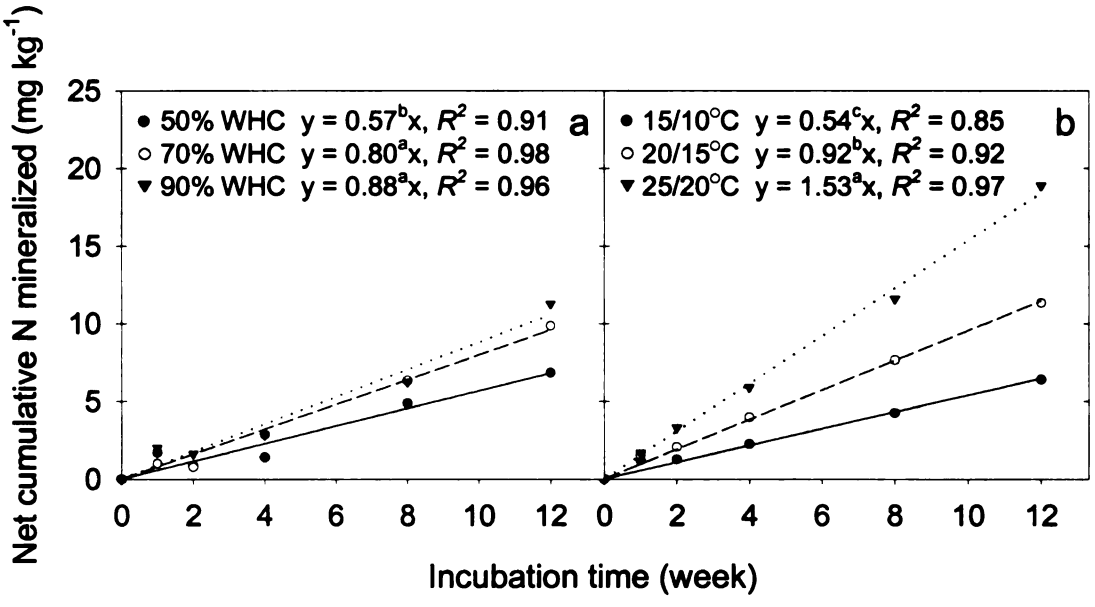

Figure 1.3. Net cumulative N mineralized from soil OM at different soil moistures (a) or temperatures (b). Slopes in regression lines followed by the same letter are not significantly different at $\alpha = 0.05$.

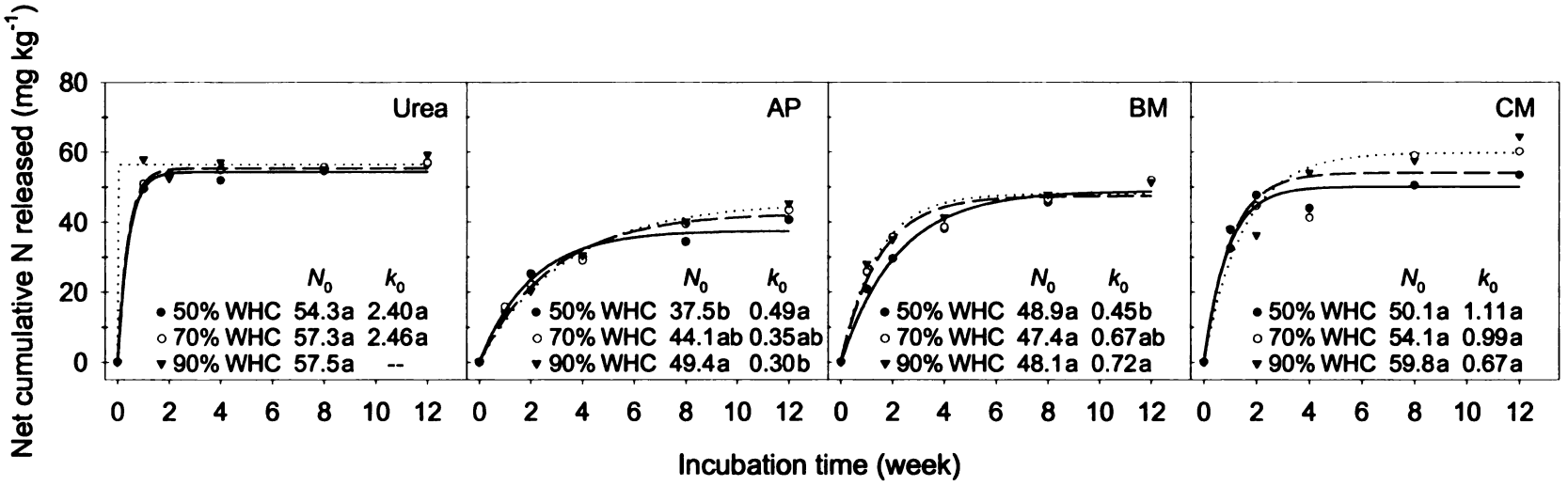

Figure 1.4. Net cumulative N released from four organic N sources at different soil moistures. The N_0 or k_0 values followed by the same letter are not significantly different at $\alpha = 0.05$.

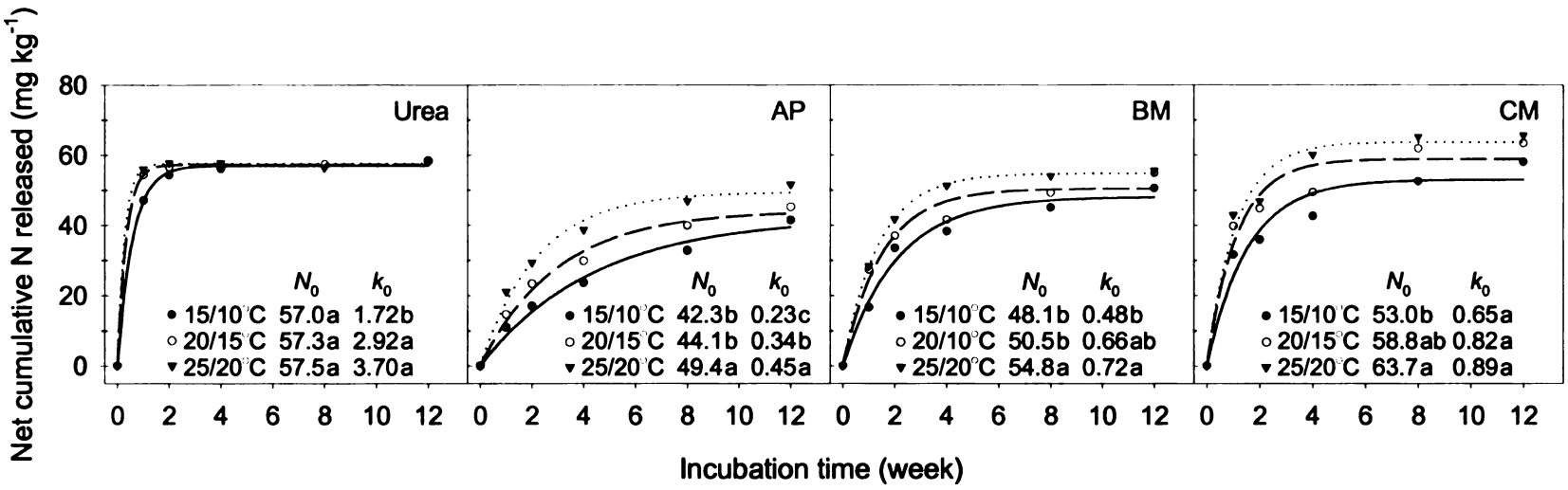

Figure 1.5. Net cumulative N released from four organic N sources at different temperatures. The N_0 or k_0 values followed by the same letter are not significantly different at $\alpha = 0.05$.

In the control, cumulative mineralized N was linearly correlated with time; the R^2 values were greater than 0.85 at all soil moisture and temperature levels (Figure 1.3). Soil N mineralization proceeded slowly throughout incubation, and only a small portion of soil organic N (< 0.5%) was mineralized at the end of incubation.

Urea hydrolysis proceeded very rapidly with over 75% of urea hydrolyzed in the first week (Table 1.4 and 1.5). The rate of hydrolysis in weeks 2-12 was very slow. Over 90% of urea was hydrolyzed at the end of incubation (Table 1.4 and 1.5). This N release pattern demonstrated the typical urea hydrolysis reported in previous studies (Tomar and Soper, 1981; Vlek and Carter, 1983; MacLean and MacRae, 1987).

The N release patterns of AP, BM, and CM showed two distinct phases during incubation; a rapid phase in the first 2 weeks followed by a slow phase in weeks 2-12 (Figure 1.4 and 1.5). This indicates that the organic N fraction in the natural organic materials is composed mainly of unstable forms that are readily mineralizable and stable forms that are more resistant to mineralization. The mineralization rate in the rapid and slow phases varied among N sources. Although mineralization occurred most rapidly in the first week, mineralization rate was slower with AP than with BM and CM (Figure 1.4 and 1.5). During the slow mineralization phase, AP and BM showed a steady N release until the end of incubation, whereas CM showed a relatively slow N release with a plateaued phase in weeks 8-12. Net N released from AP, BM, and CM averaged 43.7, 57.3, and 40.7%, respectively, in the soil moisture study (Table 1.3), and 46.8, 58.9, and 42.7%, respectively, in the temperature study (Table 1.4).

The differences in N release patterns among AP, BM, and CM could be explained by their chemical composition. Firstly, C/N ratio has been identified as a good indicator

Table 1.3. Net cumulative N released, as a percentage of organic N, from four organic N sources incubated in soil at different soil moistures.[†]

N source	Soil moisture	Incubation time (week)				
		1	2	4	8	12
	% WHC	---------- % ----------				
Urea	50	79.6 b	86.3 a	83.7 b	87.8 a	91.2 a
	70	82.0 b	85.7 a	88.4 a	89.7 a	91.7 a
	90	93.4 a	84.6 a	92.1 a	88.8 a	95.5 a
	Mean	85.0	85.5	88.0	88.8	92.8
AP	50	15.3 e	25.5 d	30.4 e	34.8 d	41.1 d
	70	15.9 e	22.4 de	29.5 e	40.0 c	44.0 cd
	90	14.0 e	20.6 e	30.9 e	40.7 c	45.9 c
	Mean	15.1	22.8	30.3	38.5	43.7
BM	50	23.1 d	32.7 c	40.2 d	48.5 b	57.4 b
	70	28.5 c	39.5 b	40.7 cd	49.5 b	57.4 b
	90	31.0 c	38.8 b	43.9 c	51.1 b	56.9 b
	Mean	27.5	37.0	41.6	49.7	57.3
CM	50	22.3 d	32.8 c	30.7 de	35.3 cd	36.7 d
	70	26.0 cd	30.7 cd	28.8 e	41.2 cd	41.3 cd
	90	26.1 cd	25.0 d	37.8 d	40.2 cd	44.3 c
	Mean	24.8	29.5	32.4	38.9	40.7

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

Table 1.4. Net cumulative N released, as a percentage of organic N, from four organic N sources incubated in soil at different temperatures.[†]

N source	Temperature	Incubation time (week)				
		1	2	4	8	12
	°C	%				
Urea	15/10	75.1 b	86.6 b	89.5 a	90.9 a	93.2 a
	20/15	86.6 a	90.1 ab	91.0 a	91.5 a	92.6 a
	25/20	89.5 a	92.1 a	92.1 a	90.0 a	92.9 a
	Mean	83.7	89.6	90.9	90.8	92.9
AP	15/10	11.1 g	17.3 i	24.1 g	33.3 h	42.1 de
	20/15	14.8 fg	23.7 h	30.4 f	40.5 fg	45.9 d
	25/20	21.5 e	29.8 f	39.3 de	47.6 de	52.4 c
	Mean	15.8	23.6	31.2	40.5	46.8
BM	15/10	18.2 ef	36.7 de	41.9 cd	49.4 cd	55.5 c
	20/15	29.9 c	40.6 d	45.6 c	53.9 c	60.2 b
	25/20	31.2 c	45.9 c	56.2 b	59.2 b	61.0 b
	Mean	26.4	41.1	47.9	54.2	58.9
CM	15/10	21.7 de	24.6 gh	29.3 f	36.0 gh	39.7 e
	20/15	27.3 cd	30.7 f	33.9 ef	42.4 ef	43.4 d
	25/20	29.5 c	32.2 ef	41.2 cd	44.7 def	45.0 d
	Mean	26.2	29.2	34.8	41.0	42.7

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

of N availability among various organic N sources (Mtambanengwe and Kirchmann, 1995; Aulakh et al., 2000; Trinsoutrot et al., 2000; Rowell et al., 2001). The higher N supplying capacity of BM was indicated by its narrow C/N ratio (3.26) compared to AP (10.93) and CM (6.99) (Table 1.2). Secondly, it is suggested that N in lignin fraction is highly resistant to mineralization as lignin/N ratio is negatively correlated with mineralization rate (Melillo et al., 1982; Constantinides and Fownes, 1994; Kumar and Goh, 2003). The lignin content of AP is presumably higher than that of CM, whereas BM does not contain lignin. On the other hand, the majority of N in BM is present in protein (Ciavatta et al., 1997). This N form of BM also demonstrated its readiness to mineralization. However, neither C/N ratio nor lignin content could explain the differences in N release pattern between AP and CM. The lowest N supplying capacity of CM was probably due to the stabilization of OM by the composting process (Hsu and Lo, 1999; Eghball, 2000; Hartz et al., 2000). It is recognized that chicken manures initially contain urea and uric acid, which are readily mineralizable (Gordillo and Cabrera, 1997; Havlin et al., 1999; Qafoku et al., 2001). This explains that initial mineralization of CM was significantly rapid compared to that of AP.

Soil Moisture Study

Soil moisture effects on net N release

In the soil moisture study, cumulative N released was significantly affected by N source, soil moisture, incubation time, and all interactions ($P < 0.001$, data not shown) except N source × soil moisture interaction ($P > 0.05$, data not shown) based on ANOVA. None of the N sources used in this study showed consistent responses to soil moisture throughout incubation.

In the control, as mineralization proceeded after week 4, increasing soil moisture significantly enhanced mineralization (Table 1.5). Soil moisture regulates oxygen diffusion in soil and maximum aerobic microbial activity occurs between 50 and 70% WHC (Linn and Doran, 1984; Franzluebbers, 1999). In general, maximum mineralization of soil OM occurs in the same range, but some studies (Hopmans et al., 1980; Goncalves and Carlyle, 1994) have suggested that the range could be up to 100% WHC. The mineralization kinetics was best described by a linear function, and the mineralization rate according to a zero-order model was 0.567, 0.798, and 0.880 mg kg^{-1} $week^{-1}$ at 50, 70, and 90% WHC, respectively (Figure 1.3a). In this study, soil N mineralization was most enhanced at 90% WHC. However, there was no significant difference in mineralization rate between 70 and 90% WHC (Figure 1.3a). In fact, the cumulative amounts of mineralized N at 70 and 90% WHC were nearly the same in weeks 1-8 (Table 1.5). Slow mineralization rate at 50% WHC can be explained by a decline in microbial activity resulting from limited diffusion of soluble substrates to microbes (Griffin, 1981; Schjønning et al., 2003) or reduced microbial mobility that limited access to substrates (Killham et al., 1993). The net amount of N released was 6.9, 9.9, and 11.3 mg N kg^{-1}

Table 1.5. Net cumulative NH_4^+-N and NO_3^--N released from four organic N sources incubated in soil at different soil moistures.[†]

		Incubation time (week)									
		1		2		4		8		12	
N source	Soil moisture	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	% WHC	mg N kg^{-1} soil									
Control	50	0.7 f	1.0 i	0.6 b	0.3 i	0.1 a	1.3 h	0.7 a	4.2 i	0.0 a	6.9 i
	70	0.1 f	0.9 i	0.4 b	0.4 i	0.2 ab	2.7 g	0.5 a	5.9 h	0.0 a	9.9 h
	90	1.1 ef	0.9 i	0.4 b	1.2 h	0.4 abc	2.4 g	0.0 b	6.3 h	0.0 a	11.3 g
Urea	50	28.3 a	21.2 d	12.6 a	41.0 bc	-0.6 de	52.6 bc	-0.1 bc	54.6 c	-0.5 d	57.2 c
	70	22.9 b	28.0 bc	-0.4 b	53.6 a	-0.7 def	55.6 ab	-0.1 bc	55.8 bc	-0.5 d	57.4 c
	90	21.9 b	36.1 a	-0.5 b	53.0 a	-0.9 ef	58.1 a	-0.5 bcde	55.6 bc	-0.5 d	59.8 bc
AP	50	1.6 ef	13.5 fgh	-0.4 b	25.6 f	-0.4 bcde	30.4 f	-0.6 cde	34.9 g	-1.0 e	41.6 f
	70	1.7 ef	14.0 fg	-1.2 b	23.3 fg	-1.2 f	30.2 f	-0.7 de	40.2 f	-1.0 e	44.4 ef
	90	1.7 ef	12.1 gh	-0.9 b	21.3 g	-0.8 ef	31.4 f	-1.0 e	41.1 f	-1.0 e	46.3 e
BM	50	9.9 d	11.0 h	0.0 b	29.6 e	-0.4 bcde	38.4 e	-0.5 bcde	46.1 e	-0.2 c	52.1 d
	70	11.2 cd	14.5 fg	0.0 b	35.7 d	-0.5 cde	39.0 de	0.0 b	46.5 e	-0.1 b	52.0 d
	90	12.9 c	15.2 ef	-0.3 b	35.3 d	-0.2 abcd	41.6 d	-0.2 bcd	48.2 de	-0.2 c	51.7 d
CM	50	9.7 d	22.7 cde	-4.8 c	52.6 a	-5.5 g	49.4 bc	-5.2 f	55.7 bcd	-5.6 f	59.0 bcd
	70	5.3 e	32.6 abc	-4.4 c	49.1 ab	-5.2 g	46.4 cd	-5.5 f	64.4 a	-5.6 f	65.7 ab
	90	2.7 ef	35.3 ab	-5.4 c	41.8 bcd	-5.7 g	59.8 ab	-5.6 f	63.1 ab	-5.6 f	70.1 a

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$. Initial NH_4^+-N and NO_3^--N contents in the treated soils (mg N kg^{-1} soil) were: control 0.0 NH_4^+-N, 22.0 NO_3^--N; urea 0.5 NH_4^+-N, 22.0 NO_3^--N; AP 1.0 NH_4^+-N, 22.1 NO_3^--N; BM 0.3 NH_4^+-N, 22.9 NO_3^--N; CM 5.6 NH_4^+-N, 22.3 NO_3^--N.

(15, 22, and 25 kg ha^{-1}) at 50, 70, and 90% WHC, respectively (Table 1.5). These values demonstrate a high, although not rapid, contribution of soil OM to crop production. Taking this into account is important for making the appropriate N recommendation. Moreover, soil moisture is clearly the factor to consider when estimating N supplied from soil OM during the growing season.

Although urea hydrolysis was somewhat suppressed at low soil moisture in the first week, it was significantly rapid (> 80%) at all soil moisture levels compared to mineralization of the natural organic materials (Table 1.3). It is recognized that urea hydrolysis is enhanced with increasing soil moisture, but optimal soil moisture for maximum urease activity varies among previous studies. For example, Saharawat (1984) found that urease activity increased as soil moisture increased from air-dried state to field capacity in two semi-arid soils (Alfisol and Vertisol). On the other hand, Dalal (1975) reported that maximum urea hydrolysis occurred at 50% WHC and further increases in soil moisture reduced urease activity in Trinidad soils. Results in this study agreed with those reported by Saharawat (1984) to the extent that urea hydrolysis increased with increasing soil moisture to near field capacity (90% WHC). The inhibition of urea hydrolysis at low soil moisture was probably due to reduced supply of urea to soil urease resulting from limited urea diffusion. During the 12 weeks incubation, hydrolysis of residual urea was almost complete, and there was no significant difference in cumulative N released among soil moisture levels in weeks 8-12 (Table 1.3). At the end of incubation, net N released ranged from 91.2 to 95.5% (Table 1.3). For the range tested in this study, soil moisture had a small influence on urea hydrolysis throughout incubation.

Unlike hydrolysis of urea, mineralization of the natural organic materials

exhibited apparent responses to soil moisture. Among these N sources, AP and CM showed similar patterns, whereas BM was quite different (Figure 1.4). During the first 2 weeks, mineralization of AP and CM was not enhanced with increasing soil moisture, but instead was reduced significantly at 90% WHC at the week 2 (Table 1.3). From weeks 2 to 12, mineralization of AP and CM was enhanced in relation to soil moisture (Table 1.3). At the end of incubation, AP and CM showed significant increases in net mineralized N of 4.7 and 11.1 mg kg^{-1} (11 and 25 kg ha^{-1}), respectively, as soil moisture increased from 50 to 90% WHC (Table 1.5). The observed occurrence of both inhibited and enhanced mineralization at high soil moisture has been reported in several studies. Doel et al. (1990) conducted a soil incubation study with white lupin at -0.30, -0.03, and -0.01 MPa. Although initial immobilization was least at -0.30 MPa, greater net mineralization occurred at -0.03 and -0.01 MPa than at -0.30 MPa after 198 days of incubation. This was likely due to microbial activity being inhibited at low soil moisture throughout incubation. De Neve and Hofman (2002) incubated a soil amended with fresh carrot leaves at soil moistures ranging from 18 to 60% WHC for 98 days. The mineralization rates at 45-60% WHC were relatively low in days 0-16 but were high in days 63-98 compared to those at 18-36% WHC. Since the added residues had high water content (87% of fresh matter), the authors concluded that rewetting dry soil to 45-60% WHC provided excessive water in the vicinity of the residues and inhibited microbial activity during the initial phase of incubation. As soil moisture was distributed away from the residues, mineralization was enhanced at high soil moisture. However, those explanations do not apply to this study, which did not show immobilization and used moist soil and dried N sources. The explanation for our results may be that microbial activity for the initial mineralization of

unstable N and the following mineralization of stable N was differently affected by soil moisture due to differences in microbial community or chemical compositions in the two phases of mineralization.

The negative effect of soil moisture on mineralization was not observed for BM. In the first week, the cumulative amount of N mineralized from BM showed an increase of 7.2 mg kg^{-1} (16 kg ha^{-1}) at 90% WHC compared to 50% WHC (Table 1.5), which was the greatest increase throughout incubation. The positive effect of soil moisture on mineralization became gradually less noticeable with incubation time, and there was no significant difference in cumulative N released among soil moisture levels after week 8 (Table 1.3). This suggests that stable N in BM, which was mineralized in the late phase of incubation, was not affected by soil moisture. At the end of incubation, net N released ranged from 56.9 to 57.4% (Table 1.3), demonstrating significantly high N supplying capacity compared to AP and CM, regardless of soil moisture. Since CM was applied at a higher rate than the other N sources, the cumulative amount of N released from CM was greater than that from AP and BM throughout incubation (Table 1.5) despite the lowest mineralization rate of CM at the end of incubation (Table 1.3).

The N release kinetics of urea, AP, BM, and CM is illustrated in Figure 1.4 using a first-order model. The model parameters, the rate constant (k_0) and the size of mineralizable N pool (N_0), reflected the effects of soil moisture on N release pattern and N supplying capacity of the organic N sources discussed above. The k_0 values for AP and CM decreased with increasing soil moisture, whereas that for BM increased in relation to soil moisture (Figure 1.3). This indicates that increasing soil moisture slowed mineralization of AP and CM but accelerated mineralization of BM. The k_0 values for

urea could not be calculated at 90% WHC because the results did not obey a first-order model. Since this model was originally proposed for soil N mineralization (Stanford and Smith, 1972), the lack of fit for a rapid urea hydrolysis may not be surprising. The N_0 values for urea and BM were constant across soil moisture levels, whereas those for AP and CM increased in relation to soil moisture (Figure 1.3). Water stress tends to reduce microbial diversity, favoring the microbes best adapted to coping with the stress (Atlas, 1984; Botter, 1985; Schimel et al., 1999). Thus, the increases in N_0 may be related to changes in composition of microbial community, such that the microbial communities favored at high soil moisture have ability to metabolize substrates that are not utilized at lower soil moistures

The N sources used in this study demonstrated contrasting results in their N release patterns at different soil moistures. First, urea showed the least apparent responses to soil moisture of all N sources used in this study (Figure 1.3). This was likely due to the rapid hydrolysis of urea that diminished the effects of soil moisture before the first measurement was made. Second, mineralization of AP and CM was enhanced at high soil moisture during the late phase of incubation, whereas that of BM was enhanced during the initial phase of incubation (Figure 1.3). This clearly indicates that effects of soil moisture on N mineralization vary with chemical composition of N sources. Incubation time is important for determination of soil moisture effects on N availability from the organic N sources.

Soil Moisture Effects on Nitrification

Nitrification, as measured by NO_3^--N production, occurred successively in all

treatments (Table 1.5). Nitrification was significantly affected by N source, soil moisture, and incubation time based on ANOVA ($P < 0.01$, data not shown).

The NH_4^+-N and NO_3^--N contents in the control reflected the slow mineralization of soil OM and subsequent nitrification. Whereas the NH_4^+-N content remained very low (< 2.0 mg kg^{-1}) throughout incubation, the NO_3^--N content increased slowly with incubation time (Table 1.5). As a result, nitrification exhibited a very similar pattern to mineralization of soil OM at all soil moisture levels, which could be described as a linear function with time ($R^2 > 0.90$, $P < 0.001$, data not shown). Increasing soil moisture significantly increased NO_3^--N production in weeks 4-12 (Table 1.5). This increase in NO_3^--N content closely corresponded to the increase in mineralized N from soil OM (Table 1.5). Hence, it is apparent that the limited NH_4^+ supply reduced NO_3^--N production at low soil moisture.

Addition of the organic N sources resulted in significant increases in both NH_4^+-N and NO_3^--N content in the first week (Table 1.5). It has been reported that active nitrification of added NH_4^+ starts with a time lag of 4-10 days following rewetting of dry soil in several incubation studies (MacLean and McRae, 1987; Mulvaney et al. 1997; Williams et al. 1998). The occurrence of the lag phase of NO_3^- accumulation was not apparent. In this study, the soil was stored under field moisture condition (10% w/w) at room temperature (20-23°C) until used, thereby likely maintaining a high population of nitrifying bacteria. The NH_4^+-N content in the first week was closely associated with the amount of N released. For example, the urea-treated soil showed a significantly greater NH_4^+-N content than the soils treated with the natural organic materials at all soil moisture levels, due to the rapid hydrolysis of urea (Table 1.5). Conversely, the AP-

treated soil, with an initially slow mineralization rate, showed the lowest NH_4^+-N content, with more than 90% of inorganic N recovered in NO_3^- form (Table 1.5). Apparently the NH_4^+ was nitrified to NO_3^- as quickly as it was mineralized. Similarly, the NO_3^--N content in the first week was significantly high in the urea and CM-treated soils, which released a greater amount of N than AP and BM (Table 1.5). Malhi and McGill (1982) reported that increasing NH_4^+ supply up to 200 mg kg^{-1} enhanced nitrification. The NO_3^--N production in the first week was positively correlated ($R^2 = 0.72$, $P < 0.001$, data not shown) with NH_4^+ supply (initial and released NH_4^+-N). Hence, high NO_3^--N production from urea and CM can be explained in part by high rate of NH_4^+ formation.

Nitrification in the N-treated soils was also affected by soil moisture in the first week. Soil moisture regulates activity of nitrifying bacteria by controlling substrate (NH_4^+) and oxygen diffusion in soil (Granli and Bockman, 1984; Sierra and Renault, 1998; Skopp et al., 1999). Nitrification rate is generally highest at 60-80% WHC (Linn and Doran, 1984; Havlin et al., 1999). In the urea-, BM-, and CM-treated soils, NO_3^--N production significantly increased in relation to soil moisture and was highest at 90% WHC (Table 1.5). Stark and Firestone (1995) studied the mechanism of decline in activity of nitrifying bacteria at low soil moisture. They clearly explained that diffusional limitation of substrate is the major limiting factor at > -0.6 MPa, but adverse physiological effects associated with cell dehydration are more inhibiting nitrification at < -0.6 MPa. Since 50% WHC is considered to be greater than -0.6 MPa for the soil used in this study, the reduction in NO_3^--N production was mainly attributed to the diffusional limitation of substrate. In addition, since urea and BM released greater amount of NH_4^+ at high soil moisture, increasing soil moisture may enhance nitrification by increasing NH_4^+

supply (Malhi and McGill, 1981; Stark and Firestone, 1995), accounting for the differences in NO_3^--N production among soil moisture levels in the urea- and BM-treated soils. However, NO_3^--N production in the AP-treated soil was not related to soil moisture (Table 1.5). This was likely due to the slow mineralization of AP regardless of soil moisture.

At week 2, continued nitrification in the N-treated soils was indicated by both NH_4^+-N disappearance and subsequent NO_3^--N production. In the urea-treated soil, although the NH_4^+-N contents at 70 and 90% WHC were less than 1.0 mg kg^{-1}, a significantly large amount of N (12.6 mg kg^{-1}) still remained as NH_4^+ at 50% WHC (Table 1.5). On the other hand, the cumulative amount of N released in the urea-treated soil did not differ among soil moisture levels (Table 1.5), suggesting that nitrification was more likely to be inhibited than urea hydrolysis at low soil moisture. In contrast, the NH_4^+-N contents in the soils treated with the natural organic materials were very low (< 1.0 mg kg^{-1}) at all soil moisture levels (Table 1.5). It was particularly notable that, although urea and CM released almost equal amounts of N, nitrification in the CM-treated soil was almost complete even at 50% WHC (Table 1.5). This suggests that activity of nitrifying bacteria was stimulated in the CM-treated soil. In addition to soil moisture, soil pH also influences nitrification rate. Nitrification occurs over a wide range in pH (4.5-10), but the optimum pH is 8.5 and decreasing pH reduces nitrification rate (Montagnini et al., 1989; Paavolainen and Smolander, 1997; Ste-Marie and Paré, 1999; Havlin et al., 1999). Addition of urea and CM can be expected to cause different changes in soil pH. First, although urea application raises soil pH temporarily, it ultimately lowers soil pH below the original value (Mulvaney et al., 1997). This is caused by the hydrolysis

of urea which neutralizes H^+ when releasing $2NH_4^+$, but subsequent nitrification of NH_4^+ produces $2H^+$ (Havlin et al., 1999). In contrast to urea, chicken manures are effective in raising soil pH (Hue, 1992; Kingery et al., 1993; Wheatley et al., 1997). Because layers are fed ground limestone, their manures contain calcium carbonate that neutralizes H^+ in soil (Mokolobate, 2002). In fact, CM had the highest pH (8.69) and Ca content (14.4%) among the natural organic materials used in this study (Table 1.2), indicating a high ability to neutralize soil acidity. Therefore, it could be expected that addition of CM raised soil pH, thereby stimulating the activity of nitrifying bacteria.

After week 4, the NH_4^+-N content was very low (< 1.0 mg kg^{-1}), whereas the NO_3^--N content increased slowly in all treatments (Table 1.5). It seems that newly released NH_4^+ from the organic N sources was quickly nitrified after their N release rates slowed down. The differences in NO_3^--N production among treatments in weeks 4-12 were attributed to the differences in NH_4^+ released from the organic N sources.

Temperature Study

Temperature effects on net N release

In the temperature study, net cumulative N release was significantly affected by N source, temperature, incubation time, and all interactions based on ANOVA ($P < 0.001$, data not shown). Increasing temperature enhanced N release from all N sources used in this study, but the magnitude of the response to temperature varied among source of N and incubation time.

In the control, mineralization of soil OM was enhanced with increasing temperature throughout incubation (Figure 1.3b). The temperature coefficient, Q_{10}, of approximately 2 over the range 5 to 35°C is generally accepted to describe the relationship between soil N mineralization and temperature (Campbell and Biederbeck, 1972; Stanford et al., 1973; Sierra, 1997; Kätterer et al., 1998). That is, a twofold increase in mineralization rate is associated with a shift of 10°C. In this study, the mineralization kinetics was best described by a linear function, and the mineralization rate according to a zero-order model was 0.54, 0.95, and 1.53 mg kg^{-1} $week^{-1}$ at 15/10, 20/15, and 25/20°C, respectively (Figure 1.3b). Although Q_{10} was not estimated due to the narrow range of temperature studied, its tendency was seen in our results. The net amount of N released was 6.4, 11.3, and 18.9 mg N kg^{-1} (14, 25, and 42 kg ha^{-1}) at 15/10, 20/15, and 25/20°C, respectively (Table 1.6), demonstrating significant increases in the pool size of mineralized N. Increases in temperature induce a shift in the composition of microbial communities (Richards et al., 1985; Carreiro and Koske, 1992; Zogg et al., 1997). Furthermore, Zogg et al. (1997) found that the shift in microbial community composition paralleled an increase in microbial respiration at temperatures between 5 and 25°C. Thus,

Table 1.6. Net cumulative NH_4^+-N and NO_3^--N released from four organic N sources incubated in soil at different temperatures.[†]

N source	Temperature	Incubation time (week)									
		1		2		4		8		12	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	°C	mg N kg^{-1} soil									
Control	15/10	0.4 g	0.8 f	0.3 c	1.0 i	0.2 a	2.0 h	0.4 a	3.8 h	0.1 a	6.3 j
	20/15	0.0 g	1.5 f	0.2 c	1.9 i	0.1 a	3.9 g	0.4 a	7.2 g	0.2 a	11.1 i
	25/20	0.2 g	1.5 f	0.1 c	3.2 i	0.1 a	5.8 g	0.2 a	11.4 f	0.2 a	18.7 h
Urea	15/10	33.8 a	13.3 d	7.8 a	46.5 c	-0.6 a	56.7 b	0.0 a	56.9 b	-0.4 a	58.8 cd
	20/15	20.6 b	33.7 b	-0.3 c	56.7 a	-0.4 a	57.4 b	-0.1 a	57.5 b	-0.2 a	58.3 cd
	25/20	6.4 de	49.7 a	-0.2 c	58.0 a	-0.2 a	57.9 b	-0.2 a	56.6 b	-0.5 a	58.8 cd
AP	15/10	2.8 fg	8.1 ef	-0.7 c	17.8 h	-1.1 b	24.9 f	-0.8 b	33.7 f	-1.1 a	42.6 g
	20/15	0.9 g	13.7 d	-1.1 c	24.4 g	-1.1 b	31.0 e	-0.8 b	40.7 e	-0.7 b	45.9 g
	25/20	0.4 g	20.7 c	-1.0 c	30.5 f	-0.9 b	39.6 d	-0.9 b	47.8 cd	-1.1 b	52.7 ef
BM	15/10	9.4 cd	7.2 f	5.1 b	28.5 f	-0.1 a	38.4 d	0.0 a	45.1 d	0.1 a	50.5 f
	20/15	10.8 c	16.5 d	0.1 c	36.9 e	-0.2 a	41.8 d	-0.2 a	49.4 c	-0.2 a	55.1 de
	25/20	3.9 efg	24.6 c	-0.1 c	41.9 d	0.0 a	51.3 c	0.0 a	54.1 b	-0.1 a	55.7 de
CM	15/10	18.0 b	13.6 de	-4.9 d	40.8 de	-6.4 c	49.1 c	-5.7 c	58.2 b	-6.0 c	64.0 bc
	20/15	6.1 def	33.7 b	-5.4 d	50.2 bc	-6.1 c	55.5 bc	-5.9 c	67.8 a	-6.4 c	69.7 ab
	25/20	-3.8 h	46.8 a	-6.1 d	53.1 ab	-6.3 c	66.4 a	-5.8 c	71.1 a	-6.1 c	71.8 ab

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$. Initial NH_4^+-N and NO_3^--N contents in the treated soils (mg N kg^{-1} soil) were: control 0.2 NH_4^+-N, 19.9 NO_3^--N; urea 0.6 NH_4^+-N, 19.9 NO_3^--N; AP 1.3 NH_4^+-N, 20.3 NO_3^--N; BM 0.4 NH_4^+-N, 20.4 NO_3^--N; CM 6.6 NH_4^+-N, 20.6 NO_3^--N.

the increase in the pool size of mineralized N at high temperature was likely due to the microbial communities favored at high temperature metabolizing substrates that were not utilized at lower temperatures. Temperature is clearly the factor to consider when estimating N supplied from soil OM during the growing season.

Although urea hydrolysis was somewhat suppressed at 15/10°C in the first 2 weeks, it was significantly rapid (> 75%) at all temperature levels compared to mineralization of the natural organic materials (Table 1.4). Hydrolysis of residual urea was almost complete by the end of week 4. Inhibited urease activity (Overrein and Moe, 1967; Saharawat, 1984; Moyo et al., 1989; Lai and Tabatabai, 1992) and slow urea diffusion (Pang et al., 1977; Sadeghi et al., 1988) at low temperature seems to retard the hydrolysis of urea at 15/10°C. In weeks 4-12, there was no significant difference in cumulative N released among temperature levels. At the end of incubation, net N released ranged from 92.6 to 93.2% (Table 1.4). For the range tested in this study, temperature had a small influence on urea hydrolysis throughout incubation. The results were consistent with those reported by MacLean and McRae (1987). In their soil incubation study, the rate of urea hydrolysis was proportional to temperature over the range of 9 to 18°C in first 3 days but showed no difference among temperature levels after 5 days due to the rapid hydrolysis of urea.

In contrast to hydrolysis of urea, mineralization of the natural organic materials was significantly enhanced with increasing temperature throughout incubation. Maximum differences in the cumulative amount of mineralized N between temperatures 15/10 and 25/20°C were observed at week 4, which were 14.9, 13.0, and 17.4 mg kg^{-1} (34, 29, and 39 kg ha^{-1}) for AP, BM, and CM, respectively (Table 1.6). From week 4 to 12,

AP showed a relatively constant increase in cumulative N released with increasing temperature (Table 1.4). In contrast, BM and CM showed a decline in response to increased temperature (Table 1.4). This was due to the mineralization at 25/20°C slowing down after week 4, with less than 5% of organic N released in weeks 4-12 (Table 1.4). At the end of incubation, AP, BM, and CM showed significant increases in the net amount of mineralized N of 10.1, 5.0, and 7.7 mg kg^{-1} (23, 11, and 17 kg ha^{-1}), respectively, as temperature increased from 15/10 to 25/20°C (Table 1.6). These values account for 10.3, 5.5, and 5.3% of the applied organic N in AP, BM, and CM, respectively, suggesting that mineralization of stable N in AP was more influenced by temperature than BM and CM (Table 1.4). In fact, net N released at 25/20°C was significantly higher with AP than with CM, but there was no significant difference in net N released at lower temperatures (Table 1.4). Among the natural organic materials used in this study, BM showed a significantly higher net N released than AP and CM in weeks 2-12 at all temperature levels, demonstrating the highest N supplying capacity regardless of temperature (Table 1.4). Since CM was applied at a higher rate than the other N sources, the cumulative amount of N released from CM was greater than that from AP and BM throughout incubation (Table 1.6) despite the lowest mineralization rate of CM at the end of incubation (Table 1.4).

The N release kinetics of urea, AP, BM, and CM is illustrated in Figure 1.5 using a first-order model. The model parameters, k_0 and N_0, reflected the effects of temperature on N release pattern and N supplying capacity of the organic N sources discussed above. Regardless of N source, the k_0 and N_0 values increased with increasing temperature. With exception of k_0 for CM, the relationships between these parameters and temperature were

significant at level of $\alpha = 0.05$ (Figure 1.5). Increases in k_0 with increasing temperature may be explained by stimulated microbial activity or accelerated diffusion at high temperature (Nicolardot et al., 1994; MacDonald et al., 1995; Zak et al., 1999). As discussed earlier for soil N mineralization, apparent increases in N_0 may be related to a shift in the composition of microbial communities, such that the microbial communities favored at high temperature have ability to metabolize substrates that are not utilized at lower temperatures (Zogg et al., 1997).

The N sources used in this study demonstrated contrasting results in their N release patterns at different temperatures. First, hydrolysis of urea was enhanced at high temperature only in the first 2 weeks, but mineralization of the natural organic materials was enhanced at high temperature throughout incubation (Figure 1.5). Considering temperature is important for the natural organic materials to make an appropriate N recommendation based on estimation of the N availability. For example, more N may have to be applied in a cool climatic condition because less N can be expected to be released. Second, mineralization of AP responded to temperature to a greater degree than that of BM and CM in the late phase of incubation (Figure 1.5). Similar results have been reported in previous studies. Cookson et al. (2002) conducted a soil incubation study using clover residues at different temperatures, and reported that 22, 33, 41, and 60% of N was mineralized at 2, 5, 10, and 15°C, respectively after 160 days incubation. Griffin and Honeycutt (2000) incubated soil amended with dairy, poultry, and swine manures for 112 days, and found that increasing temperature from 10 to 24°C accelerated the mineralization rate, but did not affect the net mineralized N after 28 days. Although these results were not comparable due to different experimental conditions, our results clearly

indicate that the effects of temperature on N mineralization vary with chemical composition of the organic N sources.

Temperature Effects on Nitrification

Nitrification, as measured by NO_3^--N production, occurred successively in all treatments. Nitrification was significantly affected by N source, temperature, incubation time, and all interactions based on ANOVA ($P < 0.01$, data not shown).

The NH_4^+-N and NO_3^--N contents in the control reflected the slow mineralization of soil OM and subsequent nitrification. Whereas the NH_4^+-N content remained very low (< 1.0 mg kg^{-1}) throughout incubation, the NO_3^--N content increased slowly with incubation time (Table 1.6). As a result nitrification exhibited a very similar pattern to mineralization of soil OM at all temperature levels, which could be described as a linear function with time ($R^2 > 0.80$, $P < 0.001$, data not shown). The NO_3^--N production was proportional to temperature throughout incubation (Table 1.6). This increase in NO_3^--N content closely corresponded to the increase in mineralized N from soil OM (Table 1.6). Hence, it is apparent that the limited NH_4^+ supply reduced NO_3^--N production at low temperature.

Considerable nitrification occurred in all N-treated soils throughout incubation as significantly high NO_3^--N content was recovered compared to the control (Table 1.6). In the first week, the NH_4^+-N and NO_3^--N contents in the N-treated soils were closely associated with the amount of N released. For example, the urea-treated soil showed the highest NH_4^+-N content at all temperature levels due to the rapid hydrolysis of urea (Table 1.6). Conversely, the AP-treated soil, with an initially slow mineralization rate,

showed very low NH_4^+-N contents ranging from 0.4 to 2.8 mg kg^{-1} (Table 1.6). Apparently NH_4^+ was nitrified as quickly as it was mineralized. The NO_3^--N content was significantly high in the urea and CM-treated soils, which released greater amount of N than AP and BM (Table 1.6). Linear regression analysis revealed a positive correlation between the NO_3^--N production and NH_4^+ supply ($R^2 = 0.55$, $P < 0.001$, data not shown); however, only 55% of the variability in NO_3^--N production could be explained by substrate availability. This was due to a great influence of temperature on NO_3^--N production. Previous studies show that increasing temperature stimulates the activity of nitrifying bacteria with maximum nitrification occurring between 25 and 35°C (Justice and Smith, 1962; Kowalenko and Cameron, 1976; Malhi and McGill, 1981; Breuer et al., 2002). In this study, the NH_4^+-N content was inversely and the NO_3^--N content was directly correlated with temperature (Table 1.6), indicating that activity of nitrifying bacteria was stimulated at high temperature. In addition, enhanced nitrification at high temperature can be explained in part by high rate of NH_4^+ formation (Malhi and McGill, 1981; Stark and Firestone, 1995).

At week 2, continued nitrification in the N-treated soils was indicated by both NH_4^+-N disappearance and subsequent NO_3^--N production (Table 1.6). In the urea-treated soil, although the NH_4^+-N contents at 20/15 and 25/20°C were nearly zero, relatively high NH_4^+-N content (7.8 mg kg^{-1}) was recovered at 15/10°C (Table 1.4). On the other hand, the difference in the cumulative amount of N released between temperatures 15/10°C and 25/20°C was relatively small (3.5 mg kg^{-1}) (Table 1.6), suggesting that nitrification was more likely to be inhibited than urea hydrolysis at low temperature. Among the soil treated with the natural organic materials, the BM-treated soil showed a significantly high

NH_4^+-N content at 15/10°C (5.1 mg kg^{-1}), whereas the AP- and CM-treated soils showed very low NH_4^+-N contents at all temperature levels (Table 1.6). It is notable that nitrification in the CM-treated soil was almost complete even at 15/10°C, though CM released greater amount of N than BM (Table 1.6). As discussed earlier, it could be expected that addition of CM raised soil pH during incubation, thereby stimulating the activity of nitrifying bacteria.

After week 4, the NH_4^+-N content was very low (< 1.0 mg kg^{-1}), whereas the NO_3^--N content increased slowly in all treatments (Table 1.5). It seems that newly released NH_4^+ from the organic N sources was quickly nitrified after their N release rates slowed down. The differences in NO_3^--N production among treatments in weeks 4-12 were attributed to the differences in NH_4^+ released from the organic N sources.

CONCLUSIONS

Four organic N sources used in this study, with different characteristics in chemical composition, varied in N release patterns and N supplying capacities. Soil moisture influences N availability from the organic N sources differently depending on source of N and incubation time. Temperature influences N availability from the organic N sources differently depending on source of N and incubation time. These interactions must be considered to determine an appropriate rate and timing of fertilization for efficient use of N inputs, especially in greenhouse production systems when controlling irrigation or temperature.

Testing both NH_4^+-N and NO_3^--N is necessary to estimate available N released from the organic N sources in an initial period of growing season. At high soil moisture or temperature conditions, a high concentration of NO_3^--N accumulated in the soil immediately after urea or CM was applied, thus nitrate leaching may be a concern.

It appears that the differences in N release response to soil moisture or temperature among N sources and incubation time are related to chemical composition of the organic N sources applied. Further research on which chemical compositions are more or less resistant to the effects of soil moisture and temperature is needed to better understand availability of N from organic N sources.

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CHAPTER 2

AVAILABILITY OF NITROGEN FROM ORGANIC NITROGEN SOURCES AS AFFECTED BY SOIL MOISTURE: BIOASSAY USING KALE (*Brassica oleracea* L.)

INTRODUCTION

Soil moisture is one of the major environmental factors affecting nitrogen (N) availability from organic N sources. Soil moisture regulates oxygen diffusion in soil with maximum aerobic microbial activity occurring between 50 and 70% of water holding capacity (WHC) (Linn and Doran, 1984; Franzluebbers, 1999). On the other hand, low soil moisture inhibits microbial activity by reducing diffusion of soluble substrates (Griffin, 1981; Schjønning et al., 2003), microbial mobility (Killham et al., 1993), and intracellular water potential (Csonka, 1989; Stark and Firestone, 1995). Low soil moisture also reduces microbial diversity, favoring the microbes best adapted to coping with water stress (Atlas, 1984; Botter, 1985; Schimel et al., 1999).

The effects of soil moisture on N availability have been evaluated for various organic N sources under laboratory conditions. Vlek and Carter (1983) incubated urea in four different soils (Uvalde, Houston, Vernon, and Crowley soils) at soil moistures ranging from 10 to 40% (w/w). All the four soils responded similarly to an increase in soil moisture, with a drastic reduction in urease activity below the permanent wilting point. Saharawat (1984) reported that urea hydrolysis rate increased with increasing soil moisture from air-dried state to field capacity in two semi-arid soils (Alfisol and Vertisol). Doel et al. (1990) conducted a 198-day soil incubation study with white lupin at -0.30, -0.03, and -0.01 MPa. Although immobilization was not overcome at -0.30 MPa throughout incubation, net mineralization occurred at -0.03, and -0.01 MPa after 168 and 187 days of incubation, respectively. De Neve and Hofman (2002) incubated fresh carrot leaves in soil at different soil moistures ranging from 18 to 60% WHC for 98 days. Net mineralized N increased with increasing soil moisture from 18 to 45% WHC, and was

constant with further increases to 60% WHC. Ultimately, the question of the interest is how accurately such information estimate actual N availability during the growing season.

Previously, soil moisture effects on N availability from four organic N sources, including urea, alfalfa pellets, blood meal, and partially composted chicken manure, were examined in a soil incubation study. The objectives of this study were to: (1) examine the effects of soil moisture on plant growth, N uptake, and N use efficiency by kale treated with the organic N sources, and (2) evaluate the correlation between N availability from the organic N sources determined in the previous soil incubation study and N use efficiency by kale.

MATERIALS AND METHODS

Soil

The soil used in this study was a Granby sandy clay loam (sandy, mixed, mesic Typic Haplaquolls). Approximately 400 kg of surface soil (15 cm) was collected from the Michigan State University Horticulture Teaching and Research Center in East Lansing MI, in October 2002. The soil was passed through a 5 mm sieve, thoroughly mixed to ensure uniformity, and stored in a covered plastic container under field moisture condition (5%, w/w) at room temperature (20-23°C) until the experiment was initiated to minimize disturbance of the microbial population (Pramer and Bartha, 1972; Honeycutt, 1999). The chemical and physical properties of the soil are listed in Table 2.1. All soil analyses were done by the same procedures used in the previous soil incubation study.

Nitrogen sources

Urea [$CO(NH_2)_2$] was used as a synthetic organic N fertilizer. Three natural organic materials were used. Alfalfa pellets (AP), which were obtained from Bradfield Industries, Inc. (Springfield, MI), are alfalfa-based fertilizers blended with animal protein, natural sulfate of potash, and molasses. Blood meal (BM) was obtained from Glorious Gardens Blood Meal Growing Markets, Inc. (West Des Moines, IA). Partially composted chicken manure (CM) was obtained from Herbruck's Poultry Ranch, Inc. (Saranac, MI). Chemical properties of these N sources are listed in Table 2.2. All nutrient analyses were done by the same procedures used in the previous soil incubation study.

Table 2.1. Chemical and physical properties of soil used in this study.

pH	Sand	Silt	Clay	CEC	OM	Total C	Total N	NH_4^+	NO_3^-	P	K	Ca	Mg
	------ % ------			cmol kg^{-1}	%	-- g N kg^{-1} --		---------- mg N kg^{-1} ----------					
6.20	54.7	15.4	29.8	75.2	3.4	19.6	4.3	0.1	17.2	179	219	1050	119

Table 2.2. Chemical characteristics of four organic N sources used in this study.†

N source	Moisture	pH	Total C	Total N	C/N ratio	NH_4^+	NO_3^-	P	K	Ca	Mg	Fe
	%		---- % ----			-- g N kg^{-1} --		---------- % ----------				
Urea	--	--	--	46.00	--	--	--	--	--	--	--	--
AP	4.46	5.73	39.3	3.60	10.93	0.49	0.15	0.58	4.55	1.69	0.16	0.06
BM	5.01	7.30	41.3	12.65	3.26	0.15	0.02	0.51	0.64	1.12	0.11	0.11
CM	9.11	8.78	26.6	3.03	8.79	1.42	0.12	2.96	3.62	18.41	0.87	0.09

† All values are expressed on a dry weight basis (105°C).

Experimental design

A pot experiment with kale (*Brassica oleracea* L. cv. Winterbor F1) was conducted using growth chambers during October to December 2002. The experimental design was a randomized complete block design with three replications. Treatments consisted of a factorial combination of three soil moisture levels (50, 70, and 90% WHC), five N sources (control, urea, AP, BM, and CM), and four growth stages [20, 35, 50, and 60 days after transplanting (DAT)].

Experimental procedure and growing conditions

Kale was seeded in a 2.5 cm depth plastic cell tray filled with a commercial seedling/propagating mix and placed in a greenhouse on 4 October 2002. Twenty-day-old seedlings were transplanted singly in 1.6-litter pots that measured 15 cm in diameter. One day before transplanting, 1900 g of soil (oven dry basis) was mixed with urea, AP, BM, or CM at the rate of 63, 100, 92, and 150 mg N per 1 kg of soil (oven dry basis), respectively, and filled into the pots. Pots were also filled with 1900 g of soil with no N source as a control. The application rates were calculated to provide approximately equal amounts of 60 mg available N kg^{-1} soil (134 kg available N ha^{-1}) using Equation [1].

$$\text{Estimated available N} = N_i + fN_o \quad [1]$$

where N_i is the inorganic N (NH_4^+-N + NO_3^--N) content, N_o is the organic N (total N – inorganic N) content, and f is the proportion of organic N fraction expected to be released during the growing period (Griffin and Honeycutt, 2000). As in the previous soil incubation study, coefficient f of 0.95, 0.59, 0.65, and 0.39 was applied for urea, AP, BM, and CM, respectively. Additional nutrients (P, K, Ca, and Mg) were not applied, since

there were sufficient amounts of these nutrients for optimum crop production (Table 1) according to soil tests and fertilizer recommendations for vegetable crops in Michigan (Warncke et al., 1992).

Amount of available N released from the organic N sources was estimated also by using a first-order model [2] determined in the previous soil incubation study (Table 2.3);

$$N_{rel} = N_0(1 - \exp^{-k_0 t}) \qquad [2]$$

where N_{rel} (mg N kg^{-1}) is the cumulative N released from an applied N source at time t, N_0 (mg N kg^{-1}) is the size of potentially mineralizable N, exp is the exponential constant with numerical value ≅ 2.718, and k_0 ($week^{-1}$) is the first-order rate constant. Availability of N from the organic N sources, as a percentage of applied N, was also estimated (Table 2.3).

Table 2.3. Available N as a function of applied N.

N source	Soil moisture	Applied N	Estimated available N	Estimated N availability
	% WHC	-- mg N kg^{-1} soil --		%
Urea	50	63	54	86
	70	63	57	91
	90	63	58	91
AP	50	100	37	37
	70	100	40	41
	90	100	42	42
BM	50	92	48	53
	70	92	47	52
	90	92	48	53
CM	50	150	50	34
	70	150	54	37
	90	150	60	41

The pots were watered with distilled water to 50, 70, and 90% WHC by weighing, and randomly transferred into growth chambers. Growth chambers were maintained at 20/15°C (± 0.5°C) day/night temperatures and 70% relative humidity. Lighting was provided by fluorescent and incandescent lamps that produced 420 ± 20 μmol m^{-2} s^{-1} PPF at plant height with a 14-hr photoperiod. In order to maintain the soil moisture levels during the experiment, the pots were watered by weighing and adding the required amount of distilled water every two days until 20 DAT and daily thereafter. Corrections for increasing plant weight were made on the basis of shoot fresh weight determined at each sampling time. The pots were covered by a plastic plate, which allowed passage of water into soil but reduced evaporation from surface soil.

Sampling, measurements, and chemical analyses

At 20, 35, 50, and 60 DAT, kale shoots were separated from soil by hand, and divided into leaves and stem for determination of fresh matter weight. Dry matter weight was determined after dried at 60°C for 48 hr. The dried tissues were then ground to pass 1 mm screen and analyzed for total Kjeldahl-N (TKN) content.

Soil was passed through a 2 mm sieve to remove roots. The sieved soil was dried at 38°C for 48 hr, thoroughly mixed and analyzed for pH and inorganic N content (NH_4^+-N and NO_3^--N). Roots were separated from the remaining soil by washing over 0.5 mm sieve in a hydropneumatic root elutriator (Gillison's Variety Fabrication, Inc., Benzonia, MI) as described by Smucker et al. (1982). After washing, debris and dead roots were manually removed from vital roots. The roots were then rinsed with distilled water and dried at 60°C for 48 hr for determination of dry matter weight. The dried roots were

ground to pass 1 mm sieve and analyzed for TKN content.

Apparent N use efficiency

Apparent N use efficiency (ANUE) was calculated using the difference method as follows (Motavalli et al., 1989):

$$ANUE\ (\%) = \{[N_{uptake}\ (\text{N-treated plant}) - N_{uptake}\ (\text{control})] / N_{applied}\} \times 100 \quad [2]$$

where N_{uptake} (mg pot^{-1}) is the total N uptake by kale calculated as the sum of TKN in leaves, stem, and roots, and $N_{applied}$ (mg pot^{-1}) is the total N in an applied N source. The difference method assumes that soil N transformations remain constant in both the soil that received N source and the soil that received no N source. Thus, the difference in total N uptake between the two soils is assumed to be the amount of N from the applied N source taken up by the plant.

Statistical analysis

A three-way analysis of variance (ANOVA) was conducted to test significant differences in main effects and interactions using the MIXED procedure of Statistical Analysis System (SAS) (SAS Institute, 1990). When statistically significant differences existed, treatment means were separated using the LSMEANS procedure, and then tested using paired *t* test at $\alpha = 0.05$. Data presented are the means of three replications.

RESULTS AND DISCUSSION

Dry matter production and partitioning among leaves, stem, and roots

Dry matter production of leaves, stem, and roots was significantly affected by N source, soil moisture, and growth stage based on ANOVA ($P < 0.001$, data not shown). Patterns of dry matter partitioning among the tissue types were also influenced by N source, soil moisture, and growth stage.

Leaf dry matter increased with time in a sigmoidal manner (Figure 2.1). At 20 DAT, leaf dry matter did not show apparent responses to N application (Figure 2.1). Among the N-treated plants significant difference was seen only with the CM-treated plants that produced greater leaf dry matter than the AP- and BM-treated plants at 70% WHC (Table 2.4). Increasing soil moisture inhibited plant growth in the control, with leaf dry matter reduced by half at 90% WHC (Table 2.4). The N-treated plants produced the greatest leaf dry matter at 70% WHC. At 35 DAT, leaf dry matter was significantly greater in the N-treated plants than in the control at all soil moisture levels, and differences continued to increase with growth (Figure 2.1). The N-treated plants

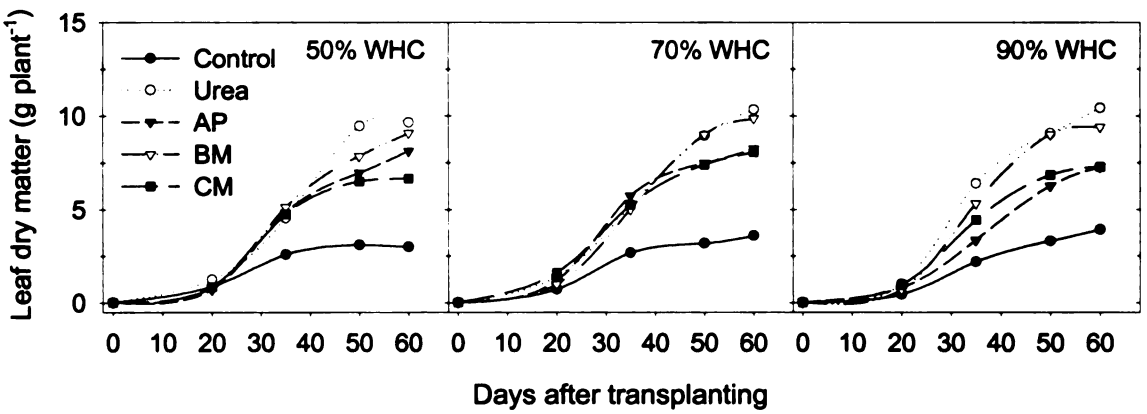

Figure 2.1. Temporal changes in leaf dry matter of kale treated with different organic N sources grown at different soil moistures.

Table 2.4. Temporal changes in dry matter production of leaves, stem, and roots in kale treated with different organic N sources grown at different soil moistures.†

		Days after transplanting											
		20			35			50			60		
N source	Soil moisture	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
	% WHC	g plant^{-1}											
Control	50	0.89 bc	0.03 ef	0.07 bcd	2.60 e	0.13 d	0.56 f	3.11 g	0.24 h	0.70 f	3.00 g	0.33 f	0.88 e
	70	0.72 cd	0.03 def	0.09 bc	2.68 e	0.15 d	0.54 f	3.19 fg	0.26 h	0.69 f	3.60 f	0.38 f	0.82 e
	90	0.45 d	0.02 f	0.06 cd	2.20 f	0.13 d	0.33 g	3.31 f	0.29 h	0.66 f	3.93 f	0.40 f	0.69 f
Urea	50	1.26 abc	0.07 abc	0.06 cd	4.55 cd	0.27 b	0.67 def	9.47 a	0.83 a	1.17 d	9.66 a	1.14 a	1.58 abc
	70	1.40 ab	0.07 a	0.12 ab	5.19 abc	0.30 ab	0.81 bcd	8.95 ab	0.88 a	1.27 bcd	10.35 a	1.14 a	1.52 abc
	90	1.01 bcd	0.06 abcd	0.09 bc	6.40 a	0.37 a	0.93 abc	9.07 ab	0.82 a	1.18 cd	10.42 a	1.08 a	1.41 cd
AP	50	0.72 cd	0.03 def	0.04 d	4.84 bc	0.25 bc	0.76 cde	6.97 de	0.64 cd	1.32 bc	8.14 c	0.90 c	1.82 ab
	70	1.17 b	0.05 abcde	0.09 bc	5.72 ab	0.33 ab	0.99 ab	7.48 cde	0.65 cd	1.37 b	8.25 bc	0.87 cd	1.80 ab
	90	0.81 bc	0.04 def	0.08 bcd	3.37 de	0.17 cd	0.60 ef	6.26 e	0.49 g	0.90 e	7.29 d	0.68 e	1.44 cd
BM	50	0.84 bc	0.04 cde	0.06 cd	5.13 abc	0.29 ab	0.92 abcd	7.87 c	0.68 bcd	1.31 bcd	9.09 ab	0.96 bc	1.87 a
	70	0.99 bc	0.05 bcde	0.07 bcd	5.00 abc	0.28 b	0.97 abc	9.01 b	0.70 bc	1.40 ab	9.87 a	1.05 ab	1.68 abc
	90	0.70 cd	0.03 def	0.06 cd	5.33 abc	0.31 ab	0.80 bcde	9.01 b	0.74 b	1.15 d	9.42 ab	0.95 c	1.21 d
CM	50	0.86 bc	0.04 def	0.07 bcd	4.75 c	0.26 b	1.00 a	6.51 e	0.53 fg	1.41 ab	6.67 e	0.67 e	1.59 abc
	70	1.61 a	0.07 ab	0.16 a	5.24 ab	0.32 ab	0.89 abc	7.41 d	0.62 de	1.65 a	8.08 c	0.87 cd	1.86 ab
	90	0.96 bc	0.04 bcde	0.08 bcd	4.45 c	0.25 bc	0.86 abc	6.85 e	0.56 ef	1.32 bcd	7.30 d	0.80 d	1.55 bc

† Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

exhibited a similar growth pattern at 50 and 70% WHC, but showed significant differences at 90% WHC (Table 2.4). Leaf dry matter in the urea-treated plants increased in relation to soil moisture, whereas that in the AP- and CM-treated plants decreased significantly at 90% WHC (Table 2.4). At 50 and 60 DAT, the urea- and BM-treated plants produced significantly greater leaf dry matter than the AP- and CM-treated plants at all soil moisture levels (Table 2.4). Maximum dry matter production of leaves (10.4 g $plant^{-1}$) occurred in the urea-treated plants at 90% WHC. However, this was not significantly different than leaf dry matter in the urea- and BM-treated plants across soil moisture levels. Among the N-treated plants, the lowest dry matter production of leaves (6.7 g $plant^{-1}$) occurred in the CM-treated plants at 50% WHC. As soil moisture increased from 50 to 90% WHC, the control, urea-, BM-, and CM-treated plants increased leaf dry matter by 31, 8, 4, and 10%, respectively, but the AP-treated plants decreased leaf dry matter by 10%. Increasing soil moisture from 70 to 90% WHC had a negative influence on leaf dry matter production by the AP- and CM-treated plants.

Stem dry matter increased with time in a linear manner. Relative differences among treatments were similar between leaf and stem dry matter at all growth stages (Table 2.4). As plants matured, the percentage of dry matter partitioned in stem increased.

Root dry matter also increased with time and by N application; however, root growth was influenced by N source and soil moisture differently from shoot growth. Only at 20 DAT were relative differences among treatments similar between leaf and root dry matter. From 35 to 60 DAT, the N-treated plants showed a similar root growth within each soil moisture level (Table 2.4). With exception of the urea-treated plants, root growth was inhibited at 90% WHC throughout the growing period. Oxygen diffusion in

soil is regulated by soil moisture (Sierra and Renault, 1998). The negative influence of soil moisture on root growth may be explained by limited oxygen diffusion in soil. This occurred primarily in the AP, BM, and CM treatments probably because addition of the natural organic materials stimulated microbial activity and increased oxygen consumption by soil microorganisms. The increased soil microbial activity and respiration after addition of animal manures or plant residues have been reported in previous studies (Bremer and van Kessel, 1992; Henriksen and Breland, 1999; Bhattacharyya et al., 2001).

The ratio of shoot to root dry matter was determined as an indicator of dry matter partitioning in roots. In all treatments the shoot/root ratio was highest at 20 DAT (data not shown). After a sharp decline at 35 DAT, the shoot/root ratio remained constant from 35 to 60 DAT (data not shown). At 60 DAT, the urea- and BM-treated plants showed higher shoot/root ratios compared to the AP- and CM-treated plants at all soil moisture levels (Figure 2.2). This was due to less shoot production in the AP- and CM-treated plants. Because shoot growth depends on roots for water and nutrients, water stress restricts shoot growth sooner than root growth (Brouwer, 1962; Khurana and Singh, 2000). In this study, the control, urea- and BM-treated plants decreased shoot/root ratio as soil moisture decreased (Figure 2.2). However, this was due to inhibited root growth at high soil moisture rather than to inhibited shoot growth at low soil moisture. Since both shoot and root growth responded to soil moisture similarly in the AP- and CM-treated plants, their shoot/root ratios were relatively constant across soil moisture levels (Figure 2.2).

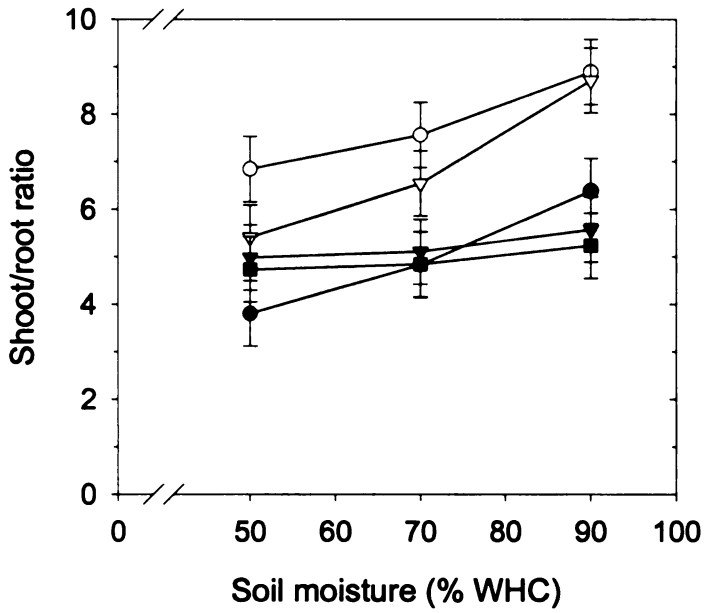

Figure 2.2. Effects of soil moisture on shoot/root ratio of kale treated with different organic N sources at 60 DAT. The symbols correspond to (●) control; (○) urea; (▼) AP; (▽) BM; (■) CM. Error bars represent one standard error (1 SE).

Nitrogen concentration and partitioning among leaves, stem, and roots

Nitrogen concentration in leaves, stem, and roots was significantly affected by N source, soil moisture, and growth stage based on ANOVA ($P < 0.05$, data not shown). Partitioning patterns of N concentration among the tissue types differed between growth stages.

At 20 DAT, N concentration was highest in the order: leaves > stem > roots (Table 2.5). The relationship between N concentration and available soil N level was analyzed for each tissue type. Leaf N concentration decreased steeply below 10 mg available N kg^{-1} (Figure 2.3). For example, the control at 50 and 70% WHC and the CM-treated plants at 70% WHC had relatively low leaf N concentrations (< 4.5 %) compared

Table 2.5. Temporal changes in N concentration in leaves, stem, and roots of kale treated with different organic N sources grown at different soil moistures.[†]

		Days after transplanting											
		20			35			50			60		
N source	Soil moisture	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
	% WHC	% N											
Control	50	3.35 f	2.34 e	1.80 e	0.84 e	1.08 d	1.43 e	0.51 e	0.60 f	1.59 bc	0.46 g	0.60 d	1.34 c
	70	4.14 de	2.68 de	1.82 e	0.86 e	1.02 d	1.45 e	0.55 de	0.69 e	1.61 abc	0.45 g	0.57 d	1.46 bc
	90	4.66 cd	3.13 abcd	1.97 de	1.12 de	1.73 a	1.63 de	0.56 de	0.69 e	1.55 bc	0.49 fg	0.73 a	1.45 bc
Urea	50	5.63 a	2.98 bcd	1.84 e	1.94 a	1.70 a	1.84 abc	0.67 ab	0.82 cd	1.62 abc	0.50 def	0.60 d	1.55 ab
	70	5.42 ab	2.87 cde	2.23 abc	1.76 ab	1.52 ab	1.73 abcd	0.65 bc	0.84 bcd	1.58 bc	0.56 abc	0.69 abc	1.63 ab
	90	5.75 a	2.96 bcd	2.32 ab	1.22 cde	1.19 bcd	1.87 ab	0.72 a	0.90 abc	1.79 a	0.59 ab	0.68 abc	1.70 a
AP	50	5.60 a	3.54 a	1.97 de	1.64 abc	1.49 abc	1.72 abcd	0.68 ab	0.95 a	1.52 c	0.52 cdef	0.62 cd	1.44 bc
	70	5.33 ab	3.28 abc	2.23 abc	0.93 e	1.18 cd	1.59 de	0.59 cd	0.96 a	1.50 c	0.55 abcde	0.65 bcd	1.49 bc
	90	4.52 cde	2.95 cd	2.22 abc	1.19 cde	1.18 cd	1.66 cd	0.68 ab	0.94 a	1.52 c	0.54 abcde	0.73 ab	1.45 bc
BM	50	5.33 ab	3.20 abcd	2.06 cd	1.30 bcde	1.17 cd	1.62 de	0.69 ab	0.85 bcd	1.61 abc	0.55 abcde	0.60 d	1.48 bc
	70	5.35 ab	3.51 ab	2.21 abc	1.57 abcd	1.35 abcd	1.59 de	0.69 ab	0.91 ab	1.63 abc	0.53 bcdef	0.60 d	1.48 bc
	90	5.75 a	3.30 abc	2.27 ab	1.21 cde	1.23 bcd	1.63 de	0.67 ab	0.97 a	1.74 ab	0.60 a	0.75 a	1.73 a
CM	50	5.37 ab	3.35 abc	2.13 bcd	1.12 de	1.14 cd	1.87 a	0.57 de	0.80 d	1.60 abc	0.52 cdef	0.62 cd	1.48 bc
	70	3.88 ef	2.69 de	2.07 cd	0.94 e	1.04 d	1.61 de	0.62 bcd	0.84 bcd	1.62 abc	0.50 efg	0.63 cd	1.51 bc
	90	4.84 bc	3.05 abcd	2.40 a	0.99 e	1.00 d	1.67 bcd	0.59 cd	0.93 a	1.68 abc	0.56 abcd	0.64 cd	1.51 bc

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

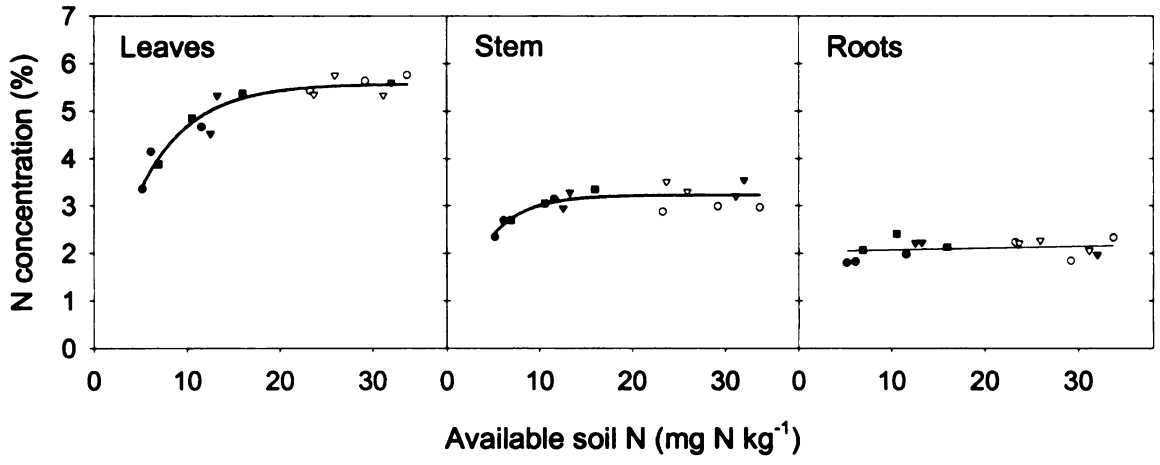

Figure 2.3. Relationship between tissue N concentration and available soil N level at 20 DAT. The symbols correspond to (●) control; (○) urea; (▼) AP; (▽) BM; (■) CM.

to the other treatments (Table 2.5). Stem N concentration decreased slightly below 10 mg available N kg^{-1} (Figure 2.3). Relative differences among treatments were similar between leaf and stem N concentration, but variation among treatments was smaller with stem N concentration (Table 2.5). No correlation between root N concentration and available soil N level was observed (Figure 2.3). Regardless of available soil N level, root N concentration increased slightly in relation to soil moisture (Table 2.5). The explanation for the contrasting responses to available soil N level among the three tissue types may be that N was translocated from leaves into stem and roots under N deficiency (Gastal and Lemaire, 2002).

From 20 to 35 DAT, leaf and stem N concentrations decreased rapidly, whereas root N concentration decreased slightly (Table 2.5). Limited N supply induced chlorosis on the lower leaves in all treatments, indicative of N deficiency (Figure 2.4). At 35 DAT, available soil N was reduced below 10 mg kg^{-1} in all treatments (Table 2.6), and no

Table 2.6. Temporal changes in NH_4^+- and NO_3^--N contents in soil treated with different organic N sources used to grow kale at different soil moistures.†

		Days after transplanting									
		0		20		35		50		60	
N source	Soil moisture	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	% WHC	mg N kg^{-1}soil									
Control	50	1.0 b	17.2 b	1.8 bc	3.4 e	0.9 e	2.4 e	1.0 f	2.3 d	1.3 h	2.5 g
	70	--	--	1.8 bc	4.3 de	1.3 cd	2.7 e	1.2 ef	2.5 cd	1.4 gh	2.7 g
	90	--	--	1.9 bc	9.7 cde	1.2 cde	3.1 de	1.6 cd	4.5 ab	1.5 fg	3.1 f
Urea	50	1.8 b	17.3 b	2.2 abc	27.0 ab	1.1 de	6.3 a	1.5 de	4.1 abc	1.5 fg	4.8 a
	70	--	--	1.7 c	21.6 abc	1.3 cd	4.3 bcd	1.6 cd	3.1 bcd	1.7 ef	3.2 ef
	90	--	--	2.2 abc	31.6 a	1.7 ab	4.2 bcd	1.8 bc	3.8 bc	2.2 ab	3.8 cd
AP	50	2.1 b	19.2 a	2.0 abc	30.1 a	1.2 cde	4.4 bcd	1.4 de	3.9 bc	1.6 f	4.3 b
	70	--	--	1.8 bc	11.5 bcd	1.4 bcd	3.1 de	1.9 bc	3.3 bcd	2.2 ab	3.3 def
	90	--	--	2.3 ab	10.3 bcde	1.5 bc	5.5 ab	2.0 b	4.5 ab	1.8 de	3.6 cde
BM	50	1.5 b	18.4 ab	2.3 ab	28.9 a	1.5 bc	5.1 bc	2.0 b	4.5 ab	1.5 fg	4.5 ab
	70	--	--	2.1 abc	21.6 abc	1.8 a	3.9 cd	1.9 bc	3.5 bcd	2.0 bc	3.8 cde
	90	--	--	2.5 a	23.5 ab	1.8 a	4.6 bc	2.1 ab	4.7 a	2.0 c	4.0 bc
CM	50	14.6 a	18.6 ab	2.1 abc	13.9 b	1.5 bc	3.5 cde	1.7 cd	3.6 bcd	1.7 ef	3.4 def
	70	--	--	2.1 abc	4.8 de	1.9 a	3.2 cde	1.9 bc	3.4 bcd	1.9 cd	3.3 ef
	90	--	--	2.3 ab	8.3 cde	1.7 ab	5.2 abc	2.4 a	4.0 b	2.3 a	3.5 cde

† Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

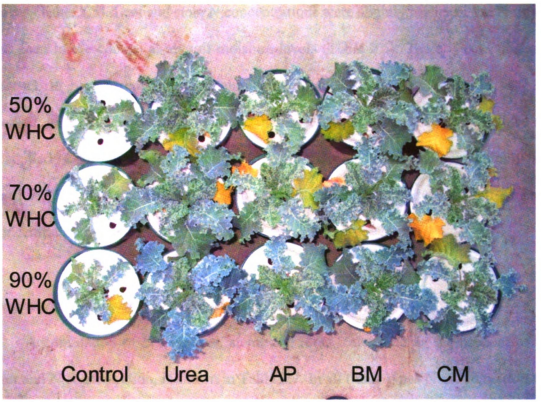

Figure 2.4. Kale plants treated with different organic N sources grown at different soil moistures at 33 DAT.

relationship between N concentration and available soil N level was found. Leaf and stem N concentrations were higher in the N treated plants than in the control at 50 and 70% WHC (Table 2.5). Among the N-treated plants, leaf and stem N concentrations were relatively high in the urea-treated plants and were lowest in the CM-treated plants at all soil moisture levels. Among soil moisture levels, decreases in leaf and stem N concentrations were related to increases in dry matter production (Table 2.4 and 2.5), suggesting that increased growth rate accelerated N deficiency. Root N concentration was slightly higher in the N-treated plants than in the control at all soil moisture levels (Table 2.5). Soil moisture had a small influence on root N concentration.

At 50 and 60 DAT, N concentration was highest in the order: roots > stem

> leaves (Table 2.5). Leaf and stem N concentrations were slightly higher in the N-treated plants than in the control at all soil moisture levels (Table 2.5). There was no apparent difference in root N concentration among treatments. At 60 DAT, as soil moisture increased, leaf and stem N concentrations increased slightly in all treatments. Since soil and applied N did not supply sufficient N, N concentration decreased considerably with growth in all treatments. As a result, N source and soil moisture had a small influence on N concentration at the late growth stage.

Nitrogen accumulation and partitioning among leaves, stem, and roots

Accumulation of N in leaves, stem, and roots, calculated as the product of dry matter and N concentration, is shown in Table 2.7. In all tissue types N accumulation was significantly affected by N source, soil moisture, and growth stage based on ANOVA ($P < 0.01$, data not shown). Partitioning patterns of accumulated N among the tissue types were also influenced by N source, soil moisture, and growth stage.

At 20 DAT, even though there were significant differences in N concentration among treatments (Table 2.5), N accumulation was more closely associated with dry matter production in all tissue types (Table 2.4 and 2.7). This was due to greater variation among treatments in dry matter than in N concentration. Since N application increased N concentration, the positive effect of N application on N accumulation was more noticeable than that on dry matter production (Table 2.4 and 2.7).

From 20 to 35 DAT, leaf N accumulation decreased in the treatments with leaf N concentration below 1%, whereas stem and root N accumulation increased in all treatments (Table 2.5 and 2.7). The N depletion in leaves can be explained by

Table 2.7. Temporal changes in N accumulation in leaves, stem, and roots of kale treated with different organic N sources grown at different soil moistures.†

		Days after transplanting											
		20			35			50			60		
N source	Soil moisture	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
	% WHC	N mg											
Control	50	29.6 ef	0.7 cd	1.3 cd	21.8 g	1.4 f	8.0 d	15.7 f	1.4 f	11.1 f	13.7 g	2.0 i	11.7 d
	70	28.8 f	0.8 bcd	1.6 bcd	23.2 g	1.5 f	7.9 de	17.6 f	1.8 ef	11.1 f	16.2 i	2.2 i	12.0 d
	90	20.8 g	0.6 d	1.2 cd	23.8 g	2.2 ef	5.3 e	18.4 f	2.0 e	10.2 f	19.3 h	3.0 h	10.0 d
Urea	50	70.7 abc	1.9 a	1.2 cd	88.2 a	4.5 a	12.3 bc	63.6 a	6.9 ab	18.9 d	48.7 cd	6.8 abc	24.6 abc
	70	75.8 ab	2.1 a	2.7 ab	83.4 a	4.3 a	14.0 bc	58.2 ab	7.4 a	20.2 bcd	58.2 a	7.9 a	24.8 abc
	90	58.7 abcd	1.6 ab	2.2 bc	77.9 b	4.4 a	17.4 a	65.0 a	7.4 a	21.2 bc	61.5 a	7.4 ab	23.7 abc
AP	50	40.3 cdef	1.2 abc	0.8 d	76.2 bc	3.6 abc	13.1 bc	46.8 c	6.1 b	20.1 cd	42.6 e	5.6 de	26.1 ab
	70	61.5 a	1.6 a	2.1 bc	53.2 e	3.9 ab	15.8 ab	44.4 cd	6.2 b	20.5 bcd	45.8 d	5.6 de	26.6 ab
	90	35.6 def	1.1 abc	1.8 bcd	38.7 f	1.9 ef	10.0 cd	42.6 cde	4.6 d	13.6 e	39.6 f	4.9 f	20.8 c
BM	50	45.0 cdef	1.3 abc	1.2 cd	66.8 cd	3.4 abc	14.8 ab	54.0 b	5.8 bc	21.1 bcd	50.3 cd	5.8 cde	27.7 a
	70	53.3 abcd	1.7 a	1.6 bcd	71.7 bcd	3.7 abc	15.1 ab	61.9 a	6.4 ab	22.9 ab	51.8 bc	6.3 bcd	24.8 abc
	90	40.5 cdef	1.1 abc	1.4 cd	64.2 d	3.8 ab	13.1 bc	60.3 a	7.2 a	20.1 cd	56.7 ab	7.0 ab	20.7 c
CM	50	46.4 bd	1.3 abc	1.5 cd	52.9 e	2.9 cd	18.6 a	37.2 e	4.3 d	22.5 bc	34.7 g	4.1 g	23.4 bc
	70	60.8 a	1.8 a	3.3 a	49.2 e	3.3 bc	14.1 abc	45.7 c	5.2 c	26.5 a	40.4 ef	5.5 ef	28.1 a
	90	45.8 cd	1.3 abc	1.9 bc	44.0 f	2.5 de	14.4 abc	40.6 de	5.3 c	22.1 bc	40.6 ef	5.1 ef	23.5 bc

† Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

translocation of N from leaves into stem and roots under severe N deficiency. At 35 DAT, greater N accumulation in leaves and stem did not consistently correlate with greater dry matter production. Leaf and stem N accumulation was greatest in the urea-treated plants at all soil moisture levels, though there was no difference in dry matter among the N-treated plants at 50 and 70% WHC (Table 2.4 and 2.7). Among soil moisture levels, N accumulation in leaves and stem was closely associated with N concentration rather than with dry matter production (Table 2.5 and 2.7). It was likely that increased growth rate resulted in decreased N concentration and consequent N depletion to a greater degree. In roots, with relatively small variation in N concentration, N accumulation was more closely associated with dry matter production (Table 2.4 and 2.7).

From 35 to 60 DAT, N depletion in leaves occurred in almost all treatments (Table 2.7). Continuous increases in N accumulation were observed only in roots, suggesting that N translocation occurred from leaves and stem into roots at this late growth stage. At 50 and 60 DAT, N accumulation was closely associated with dry matter production in all tissue types (Table 2.4 and 2.7). At 60 DAT, since N concentration increased with increasing soil moisture, the positive effect of soil moisture on N accumulation was more noticeable than that on dry matter production (Table 2.4 and 2.7).

The percentage of N partitioned in leaves was highest (> 90%) at 15 DAT and decreased with time (Table 2.7). At 40 DAT, the greatest N partitioning (67%) in leaves was obtained in the BM-treated plants at 90% WHC. The lowest N partitioning (50%) in leaves was obtained in the control at 50% WHC. Greater N partitioning in leaves consistently correlated with greater leaf N concentration. Therefore, it was likely that N translocation from leaves into stem and roots decreased N partitioning in leaves.

Net N uptake

Net N uptake, calculated as the sum of N accumulated in leaves, stem, and roots, is shown in Table 2.8. Net N uptake was significantly affected by N source, soil moisture, and growth stage based on ANOVA ($P < 0.05$, data not shown). The sum of N recovered as available soil N and net N uptake is also shown in Table 2.8. Greater N recovery may indicate greater N released from an applied N source. In general, N recovery was greatest in the treatment order: urea > BM > AP > CM > control.

At 20 DAT, greater net N uptake did not consistently correlate with greater N recovery. The CM-treated plants accumulated greater N than the AP- and BM-treated plants, though CM released less N (Table 2.8). This indicates that the CM-treated plants effectively utilized inorganic N initially contained in CM (Table 2.8). The urea-treated plants accumulated the greatest N, which coincided with the greatest N recovery (Table 2.8). In the urea treatment, rapid N release likely enhanced N uptake (Table 2.8). Soil moisture had both positive and negative influences on net N uptake. Increasing soil moisture from 50 to 70% WHC increased net N uptake by the N-treated plants, though N recovery data shows that it did not enhance N release from applied N sources (Table 2.8). The explanation for the increased N uptake may be that increasing soil moisture enhanced movement of soil N to roots. Firstly, mass flow of N to roots increased by accelerated water movement and increased growth rate (Havlin et al., 1999). Secondly, root interception of soil N increased by extensive root growth (Havlin et al., 1999). Further increases to 90% WHC decreased net N uptake in all treatments (Table 2.8). This decrease may be due to low N demands by kale resulting from the inhibited plant growth at 90% WHC (Table 2.4). In addition, N recovery data shows that N released from AP

Table 2.8. Temporal changes in net N uptake by kale treated with different organic N sources grown at different soil moistures.[†]

		Days after transplanting								
		0	20		35		50		60	
N source	Soil moisture	Soil N	N uptake	N uptake + soil N	N uptake	N uptake + soil N	N uptake	N uptake + soil N	N uptake	N uptake + soil N
	% WHC	mg pot^{-1}								
Control	50	35 b	32 ef	42 g	31 i	38 j	28 i	34 j	27 h	35 k
	70	--	31 fg	43 fg	33 i	40 j	30 h	38 ij	30 g	38 j
	90	--	23 g	45 f	31 i	39 j	31 h	42 i	32 g	41 i
Urea	50	36 b	74 abc	129 a	105 a	119 a	89 a	100 abc	80 b	92 cd
	70	--	81 a	125 a	102 ab	112 b	86 ab	95 bcd	91 a	100 ab
	90	--	63 bcde	127 a	100 b	111 b	94 a	104 a	93 a	104 a
AP	50	40 b	42 cdef	103 b	93 c	104 c	73 de	83 ef	74 c	86 ef
	70	--	65 b	90 cd	73 ef	82 fg	71 de	81 ef	78 bc	89 de
	90	--	39 def	62 e	51 h	64 i	61 g	73 h	65 ef	76 h
BM	50	38 b	48 cdef	107 b	85 cd	98 cd	81 bc	93 cd	84 b	95 bc
	70	--	57 bcd	102 bc	90 cd	101 cd	91 a	101 ab	83 b	94 cd
	90	--	43 cdef	92 bcd	81 de	93 de	88 a	101 ab	84 b	96 bc
CM	50	63 a	49 cd	80 d	74 e	84 ef	64 fg	74 gh	62 f	72 h
	70	--	66 b	79 d	67 fg	76 gh	77 cd	87 de	74 cd	84 fg
	90	--	49 cd	69 e	61 g	74 h	68 ef	80 fg	69 de	80 g

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

and CM significantly decreased at 90% WHC, which may partly account for the decreases in net N uptake by the AP- and CM-treated plants.

From 20 to 35 DAT, the control increased net N uptake only at 90% WHC, whereas the N-treated plants increased net N uptake at all soil moisture levels (Table 2.8). At 35 DAT, net N uptake was limited by N supply, thus positively correlating with N recovery (Table 2.8). The urea-treated plants accumulated significantly greater N than the plants treated with the natural organic materials at all soil moisture levels. Increasing soil moisture did not increase net N uptake, but significantly decreased net N uptake by the AP and CM-treated plants (Table 2.8). Since N was a limiting factor at all soil moisture levels, the decreases in net N uptake can be attributed to less N released from AP and CM rather than to adverse physiological effects at high soil moisture.

From 35 to 60 DAT, the urea-treated plants decreased net N uptake at all soil moisture levels, and the AP- and CM-treated plants decreased net N uptake at 50% WHC (Table 2.8). This N depletion was due to senescent leaf-fall under severe N deficiency. At 50 and 60 DAT, the urea- and BM-treated plants accumulated significantly greater N compared to the AP- and CM-treated plants at all soil moisture levels. At 60 DAT, maximum net N uptake (91 and 93 mg $plant^{-1}$) occurred in the urea-treated plants at 70 and 90% WHC. Among the N-treated plants the least net N uptake (65 and 62 mg $plant^{-1}$) occurred in the AP-treated plants at 90% WHC and the CM-treated plants at 50% WHC. As soil moisture increased from 50 to 90% WHC, the control, urea-, BM-, and CM-treated plants increased net N uptake by 18, 16, 1, and 11%, respectively, but the AP-treated plants decreased net N uptake by 12%. This increase in the urea- and CM-treated plants was due to rapid senescence at 50% WHC rather than to increased N released from

urea and CM. Increasing soil moisture from 70 to 90% WHC had a negative influence on net N uptake by the AP- and CM-treated plants. This was consistent throughout the growing period, and likely due to less N released from AP and CM at 90% WHC.

Apparent N use efficiency (ANUE)

According to ANUE determined at 60 DAT, the proportion of applied N that was utilized by kale was greatest in the order: urea (47-53%) > BM (32-34%) > AP (18-26%) > CM (13-16%), regardless of soil moisture (Figure 2.5).

Using data from the previous soil incubation study, N availability from the organic N sources was estimated and compared with ANUE (Table 2.3). Apparently, greater ANUE was related to greater N availability.

Increase in soil moisture from 50 to 70% WHC increased ANUE by 15 and 25% for urea and CM, respectively, but did not significantly affect AUNE for AP and BM (Figure 2.5). Further increases to 90% WHC decreased ANUE by 31 and 15% for AP and CM, but did not significantly affect ANUE for urea and BM. The results show that estimated N availability did not accurately predict variations in N availability among soil moisture levels. This could be explained in part by insufficient N supply that resulted in plant senescence. The other explanation may be that soil moisture influenced N availability differently in the two studies. Although constant soil moisture was maintained in the previous soil incubation study, soil was exposed to repeated drying-rewetting cycles in this study. Fierer and Schimel (2002) found that when soil experienced a series of drying-rewetting, soil N mineralization significantly decreased compared to the unstressed control. In addition, Fierer et al. (2003) reported that drying-

rewetting cycles changed microbial community composition. Thus, a shift in microbial community composition may result in differential N availability at constant soil moisture and under drying-rewetting cycles.

Low ANUE relative to the estimated available N may be explained by N immobilization (Beauchamp, 1986; Martín-Olmedo et al., 1999; Burger and Jackson, 2003) or gaseous losses of N through denitrification (Paul and Beauchamp, 1993; Mulvaney et al., 1997; Khaili et al., 2002) and volatilization (Craig and Wollum II, 1982; Manjula and Malzer, 1994; Sullivan et al., 2003). Nitrate leaching likely did not occur, as soil moisture was maintained below field capacity throughout the growing period. No attempt was made to identify the unaccounted portion of applied N in this study.

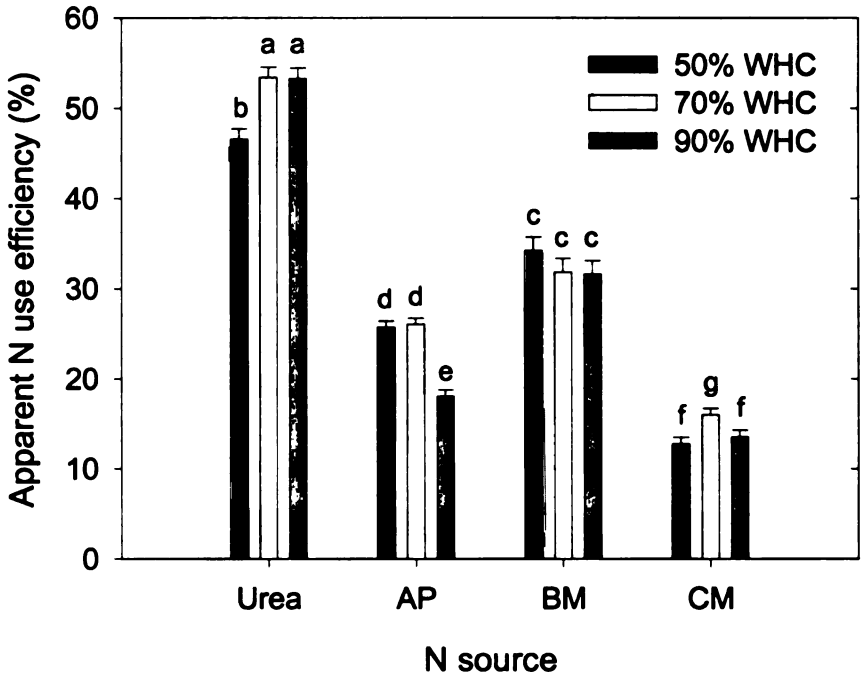

Figure 2.5. Effects of soil moisture on apparent N use efficiency by kale treated with different organic N sources at 60 DAT. Error bars represent 1 SE. Bars with the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

CONCLUSIONS

Shoot production and N uptake by kale is affected directly by the level of soil moisture, as well as indirectly by the effect of soil moisture on N availability from an applied N source. In this study, the former soil moisture effect was apparent in the early growth stage and consistent among N sources, whereas the latter soil moisture effect was apparent in the late growth stage and varied among N sources. Root production by kale is affected directly by the level of soil moisture. Root growth appears to be less dependent on N availability than shoot growth. To what extent the soil moisture effects on crop production are a result of N availability limitation versus direct soil moisture limitation is needed to be studied at various N application rates. Such information will be valuable to making decisions for the most efficient use of organic N sources especially in greenhouse production systems, where irrigation control can be easily managed.

Estimated N availability using the previous soil incubation data is a reliable indicator of N availability on a field scale. However, it will not accurately predict variations in N availability among soil moistures. In the field, although soil moisture may have a small influence on N availability from urea, BM, and CM, increasing soil moisture may significantly reduce N availability from AP. When conducting a soil incubation study, distribution of soil water has to be considered in order to better evaluate soil moisture effects on N availability.

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CHAPTER 3

AVAILABILITY OF NITROGEN FROM ORGANIC NITROGEN SOURCES AS AFFECTED BY TEMPERATURE: BIOASSAY USING KALE (*Brassica oleracea* L.)

INTRODUCTION

Temperature is one of the major environmental factors affecting nitrogen (N) availability from organic N sources. Increasing temperature enhances N release from organic N sources by stimulating microbial activity and accelerating diffusion of soluble substrates in soil (Nicolardot et al., 1994; MacDonald et al., 1995; Zak et al., 1999). In addition, increasing temperature induces a shift in the composition of microbial communities (Richards et al., 1985; Carreiro and Koske, 1992; Zogg et al., 1997), which parallels an increase in microbial activity (Zogg et al., 1997). It is suggested that microbial communities favored at high temperature have ability to metabolize substrates that are not utilized at lower temperatures (Zogg et al., 1997).

The effects of temperature on N availability have been evaluated for various organic N sources under laboratory conditions. Urea hydrolysis rate increases in relation to temperature with maximum urease activity occurring between 60 and 70°C (Overrein and Moe, 1967; Saharawat, 1984; Moyo et al., 1989; Lai and Tabatabai, 1992). MacLean and MacRae (1987) studied the rate of urea hydrolysis in soil incubated at different temperatures, and reported that 52, 67, 80, and 93% of urea was hydrolyzed at 4, 9, 13, and 18°C, respectively, after three days of incubation. Griffin and Honeycutt (2000) incubated soil amended with dairy, poultry, and swine manures for 112 days, and found that increasing temperature from 10 to 24°C significantly accelerated the mineralization rate. Cookson et al. (2002) used clover residues in a soil incubation study, and reported that 22, 33, 41, and 60% of N was mineralized at 2, 5, 10, and 15°C, respectively after 160 days of incubation. Ultimately, the question of the interest is how accurately such information estimate actual N availability during the growing season.

In the field conditions, Paul and Beauchamp (1994) compared N uptake by corn amended with fresh cow manure, composted cow manure, and ammonia sulfate [$(NH_4)_2SO_4$] at temperatures of 27/17 and 18/12°C day/night. They found a greater N uptake response to temperature by corn amended with fresh cow manure than with composted cow manure and ammonia sulfate. This may be a result of greater N mineralization response to temperature of fresh cow manure.

Previously, temperature effects on N availability from four organic N sources, including urea, alfalfa pellets, blood meal, and partially composted chicken manure, were examined in a soil incubation study. The objectives of this study were to: (1) examine the effects of temperature on plant growth, N uptake, and N use efficiency by kale treated with the organic N sources, and (2) evaluate the correlation between N availability from the organic N sources determined in the previous soil incubation study and N use efficiency by kale.

CHAPTER 3.1

AVAILABILITY OF NITROGEN FROM ORGANIC NITROGEN SOURCES AS AFFECTED BY TEMPERATURE: BIOASSAY USING KALE (*Brassica oleracea* L.) WITH A LIMITED NITROGEN SUPPLY

MATERIALS AND METHODS

Soil

The soil used in this study was a Granby sandy clay loam (sandy, mixed, mesic Typic Haplaquolls). Approximately 400 kg of surface soil (15 cm) was collected from the Michigan State University Horticulture Teaching and Research Center in East Lansing MI, in April 2003. The soil was passed through a 5 mm sieve, thoroughly mixed to ensure uniformity, and stored in a covered plastic container under field moisture condition (5%, w/w) at room temperature (20-23°C) until the experiment was initiated to minimize disturbance of the microbial population (Pramer and Bartha, 1972; Honeycutt, 1999). The chemical and physical properties of the soil are listed in Table 3.1.1. All soil analyses were done by the same procedures used in the previous soil incubation study.

Nitrogen sources

Urea [$CO(NH_2)_2$] was used as a synthetic organic N fertilizer. Three natural organic materials were used. Alfalfa pellets (AP), which were obtained from Bradfield Industries, Inc. (Springfield, MI), are alfalfa-based fertilizers blended with animal protein, natural sulfate of potash, and molasses. Blood meal (BM) was obtained from Glorious Gardens Blood Meal Growing Markets, Inc. (West Des Moines, IA). Partially composted chicken manure (CM) was obtained from Herbruck's Poultry Ranch, Inc. (Saranac, MI). Chemical properties of these N sources are listed in Table 3.1.2. All nutrient analyses were done by the same procedures used in the previous soil incubation study.

Table 3.1.1. Chemical and physical properties of soil used in this study.

pH	Sand	Silt	Clay	CEC	OM	Total C	Total N	NH_4^+	NO_3^-	P	K	Ca	Mg
	------ % ------			cmol kg^{-1}	%	-- g N kg^{-1} --		---------- mg N kg^{-1} ----------					
6.10	54.7	15.4	29.8	66.9	3.7	21.3	4.9	5.7	7.0	206	190	813	171

Table 3.1.2. Chemical characteristics of four organic N sources used in this study.[†]

N source	Moisture	pH	Total C	Total N	C/N ratio	NH_4^+	NO_3^-	P	K	Ca	Mg	Fe
	%		---- % ----			-- g N kg^{-1} --		---------- % ----------				
Urea	--	--	--	46.00	--	--	--	--	--	--	--	--
AP	4.46	5.73	39.3	3.60	10.93	0.49	0.15	0.58	4.55	1.69	0.16	0.06
BM	5.01	7.30	41.3	12.65	3.26	0.15	0.02	0.51	0.64	1.12	0.11	0.11
CM	9.11	8.78	26.6	3.03	8.79	1.42	0.12	2.96	3.62	18.41	0.87	0.09

[†] All values are expressed on a dry weight basis (105°C).

Experimental design

A pot experiment with kale (*Brassica oleracea* L. cv. Winterbor F1) was conducted using growth chambers during April to July 2003. The experimental design was a completely randomized design with three replications. Treatments consisted of a factorial combination of three temperature levels [15/10, 20/15, and 25/20°C day/night (14/10 hr)], five N sources (control, urea, AP, BM, and CM), and four growth stages [20, 35, 50, and 60 days after transplanting (DAT)].

Experimental procedure and growing conditions

Kale was seeded on a 2.5 cm depth plastic cell tray filled with a commercial seedling/propagating mix and placed in a greenhouse on 18 April 2003. Twenty-day-old seedlings were transplanted singly in 1.6-litter pots that measured 15 cm in diameter. One day before transplanting, 1900 g of soil (oven dry basis) was mixed with urea, AP, BM, or CM at the rate of 63, 100, 92, and 150 mg N per 1 kg of soil (oven dry basis), respectively, and filled into the pots. Pots were also filled with 1900 g of soil with no N source as a control. The application rates were calculated to provide approximately equal amounts of 60 mg available N kg^{-1} soil (134 kg available N ha^{-1}) using Equation [1].

$$\text{Estimated available N} = N_i + fN_o \qquad [1]$$

where N_i is the inorganic N (NH_4^+-N + NO_3^--N) content, N_o is the organic N (total N – inorganic N) content, and f is the proportion of organic N fraction expected to be released during the growing period (Griffin and Honeycutt, 2000). As in the previous soil incubation study, coefficient f of 0.95, 0.59, 0.65, and 0.39 was applied for urea, AP, BM, and CM, respectively. Additional nutrients (P, K, Ca, and Mg) were not applied, since

there were sufficient amounts of these nutrients for optimum crop production (Table 3.1.1) according to soil tests and fertilizer recommendations for vegetable crops in Michigan (Warncke et al., 1992).

Amount of available N released from the organic N sources was estimated also by using a first-order model [2] determined in the previous soil incubation study (Table 3.1.3);

$$N_{rel} = N_0(1 - \exp^{-k_0 t}) \qquad [2]$$

where N_{rel} (mg N kg^{-1}) is the cumulative N released from an applied N source at time t, N_0 (mg N kg^{-1}) is the size of potentially mineralizable N, exp is the exponential constant with numerical value ≅ 2.718, and k_0 ($week^{-1}$) is the first-order rate constant. Availability of N from the organic N sources, as a percentage of applied N, was also estimated (Table 3.1.3).

Table 3.1.3. Available N as a function of applied N.

N source	Temperature	Applied N	Estimated available N	Estimated N availability
	°C	-- mg N kg^{-1} soil --		%
Urea	15/10	63	57	90
	20/15	63	57	91
	25/20	63	58	91
AP	15/10	100	36	37
	20/15	100	42	42
	25/20	100	48	49
BM	15/10	92	47	52
	20/15	92	50	56
	25/20	92	55	61
CM	15/10	150	53	36
	20/15	150	59	40
	25/20	150	64	44

The pots were watered with distilled water to 70% water holding capacity (WHC) by weighing, and randomly transferred into the growth chambers set at 15/10, 20/15, and 25/20°C (± 0.5°C) day/night temperatures. Relative humidity was set at 58, 70, and 78%, respectively, to maintain constant vapor pressure deficit among the three temperature levels. Lighting was provided by fluorescent and incandescent lamps that produced 420 ± 20 μmol m^{-2} s^{-1} PPF at plant height with a 14-hr photoperiod. In order to maintain soil moisture at 70% WHC during the experiment, the pots were watered by weighing and adding the required amount of distilled water every two days until 20 DAT and daily thereafter. Corrections for increasing plant weight were made on the basis of shoot fresh weight determined at each sampling time. The pots were covered by a plastic plate, which allowed passage of water into soil but reduced evaporation from surface soil.

Sampling, measurements, and chemical analyses

At 20, 35, 50, and 60 DAT, kale shoots were separated from soil by hand, and divided into leaves and stem for determination of fresh matter weight. Dry matter weight was determined after dried at 60°C for 48 hr. The dried tissues were then ground to pass 1 mm screen and analyzed for total Kjeldahl-N (TKN) content.

Soil was passed through a 2 mm sieve to remove roots. The sieved soil was dried at 38°C for 48 hr, thoroughly mixed and analyzed for pH and inorganic N content (NH_4^+-N and NO_3^--N). Roots were separated from the remaining soil by washing over 0.5 mm sieve in a hydropneumatic root elutriator (Gillison's Variety Fabrication, Inc., Benzonia, MI) as described by Smucker et al. (1982). After washing, debris and dead roots were manually removed from vital roots. The roots were then rinsed with distilled water and

dried at 60°C for 48 hr for determination of dry matter weight. The dried roots were ground to pass 1 mm sieve and analyzed for TKN content.

Apparent N use efficiency

Apparent N use efficiency (ANUE) was calculated using the difference method as follows (Motavalli et al., 1989):

$$\text{ANUE (\%)} = \{[N_{uptake}\text{ (N-treated plant)} - N_{uptake}\text{ (control)}] / N_{applied}\} \times 100 \quad [2]$$

where N_{uptake} (mg pot^{-1}) is the total N uptake by kale calculated as the sum of TKN in leaves, stem, and roots, and $N_{applied}$ (mg pot^{-1}) is the total N in an applied N source. The difference method assumes that soil N transformations remain constant in both the soil that received N source and the soil that received no N source. Thus, the difference in total N uptake between the two soils is assumed to be the amount of N from the applied N source taken up by the plant.

Statistical analysis

A two-way analysis of variance (ANOVA) was conducted to test significance of N source and growth stage effects and the interaction using the MIXED procedure of Statistical Analysis System (SAS) (SAS Institute, 1990). Temperature effects could not be analyzed statistically with the experimental design in this study. When statistically significant differences existed, treatment means were separated using the LSMEANS procedure, and then tested using paired *t* test at $\alpha = 0.05$. Data presented are the means of three replications.

RESULTS AND DISCUSSION

Dry matter production and partitioning among leaves, stem, and roots

Dry matter production of leaves, stem, and roots was significantly affected by N source, growth stage, and the interaction based on ANOVA ($P < 0.001$, data not shown). Although temperature effects could not be analyzed statistically with the experimental design in this study, dry matter production was apparently affected by temperature. Patterns of dry matter partitioning among the tissue types were also influenced by N source, temperature, and growth stage.

Leaf dry matter increased with time in a sigmoidal manner (Figure 3.1.1). At 20 DAT, the N-treated plants produced greater leaf dry matter than the control, with the difference magnified as temperature increased (Figure 3.1.1). The urea-treated plants produced significantly greater leaf dry matter than the AP- and BM-treated plants at all temperature levels (Table 3.1.4). The CM-treated plants produced leaf dry matter comparable to the urea-treated plants at 15/10 and 20/15°C (Table 3.1.4). Increasing temperature enhanced plant growth in all treatments, with more than three-fold increases

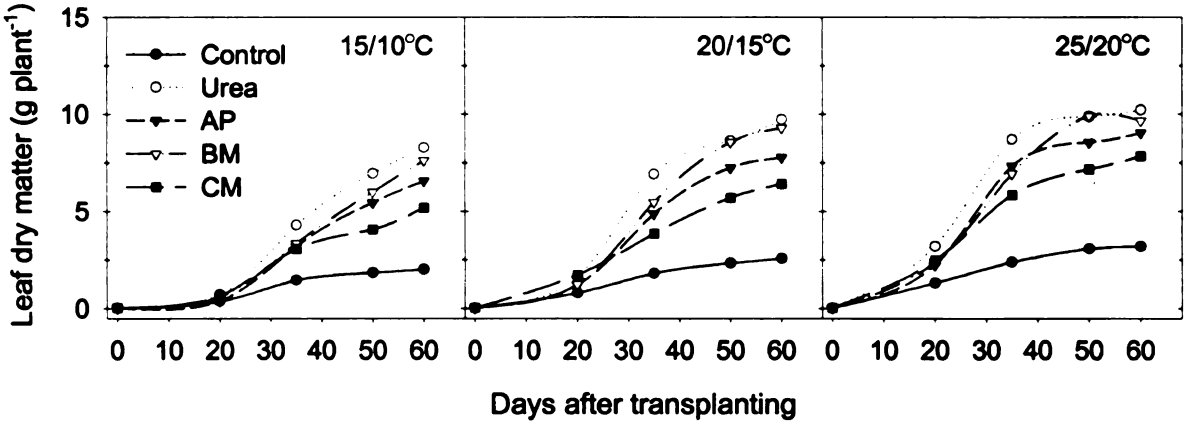

Figure 3.1.1. Temporal changes in leaf dry matter of kale treated with different organic N sources grown at different temperatures.

Table 3.1.4. Temporal changes in dry matter production of leaves, stem, and roots in kale treated with different organic N sources grown at different temperatures.[†]

		Days after transplanting											
		20			35			50			60		
Temperature	N source	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
°C		g plant^{-1}											
15/10	Control	0.37 c	0.02 b	0.09 b	1.46 c	0.07 c	0.35 d	1.84 e	0.13 d	0.50 b	2.02 e	0.21 d	0.68 b
	Urea	0.73 a	0.03 a	0.10 ab	4.28 a	0.21 a	0.72 a	6.95 a	0.45 a	1.04 a	8.28 a	0.70 a	1.47 a
	AP	0.38 c	0.02 b	0.06 c	3.20 b	0.17 b	0.59 c	5.45 c	0.38 b	1.20 a	6.57 c	0.60 b	1.65 a
	BM	0.59 b	0.03 a	0.09 b	3.34 b	0.17 b	0.61 bc	5.98 b	0.34 c	1.21 a	7.62 b	0.56 b	1.60 a
	CM	0.66 ab	0.04 a	0.11 a	3.07 b	0.15 b	0.64 b	4.06 d	0.31 c	1.31 a	5.18 d	0.46 c	1.63 a
20/15	Control	0.80 c	0.04 c	0.19 c	1.82 e	0.13 e	0.44 c	2.34 d	0.21 c	0.48 b	2.58 d	0.29 d	0.60 c
	Urea	1.61 a	0.09 a	0.24 b	6.91 a	0.46 a	0.86 ab	8.63 a	0.77 a	1.45 a	9.72 a	1.11 a	1.67 a
	AP	1.20 b	0.07 b	0.16 c	4.86 c	0.33 b	0.72 b	7.26 b	0.69 a	1.54 a	7.77 b	0.94 b	1.72 a
	BM	1.24 b	0.05 b	0.21 b	5.45 b	0.31 c	0.85 ab	8.55 a	0.67 a	1.46 a	9.30 a	0.94 b	1.42 b
	CM	1.73 a	0.09 a	0.34 a	3.85 d	0.26 d	0.89 a	5.70 c	0.48 b	1.44 a	6.41 c	0.78 c	1.77 a
25/20	Control	1.32 c	0.07 c	0.27 b	2.39 d	0.18 c	0.40 d	3.08 d	0.34 c	0.56 d	3.19 d	0.43 d	0.71 c
	Urea	3.20 a	0.18 a	0.41 a	8.71 a	0.72 a	0.81 c	9.88 a	1.07 a	1.13 c	10.21 a	1.22 b	1.31 b
	AP	2.23 b	0.13 bc	0.31 ab	7.34 b	0.64 ab	1.04 a	8.56 b	1.13 a	1.50 a	9.04 b	1.48 a	1.51 ab
	BM	2.38 b	0.14 bc	0.40 ab	6.94 bc	0.54 ab	0.93 bc	9.92 ab	1.06 a	1.21 bc	9.66 ab	1.48 a	1.43 ab
	CM	2.47 b	0.15 b	0.46 a	5.85 c	0.47 b	0.94 b	7.17 c	0.83 b	1.41 ab	7.84 c	1.05 c	1.70 a

[†] Means may be compared among N sources within temperature and growth stage. Means followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

in leaf dry matter at 25/20°C (Table 3.1.4). At 35 DAT, leaf dry matter was significantly greater in the N-treated plants than in the control at all temperature levels (Table 3.1.4), and differences continued to increase with growth (Figure 3.1.1). The urea-treated plants produced the greatest leaf dry matter at all temperature levels (Figure 3.1.1). The AP- and BM-treated plants produced greater leaf dry matter than the CM-treated plants at 20/15 and 25/20°C (Figure 3.1.1). Leaf dry matter at 25/20°C was nearly twice that at 15/10°C in all treatments (Table 3.1.4). At 50 and 60 DAT, leaf dry matter was greatest in the treatment order: urea > BM > AP > CM > control, at all temperature levels (Figure 3.1.1). Significant difference between the urea- and BM-treated plants was seen only at 15/10°C (Table 3.1.4). Maximum dry matter production of leaves (10.2 g $plant^{-1}$) occurred in the urea-treated plants at 25/10°C (Table 3.1.4). Among the N-treated plants the lowest dry matter production of leaves (5.2 g $plant^{-1}$) occurred in the CM-treated plants at 15/10°C (Table 3.1.4). The control, urea-, AP-, BM-, and CM-treated plants increased leaf dry matter by 58, 23, 38, 27, and 51%, respectively, as temperature increased from 15/10 to 25/20°C (Table 3.1.4).

Stem dry matter increased with time in a linear manner. Relative differences among treatments were similar between leaf and stem dry matter at all growth stages (Table 3.1.4). As plants matured, the percentage of dry matter partitioned in stem increased.

Root dry matter also increased with time and by N application; however, root growth was influenced by N source and temperature differently from shoot growth. At 20 DAT, relative differences among treatments were not similar between leaf and root dry matter at 25/20°C. From 35 to 60 DAT, the N-treated plants showed a similar root growth

within each temperature level (Table 3.1.4). Increasing temperature did not enhance root growth as much as shoot growth. For example, at 35 and 50 DAT, root dry matter did not increase beyond 20/15°C in all treatments (Table 3.1.4). At 60 DAT, root dry matter was relatively constant across temperature levels despite more shoot growth at higher temperatures (Table 3.1.4).

The ratio of shoot to root dry matter was determined as an indicator of dry matter partitioning in roots. In general, the shoot/root ratio at 15/10°C declined with time, whereas that at 20/15 and 25/20°C were relatively constant throughout the growing period (data not shown). At 60 DAT, the urea- and BM-treated plants showed higher shoot/root ratios compared to the AP- and CM-treated plants at all temperature levels (Figure 3.1.2). This was due to more shoot production in the urea- and BM-treated plants. Increasing temperature enhanced shoot production to a greater degree than root

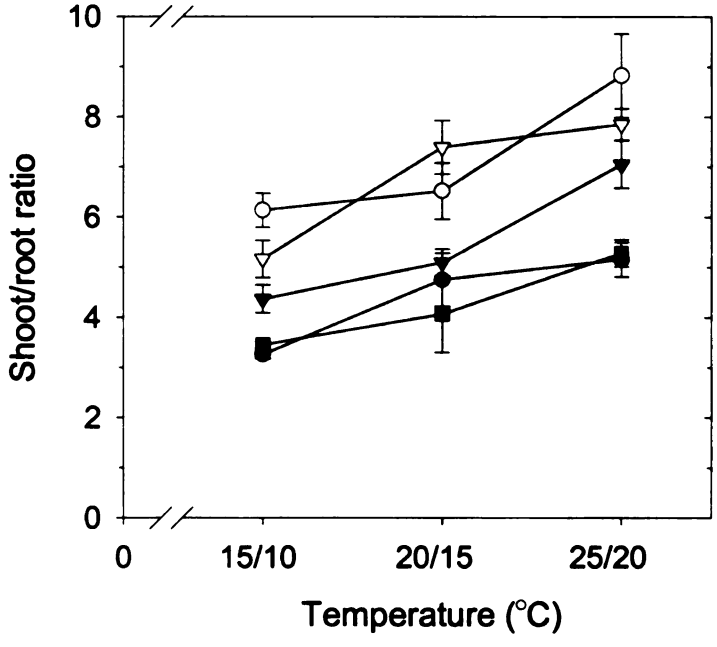

Figure 3.1.2. Effects of temperature on shoot/root ratio of kale treated with different organic N sources at 60 DAT. The symbols correspond to (●) control; (○) urea; (▼) AP; (▽) BM; (■) CM. Error bars represent 1 SE.

production, thereby increasing shoot/root ratio regardless of N source (Figure 3.1.2).

Nitrogen concentration and partitioning among leaves, stem, and roots

Nitrogen concentration in leaves, stem, and roots was significantly affected by N source, growth stage, and the interaction based on ANOVA ($P < 0.001$, data not shown). Although temperature effects could not be analyzed statistically with the experimental design in this study, N concentration was apparently affected by temperature. Partitioning patterns of N concentration among the tissue types differed between growth stages.

At 20 DAT, N concentration was highest in the order: leaves > stem > roots (Table 3.1.5). The relationship between N concentration and available soil N level was analyzed for each tissue type. Leaf and stem N concentrations decreased steeply below 5 mg available N kg^{-1} (Figure 3.1.3). For example, the control had significantly lower leaf and stem N concentrations than the N-treated plants at all temperature levels (Table 3.1.5). Among the N-treated plants the CM-treated plants had relatively low leaf and stem

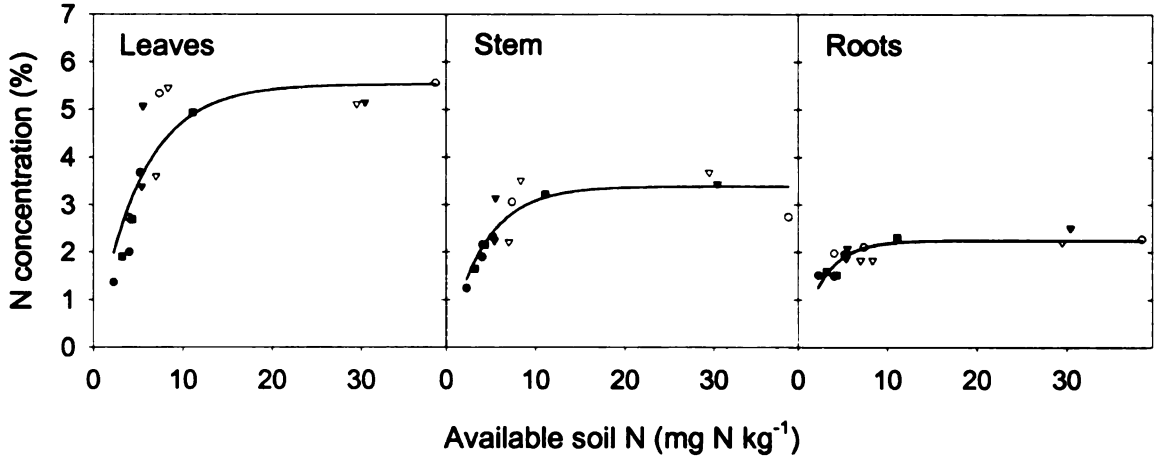

Figure 3.1.3. Relationship between tissue N concentration and available soil N level at 20 DAT. The symbols correspond to (●) control; (○) urea; (▼) AP; (▽) BM; (■) CM.

Table 3.1.5. Temporal changes in N concentration in leaves, stem, and roots of kale treated with different organic N sources grown at different temperatures.[†]

		Days after transplanting											
		20			35			50			60		
Temperature	N source	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
°C		% N											
15/10	Control	3.67 c	2.32 b	1.94 d	0.95 c	1.57 ab	1.37 c	0.70 c	0.71 c	1.49 a	0.55 c	0.63 b	1.35 a
	Urea	5.55 a	3.41 a	2.27 bc	1.85 a	1.66 a	1.83 a	1.06 a	1.36 a	1.59 a	0.67 ab	0.80 a	1.52 a
	AP	5.15 b	3.44 a	2.51 a	1.68 a	1.36 bc	1.65 b	0.92 b	1.23 a	1.52 a	0.71 a	0.77 a	1.43 a
	BM	5.11 b	3.68 a	2.21 c	1.84 a	1.30 bc	1.63 b	1.02 ab	1.23 a	1.55 a	0.66 ab	0.82 a	1.52 a
	CM	4.93 b	3.22 a	2.31 b	1.21 b	1.16 c	1.57 b	0.76 c	0.96 b	1.50 a	0.60 bc	0.69 b	1.38 a
20/15	Control	2.00 d	1.89 c	1.49 b	0.80 c	0.93 bc	1.51 b	0.58 a	0.66 b	1.51 a	0.44 b	0.59 b	1.29 d
	Urea	5.33 ab	3.05 a	2.10 a	1.12 ab	0.93 bc	1.66 a	0.66 a	0.84 a	1.66 a	0.49 a	0.58 b	1.54 ab
	AP	5.07 b	3.14 a	2.08 a	1.08 b	1.02 ab	1.63 ab	0.64 a	0.88 a	1.50 a	0.49 ab	0.58 b	1.40 cd
	BM	5.45 a	3.51 a	1.83 a	1.21 a	1.03 a	1.59 ab	0.64 a	0.82 a	1.58 a	0.51 a	0.64 a	1.57 a
	CM	2.69 c	2.15 b	1.52 b	1.03 b	0.81 c	1.54 ab	0.59 a	0.82 a	1.49 a	0.53 a	0.64 a	1.42 bc
25/20	Control	1.36 b	1.24 c	1.51 b	0.67 a	0.71 a	1.51 b	0.55 ab	0.70 b	1.52 a	0.53 a	0.63 b	1.47 a
	Urea	2.72 a	2.15 a	1.97 a	0.78 a	0.77 a	1.70 a	0.57 a	0.77 a	1.55 a	0.47 a	0.61 b	1.57 a
	AP	3.38 a	2.24 ab	1.87 a	0.76 a	0.74 a	1.48 b	0.53 b	0.77 a	1.47 a	0.51 a	0.66 a	1.43 a
	BM	3.60 a	2.21 a	1.83 a	1.04 a	0.93 a	1.77 a	0.55 ab	0.78 a	1.56 a	0.51 a	0.63 ab	1.46 a
	CM	1.90 a	1.65 b	1.59 b	0.70 a	0.79 a	1.49 b	0.52 b	0.79 a	1.50 a	0.53 a	0.65 a	1.53 a

[†] Means may be compared among N sources within temperature and growth stage. Means followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

N concentrations at 20/15 and 25/20°C (Table 3.1.5). Leaf N concentration decreased as temperature increased, probably due to reduced available soil N resulting from increased growth rate (Table 3.1.5 and 3.1.6). Root N concentration decreased slightly below 10 mg available N kg^{-1} (Figure 3.1.3). The control and CM-treated plants had significantly lower root N concentrations than the urea-, AP-, and BM-treated plants at 20/15 and 25/20°C (Table 3.1.5). As temperature increased, root N concentration decreased slightly. The explanation for the contrasting responses to available soil N level among the three tissue types may be that N was translocated from leaves into stem and roots under N deficiency (Gastal and Lemaire, 2002).

From 20 to 35 DAT, leaf and stem N concentrations decreased rapidly, whereas root N concentration decreased slightly (Table 3.1.5). At 35 DAT, available soil N was reduced below 5 mg kg^{-1} in all treatments (Table 3.1.6), and no relationship between N concentration and available soil N level was found. Leaf N concentration was significantly higher in the N-treated plants than in the control at 15/10 and 20/15°C (Table 3.1.5). The CM-treated plants had a significantly lower leaf N concentration than the urea-, AP-, and BM-treated plants at 15/10°C. As temperature increased, leaf N concentration decreased in all treatments, suggesting that rapid plant growth accelerated N deficiency. Relative differences among treatments were similar between leaf and stem N concentration, but variation among treatments was smaller with stem N concentration (Table 3.1.5). Root N concentration was significantly higher in the N-treated plants than in the control at 15/10°C (Table 3.1.5). Among the N-treated plants, the urea-treated plants had a relatively high root N concentration at all temperature levels. Temperature had a small influence on root N concentration.

Table 3.1.6. Temporal changes in NH_4^+- and NO_3^--N contents in soil treated with different organic N sources used to grow kale at different temperatures.†

		Days after transplanting									
		0		20		35		50		60	
Temperature	N source	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
°C		mg N kg^{-1}soil									
15/10	Control	5.7 b	7.0 b	1.8 c	3.4 d	1.5 b	0.6 b	1.7 c	0.9 b	1.5 a	1.9 d
	Urea	6.0 b	7.1 b	2.3 bc	36.1 a	1.8 ab	1.2 b	1.9 bc	1.1 b	1.8 a	2.4 c
	AP	7.2 b	8.5 a	2.4 bc	28.1 b	1.7 b	1.8 ab	2.4 b	1.2 b	1.6 a	2.7 bc
	BM	6.2 b	7.5 b	3.3 a	26.3 b	2.3 a	2.6 a	3.3 a	1.8 a	1.8 a	3.3 a
	CM	19.0 a	8.3 a	2.5 b	8.6 c	1.8 ab	2.4 ab	2.1 bc	1.8 a	2.0 a	3.0 b
20/15	Control	--	--	1.8 b	2.3 bc	1.3 b	0.7 b	1.7 b	1.4 bc	1.7 a	3.3 ab
	Urea	--	--	2.2 b	5.2 a	1.5 b	2.5 a	2.1 a	2.2 a	2.0 a	3.0 b
	AP	--	--	2.6 ab	3.0 b	1.8 ab	2.3 a	2.4 ab	1.4 c	1.9 a	3.0 b
	BM	--	--	2.8 a	5.6 a	1.6 b	2.8 a	2.0 a	2.0 ab	1.8 a	3.6 a
	CM	--	--	2.4 ab	1.9 c	2.7 a	2.0 a	2.0 ab	1.6 bc	2.0 a	2.8 b
25/20	Control	--	--	1.7 c	0.6 c	1.1 b	1.0 b	1.6 b	1.0 b	1.8 c	2.9 b
	Urea	--	--	1.7 bc	2.4 a	1.4 a	2.6 a	2.1 a	2.1 a	1.8 c	3.8 a
	AP	--	--	2.0 ab	3.4 a	1.4 a	1.3 b	1.9 a	1.6 ab	2.1 ab	3.1 b
	BM	--	--	2.1 a	4.9 a	1.6 a	2.5 ab	2.0 a	2.3 a	2.3 a	3.8 ab
	CM	--	--	2.3 a	1.0 b	1.6 a	1.6 b	1.8 ab	1.8 a	1.9 bc	3.2 b

† Means may be compared among N sources within temperature and growth stage. Means followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

At 50 and 60 DAT, N concentration was highest in the order: roots > stem > leaves (Table 3.1.5). Limited N supply induced chlorosis on the lower leaves in all treatments, indicative of N deficiency (Figure 3.1.4). Leaf and stem N concentrations were significantly lower in the control and CM-treated plants than in the urea-, AP, and BM-treated plants at 15/10°C. There were only small differences in leaf and stem N concentrations among treatments at 20/15 and 25/20°C. As temperature increased, leaf and stem N concentrations decreased slightly in all treatments. There was no apparent difference in root N concentration among treatments (Table 3.1.5). Since soil and applied N did not supply sufficient N, N concentration decreased considerably with growth in all treatments. As a result, N source and temperature had a small influence on N concentration at the late growth stage.

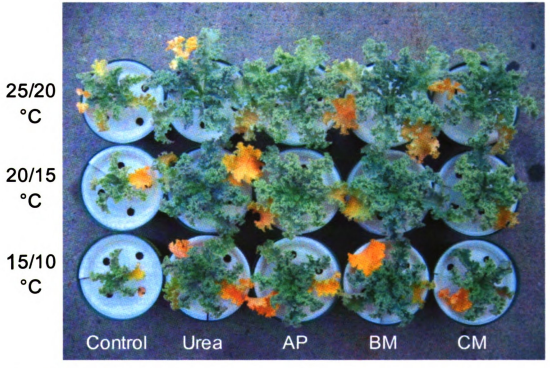

Figure 3.1.4. Kale plants treated with different organic N sources grown at different temperatures at 45 DAT.

Nitrogen accumulation and partitioning among leaves, stem, and roots

Accumulation of N in leaves, stem, and roots calculated as the product of dry matter and N concentration is shown in Table 3.1.7. In all tissue types N accumulation was significantly affected by N source, growth stage, and the interaction based on ANOVA ($P < 0.001$, data not shown). Although temperature effects could not be analyzed statistically with the experimental design in this study, N accumulation was apparently affected by temperature. Partitioning patterns of N accumulated among the tissue types were also influenced by N source, temperature, and growth stage.

At 20 DAT, greater leaf N accumulation did not consistently correlate with greater dry matter production. The CM-treated plants accumulated significantly less leaf N compared to the AP- and BM-treated plants at 20/15 to 25/20°C, though it produced greater leaf dry matter (Table 3.1.4 and 3.1.7). In addition, leaf N accumulated at 20/15 and 25/20°C was roughly equivalent in all treatments, though leaf dry matter was greater at 25/20°C (Table 3.1.4 and 3.1.7). It was likely that increased growth rate resulted in decreased N concentration and consequent N depletion to a greater degree. In stem and roots, with relatively small variation in N concentration, N accumulation was more closely associated with dry matter production (Table 3.1.4 and 3.1.7).

From 20 to 35 DAT, leaf N accumulation increased at 15/10°C but decreased at 20/15 and 25/20°C (Table 3.1.7). Stem and root N accumulation increased in all treatments. The N depletion in leaves can be explained by translocation of N from leaves into stem and roots under severe N deficiency. At 35 DAT, only among N sources was leaf N accumulation closely associated with dry matter production. Although leaf dry matter increased in relation to temperature, leaf N accumulation was relatively constant

Table 3.1.7. Temporal changes in N accumulation in leaves, stem, and roots of kale treated with different organic N sources grown at different temperatures.[†]

		Days after transplanting											
		20			35			50			60		
Temperature	N source	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
°C		N mg											
15/10	Control	13.6 c	0.4 c	1.7 b	13.8 e	1.1 d	4.7 c	12.8 e	0.9 d	7.5 b	11.2 d	1.4 c	9.2 b
	Urea	40.4 a	1.1 a	2.4 a	78.7 a	3.5 a	13.2 a	73.7 a	6.2 a	16.6 a	55.3 a	5.7 a	22.4 a
	AP	20.0 c	0.6 b	1.4 b	53.6 c	2.3 b	9.7 b	49.9 c	4.6 b	18.2 a	46.4 b	4.6 a	23.7 a
	BM	30.3 b	1.1 a	2.0 a	61.2 b	2.2 b	10.0 b	60.8 b	4.1 b	18.7 a	50.5 ab	4.6 a	24.4 a
	CM	32.7 b	1.1 a	2.5 a	37.2 d	1.7 c	10.1 b	30.8 d	3.0 c	19.7 a	31.2 c	3.2 b	22.6 a
20/15	Control	16.0 e	0.8 c	2.8 c	14.6 e	1.2 d	6.6 b	13.5 d	1.4 c	7.1 b	11.4 c	1.7 d	7.8 c
	Urea	85.7 a	2.8 a	5.0 a	77.2 a	4.2 a	14.2 a	57.0 a	6.4 a	24.0 a	47.6 a	6.4 a	25.8 a
	AP	60.7 c	2.1 b	3.3 bc	52.7 c	3.3 b	11.7 a	46.3 b	6.1 a	23.0 a	37.8 b	5.5 b	24.1 a
	BM	67.3 b	1.9 b	3.9 b	65.8 b	3.2 b	13.6 a	54.5 a	5.5 a	23.0 a	47.5 a	6.0 ab	22.1 b
	CM	46.5 d	1.9 b	5.1 a	38.9 d	2.1 c	13.7 a	33.7 c	3.9 b	21.5 a	33.9 b	4.9 c	25.2 a
25/20	Control	17.8 e	0.9 c	4.1 b	15.8 d	1.3 c	6.1 b	17.0 d	2.4 c	8.5 d	16.9 c	2.7 c	10.4 c
	Urea	86.9 a	4.0 a	8.0 a	67.7 a	5.6 a	13.7 a	56.4 a	8.2 a	17.3 c	48.2 a	7.5 b	20.5 b
	AP	66.9 c	2.7 b	5.7 ab	55.7 b	4.7 b	15.4 a	45.3 b	8.7 a	22.0 a	46.1 a	9.8 a	21.4 b
	BM	75.2 b	2.8 ab	7.4 a	66.4 ab	4.7 ab	16.3 a	54.8 a	8.3 a	18.7 bc	49.7 a	9.3 a	20.8 b
	CM	45.8 d	2.4 b	7.2 a	40.9 c	3.7 b	13.9 a	37.6 c	6.5 b	21.2 ab	41.4 b	6.8 b	26.0 a

[†] Means may be compared among N sources within temperature and growth stage. Means followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

across temperature levels due to accelerated N depletion at higher temperatures (Table 3.1.4 and 3.1.7). In stem and roots, variation in N concentration remained small, and N accumulation was more closely associated with dry matter production (Table 3.1.4 and 3.1.7).

From 35 to 60 DAT, N depletion in leaves occurred in all treatments (Table 3.1.7). Continuous increases in N accumulation were observed only in roots, suggesting that N translocation occurred from leaves and stem into roots at this late growth stage. At 50 and 60 DAT, only among N sources was leaf N accumulation closely associated with dry matter production. Although leaf dry matter increased in relation to temperature in all treatments, leaf N accumulation showed different responses to temperature among N sources applied. As temperature increased, the control and CM-treated plants accumulated more leaf N, but the urea-, AP-, and BM-treated plants accumulated less leaf N (Table 3.1.7). In stem and roots, variation in N concentration remained small, and N accumulation was more closely associated with dry matter production (Table 3.1.4 and 3.1.7).

The percentage of N partitioned in leaves was highest (> 78%) at 20 DAT and decreased with time (Table 3.1.7). At 60 DAT, the greatest N partitioning (66%) in leaves was obtained in the urea-treated plants at 15/10°C (Table 3.1.7). The lowest N partitioning (51%) in leaves was obtained in the control at 15/10°C. Greater N partitioning in leaves consistently correlated with greater leaf N concentration. Therefore, it was likely that N translocation from leaves into stem and roots decreased N partitioning in leaves.

Net N uptake

Net N uptake, calculated as the sum of N accumulated in leaves, stem, and roots, is shown in Table 3.1.8. Net N uptake was significantly affected by N source and growth stage based on ANOVA ($P < 0.05$, data not shown). Although temperature effects could not be analyzed statistically with the experimental design in this study, net N uptake was apparently affected by temperature. The sum of N recovered as available soil N and net N uptake is also shown in Table 3.1.8. Greater N recovered may indicate greater N released from an applied N source. In general, N recovery was greatest in the treatment order: urea > BM > AP > CM > control.

At 20 DAT, greater net N uptake did not consistently correlate with N recovery. At 15/10°C, the CM-treated plants accumulated greater N than the AP- and BM-treated plants, though CM released less N. This indicates that the CM-treated plants effectively utilized inorganic N initially contained in CM (Table 3.1.8). The urea-treated plants accumulated the greatest N, which coincided with the greatest N recovery (Table 3.1.8). In the urea treatment, rapid N release likely enhanced N uptake. At 20/15 and 25/20°C, net N uptake was limited by N supply, thus positively correlating with N recovery. Regardless of N source applied, net N uptake increased as temperature increased (Table 3.1.8). At higher temperatures, movement of N in soil to roots was likely enhanced for two reasons. Firstly, mass flow of N to roots accelerated by increased plant growth rate and transpiration (Havlin et al., 1999). Secondly, root interception of soil N increased by extensive root growth (Havlin et al., 1999). In addition, N recovery data shows that N released from AP, BM, and CM increased with increasing temperature, which may partly account for the increases in net N uptake by the plants treated with the natural organic

Table 3.1.8. Temporal changes in net N uptake by kale treated with different organic N sources grown at different temperatures.[†]

		Days after transplanting								
		0	20		35		50		60	
Temperature	N source	Soil N	N uptake	N uptake + soil N	N uptake	N uptake + soil N	N uptake	N uptake + soil N	N uptake	N uptake + soil N
°C		mg pot^{-1}								
15/10	Control	35 c	16 c	26 e	20 e	24 e	21 e	26 e	22 c	28 d
	Urea	36 c	44 a	117 a	95 a	101 a	96 a	102 a	83 a	91 a
	AP	40 b	22 c	80 c	66 c	72 c	73 c	79 c	75 a	83 b
	BM	38 bc	33 b	90 b	73 b	83 b	84 b	93 b	79 a	89 a
	CM	63 a	36 b	57 d	49 d	57 d	53 d	61 d	57 b	66 c
20/15	Control	--	20 e	27 e	22 e	26 d	22 d	28 d	21 c	30 c
	Urea	--	94 a	108 a	96 a	103 a	87 a	96 a	80 a	89 a
	AP	--	66 c	77 c	68 c	76 c	75 b	83 b	67 b	76 b
	BM	--	73 b	89 b	83 b	91 b	83 a	91 a	76 a	86 a
	CM	--	53 d	62 d	55 d	64 c	59 c	66 c	64 b	73 b
25/20	Control	--	23 d	27 e	23 d	27 d	28 d	33 d	30 c	39 b
	Urea	--	99 a	107 a	87 a	94 a	82 a	90 a	76 ab	87 a
	AP	--	75 b	86 c	76 b	81 b	76 b	83 b	77 a	87 a
	BM	--	85 b	99 b	87 a	95 a	82 a	90 a	80 a	91 a
	CM	--	55 c	62 d	59 c	65 c	65 c	72 c	74 b	84 a

[†] Means may be compared among N sources within temperature and growth stage. Means followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

materials. Majority of the increase occurred between 15/10 and 20/15°C due to N limitation.

From 20 to 35 DAT, apparent increases in net N uptake were observed only at 15/10°C (Table 3.1.8). The urea-treated plants decreased net N uptake at 25/20°C, probably due to senescent leaf-fall under severe N deficiency. At 35 DAT, net N uptake was greatest in the treatment order: urea > BM > AP > CM > control, positively correlating with N recovery (Table 3.1.8). The AP-, BM-, and CM-treated plants increased net N uptake in relation to temperature (Table 3.1.8). Since N was a limiting factor at all temperature levels, the increases in net N uptake can be attributed to greater N released at higher temperatures. In contrast, the urea-treated plants decreased net N uptake at 25/20°C due to accelerated senescence.

From 35 to 60 DAT, apparent increases in net N uptake were observed only in the CM-treated plants (Table 3.1.8). The urea-treated plants decreased net N uptake at all temperature levels. At 50 and 60 DAT, the urea- and BM-treated plants accumulated significantly greater N compared to the AP- and CM-treated plants at all temperature levels (Table 3.1.8). At 60 DAT, the greatest net N uptake (83 mg $plant^{-1}$) occurred in the urea-treated plants at 15/10°C. However, this was not significantly different than net N uptake by the AP- and BM-treated plants at same temperature level. Among the N-treated plants the least net N uptake (57 mg $plant^{-1}$) occurred in the CM-treated plants at 15/10°C. The positive effect of temperature on N uptake was apparent only in the control and CM-treated plants, which increased net N uptake by 37 and 30%, respectively, as temperature increased from 15/10 to 25/20°C (Table 3.1.8). In contrast, the urea-treated plants decreased net N uptake by 9% as temperature increased from 15/10 to 25/20°C. This

decrease was likely due to accelerated senescence at higher temperatures. The results indicate a greater N release response to temperature of soil OM and CM compared to urea, AP, and BM.

Apparent N use efficiency (ANUE)

According to ANUE determined at 60 DAT, the proportion of applied N that was utilized by kale was greatest in the order: urea (41-54%) > BM (30-35%) > AP (25-29%) > CM (13-16%), regardless of temperature (Figure 3.1.5).

Using data from the previous soil incubation study, N availability from the organic N sources was estimated (Table 3.1.3) and compared with ANUE. Apparently, greater ANUE was related to greater N availability.

As temperature increased from 15/10 to 25/20°C, the CM-treated plants increased ANUE by 26%, whereas the urea-, AP- and BM-treated plants decreased ANUE by 25, 11, and 14%, respectively (Figure 3.1.5). The results show that estimated N availability did not accurately predict variations in N availability among temperatures. This was probably due to rapid senescence resulting from insufficient N supply.

Low ANUE relative to the estimated available N may be explained by N immobilization (Beauchamp, 1986; Martín-Olmedo et al., 1999; Burger and Jackson, 2003) or gaseous losses of N through denitrification (Paul and Beauchamp, 1993; Mulvaney et al., 1997; Khaili et al., 2002) and volatilization (Craig and Wollum II, 1982; Manjula and Malzer, 1994; Sullivan et al., 2003). Nitrate leaching likely did not occur, as soil moisture was maintained below field capacity throughout the growing period. No attempt was made to identify the unaccounted portion of applied N in this study.

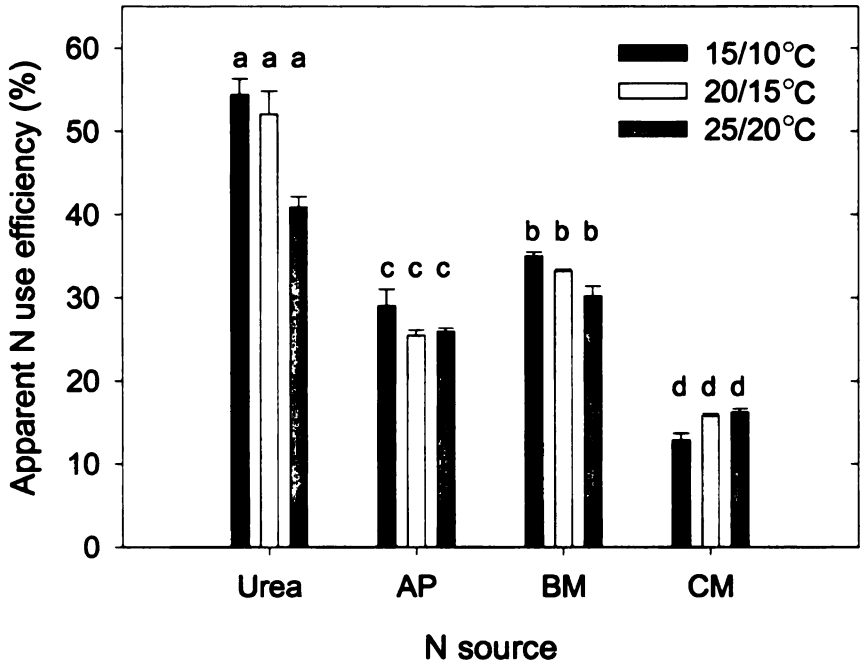

Figure 3.1.5. Effects of temperature on apparent N use efficiency by kale treated with different organic N sources at 60 DAT. Error bars represent 1 SE. Error bars represent 1 SE. Bars with the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$, comparing N source within each temperature level.

CONCLUSIONS

Shoot production and N uptake by kale is affected directly by the level of temperature, as well as indirectly by the effect of temperature on N availability from an applied N source. The former temperature effect is consistent among N sources, whereas the latter temperature effect varies among N sources. Root production by kale is affected directly by the level of temperature. Root growth appears to be less dependent on N availability than shoot growth. To what extent the temperature effects on crop production are a result of N availability limitation versus direct temperature limitation is needed to be studied at various N application rates. Such information will be valuable to making decisions for the most efficient use of organic N sources especially in greenhouse production systems, where temperature control can be easily managed.

Estimated N availability using the previous soil incubation data is a reliable indicator of N availability on a field scale. In this study, however, it did not accurately predict increases in N availability at higher temperatures due to insufficient N application. Additional study at higher N application rates is needed to better understand temperature effects on N availability on a field scale.

CHAPTER 3.2

AVAILABILITY OF NITROGEN FROM ORGANIC NITROGEN SOURCES AS AFFECTED BY TEMPERATURE: BIOASSAY USING KALE (*Brassica oleracea* L.) WITH AN ADEQUATE NITROGEN SUPPLY

MATERIALS AND METHODS

Soil

The soil used in this study was a Granby sandy clay loam (sandy, mixed, mesic Typic Haplaquolls). Approximately 400 kg of surface soil (15 cm) was collected from the Michigan State University Horticulture Teaching and Research Center in East Lansing MI, in May and August 2003. The soil was passed through a 5 mm sieve, thoroughly mixed to ensure uniformity, and stored in a covered plastic container under field moisture condition (5%, w/w) at room temperature (20-23°C) until the experiment was initiated to minimize disturbance of the microbial population (Pramer and Bartha, 1972; Honeycutt, 1999). The chemical and physical properties of the soils are listed in Table 3.2.1. All soil analyses were done by the same procedures used in the previous soil incubation study.

Table 3.2.1. Chemical and physical properties of soils used in this study.

Property	Soil (May)†	Soil (August)‡
pH	5.3	5.6
Sand (%)	54.7	54.7
Silt (%)	15.4	15.4
Clay (%)	29.8	29.8
CEC (cmol kg^{-1})	44.7	51.8
OM content (%)	3.6	3.5
Total C (g kg^{-1})	21.1	20.1
Total N (g kg^{-1})	5.3	4.2
NH_4^+-N (mg kg^{-1})	0.9	0.4
NO_3^--N (mg kg^{-1})	19.8	33.6
P (mg kg^{-1})	184	175
K (mg kg^{-1})	201	198
Ca (mg kg^{-1})	504	627
Mg (mg kg^{-1})	71	99

† Soil collected in May 2003.
‡ Soil collected in August 2003.

Nitrogen sources

Urea [$CO(NH_2)_2$] was used as a synthetic organic N fertilizer. Three natural organic materials were used. Alfalfa pellets (AP), which were obtained from Bradfield Industries, Inc. (Springfield, MI), are alfalfa-based fertilizers blended with animal protein, natural sulfate of potash, and molasses. Blood meal (BM) was obtained from Glorious Gardens Blood Meal Growing Markets, Inc. (West Des Moines, IA). Partially composted chicken manure (CM) was obtained from Herbruck's Poultry Ranch, Inc. (Saranac, MI). Chemical properties of these N sources are listed in Table 3.2.2. All nutrient analyses were done by the same procedures used in the previous soil incubation study.

Table 3.2.2. Chemical characteristics of four organic N sources used in this study.[†]

N source	Moisture	pH	Total C	Total N	C/N ratio	NH_4^+	NO_3^-	P	K	Ca	Mg	Fe
	%		----%	----		-- g N kg^{-1} --		----------%	----------			
Urea	--	--	--	46.00	--	--	--	--	--	--	--	--
AP	4.46	5.73	39.3	3.60	10.93	0.49	0.15	0.58	4.55	1.69	0.16	0.06
BM	5.01	7.30	41.3	12.65	3.26	0.15	0.02	0.51	0.64	1.12	0.11	0.11
CM	9.11	8.78	26.6	3.03	8.79	1.42	0.12	2.96	3.62	18.41	0.87	0.09

[†] All values are expressed on a dry weight basis (105°C).

Experimental design

A pot experiment with kale (*Brassica oleracea* L. cv. Winterbor F1) was conducted using growth chambers during July 7 to August 15 and August 24 to October 3 2003. Due to limited availability of growth chambers, the experiment was conducted twice to be replicated. The experimental design was a split plot design in two randomized blocks with three subsamples. Treatments consisted of a factorial combination of three temperature levels [15/10, 20/15, and 25/20°C day/night (14/10 hr)], five N sources (control, urea, AP, BM, and CM), and three growth stages [15, 30, and 40 days after transplanting (DAT)]. Temperature was designated as a main plot, and N source and growth stage were designated as subplots.

Experimental procedure and growing conditions

Kale was seeded on a 2.5 cm depth plastic cell tray filled with a commercial seedling/propagating mix and placed in a greenhouse on 21 June and 9 August 2003 in the first and second experiment, respectively. Fifteen-day-old seedlings were transplanted singly in 1.6-litter pots that measured 15 cm in diameter. One day before transplanting, 1900 g of soil (oven dry basis) was mixed with urea, AP, BM, or CM at the rate of 222, 351, 324, and 526 mg N per 1 kg of soil, respectively, and filled into the pots. Pots were also filled with 1900 g of soil (oven dry basis) with no N source as a control. The application rates were calculated to provide approximately equal amounts of 211 mg available N kg^{-1} soil (472 kg available N ha^{-1}) using Equation [1].

$$\text{Estimated available N} = N_i + fN_o \quad [1]$$

where N_i is the inorganic N (NH_4^+-N + NO_3^--N) content, N_o is the organic N (total N –

inorganic N) content, and *f* is the proportion of organic N fraction expected to be released during the growing period (Griffin and Honeycutt, 2000). As in the previous soil incubation study, coefficient *f* of 0.95, 0.59, 0.65, and 0.39 was applied for urea, AP, BM, and CM, respectively. Additional nutrients (P, K, Ca, and Mg) were not applied, since there were sufficient amounts of these nutrients for optimum crop production (Table 3.2.1) according to soil tests and fertilizer recommendations for vegetable crops in Michigan (Warncke et al., 1992).

Amount of available N released from the organic N sources was estimated also by using a first-order model [2] determined in the previous soil incubation study (Table 3.2.3);

$$N_{rel} = N_0(1 - \exp^{-k_0 t}) \qquad [2]$$

where N_{rel} (mg N kg^{-1}) is the cumulative N released from an applied N source at time t, N_0 (mg N kg^{-1}) is the size of potentially mineralizable N, exp is the exponential constant with numerical value ≅ 2.718, and k_0 ($week^{-1}$) is the first-order rate constant. Availability of N from the organic N sources, as a percentage of applied N, was also estimated (Table 3.2.3).

The pots were watered with distilled water to 70% water holding capacity (WHC) by weighing, and randomly transferred into the growth chambers set at 15/10, 20/15, and 25/20°C (± 0.5°C) day/night temperatures. Relative humidity was set at 58, 70, and 78%, respectively, to maintain constant vapor pressure deficit among the three temperature levels. Lighting was provided by fluorescent and incandescent lamps that produced 420 ± 20 μmol m^{-2} s^{-1} PPF at plant height with a 14-hr photoperiod. In order to maintain soil moisture at 70% WHC during the experiment, the pots were watered by weighing and

Table 3.2.3. Available N as a function of applied N.

N source	Temperature	Applied N	Estimated available N	Estimated N availability
	°C	-- mg N kg^{-1} soil --		%
Urea	15/10	222	201	90
	20/15	222	202	91
	25/20	222	203	91
AP	15/10	351	127	37
	20/15	351	146	42
	25/20	351	170	49
BM	15/10	324	167	52
	20/15	324	177	56
	25/20	324	192	60
CM	15/10	526	185	36
	20/15	526	206	40
	25/20	526	223	44

adding the required amount of distilled water every two days until 15 DAT and daily thereafter. Corrections for increasing plant weight were made on the basis of shoot fresh weight determined at each sampling time. The pots were covered by a plastic plate, which allowed passage of water into soil but reduced evaporation from surface soil.

Sampling, measurements, and chemical analyses

At 15, 30, and 40 DAT, kale shoots were separated from soil by hand, and divided into leaves and stem for determination of fresh matter weight. Dry matter weight was determined after dried at 60°C for 48 hr. The dried tissues were then ground to pass 1 mm screen and analyzed for total Kjeldahl-N (TKN) content (all tissue types) and NO_3^--N content (leaves).

Soil was passed through a 2 mm sieve to remove roots. The sieved soil was dried at 38°C for 48 hr, thoroughly mixed and analyzed for pH and inorganic N content (NH_4^+-N and NO_3^--N). Roots were separated from the remaining soil by washing over 0.5 mm sieve in a hydropneumatic root elutriator (Gillison's Variety Fabrication, Inc., Benzonia, MI) as described by Smucker et al. (1982). After washing, debris and dead roots were manually removed from vital roots. The roots were then rinsed with distilled water and dried at 60°C for 48 hr for determination of dry matter weight. The dried roots were ground to pass 1 mm sieve and analyzed for TKN content.

Apparent N use efficiency

Apparent N use efficiency (ANUE) was calculated using the difference method as follows (Motavalli et al., 1989):

$$\text{ANUE (\%)} = \{[N_{uptake}\text{ (N-treated plant)} - N_{uptake}\text{ (control)}] / N_{applied}\} \times 100 \quad [2]$$

where N_{uptake} (mg pot^{-1}) is the total N uptake by kale calculated as the sum of TKN in leaves, stem, and roots plus NO_3^--N content in leaves, and $N_{applied}$ (mg pot^{-1}) is the total N in an applied N source. The difference method assumes that soil N transformations remain constant in both the soil that received N source and the soil that received no N source. Thus, the difference in total N uptake between the two soils is assumed to be the amount of N from the applied N source taken up by the plant.

Statistical analysis

A three-way analysis of variance (ANOVA) was conducted to test significance of main effects and interactions using the MIXED procedure of Statistical Analysis System

(SAS) (SAS Institute, 1990). When statistically significant differences existed, treatment means were separated using the LSMEANS procedure, and then tested using paired *t* test at $\alpha = 0.05$. Data presented are the means of two replications with three subsamples.

RESULTS AND DISCUSSION

Dry matter production and partitioning among leaves, stem, and roots

Dry matter production of leaves, stem, and roots was significantly affected by N source, temperature, growth stage, and all interactions based on ANOVA ($P < 0.05$, data not shown). Patterns of dry matter distribution among the tissue types were also influenced by N source, temperature, and growth stage.

Leaf dry matter in the control increased with time in a linear function, whereas that in the N-treated plants increased in an expolinear function (Figure 3.2.1). At 15 DAT, application of AP and BM did not increase leaf dry matter, though it significantly increased available soil N level (Table 3.2.4 and 3.2.5). The urea- and CM-treated plants produced greater leaf dry matter than the control, with significant differences seen only at 25/20°C (Table 3.2.4). The soil used in this study initially contained 27 mg available N kg^{-1} (61 kg ha^{-1}). Kale effectively utilized the indigenous N, so that additional N had a small influence on early growth of kale. Increasing temperature significantly enhanced plant growth in all treatments, with leaf dry matter increasing more than three-fold at

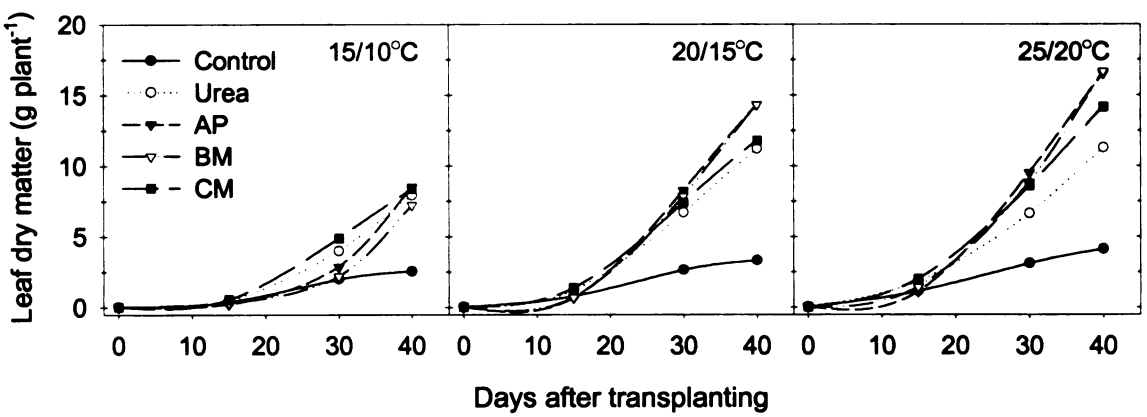

Figure 3.2.1. Temporal changes in leaf dry matter of kale treated with different organic N sources grown at different temperatures.

Table 3.2.4. Temporal changes in dry matter production of leaves, stem, and roots in kale treated with different organic N sources grown at different temperatures.[†]

		Days after transplanting								
		15			30			40		
Temperature	N source	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
°C		g plant^{-1}								
15/10	Control	0.39 e	0.02 ab	0.06 a	2.00 g	0.10 g	0.49 de	2.6 g	0.20 h	0.75 hi
	Urea	0.50 de	0.02 ab	0.05 a	4.01 de	0.21 fg	0.50 de	7.9 d	0.42 fg	0.94 gh
	AP	0.24 e	0.01 b	0.02 a	2.88 fg	0.15 fg	0.32 e	8.5 d	0.50 f	1.32 f
	BM	0.33 e	0.02 ab	0.04 a	2.19 fg	0.12 g	0.32 e	7.2 d	0.38 fg	1.18 fg
	CM	0.56 de	0.03 ab	0.06 a	4.90 d	0.26 f	0.79 cd	8.4 d	0.53 f	1.78 de
20/15	Control	0.79 cde	0.04 ab	0.15 a	2.66 fg	0.20 fg	0.52 de	3.3 f	0.33 gh	0.81 hi
	Urea	1.19 bcd	0.06 ab	0.13 a	6.70 c	0.42 e	0.78 cd	11.2 c	0.82 e	1.48 ef
	AP	0.67 de	0.03 ab	0.07 a	8.24 b	0.59 cd	1.10 abc	14.3 b	1.14 c	2.31 ab
	BM	0.70 de	0.03 ab	0.11 a	7.84 bc	0.51 de	1.00 bc	14.3 b	1.03 cd	1.92 cd
	CM	1.37 abc	0.08 ab	0.14 a	7.39 bc	0.49 de	1.30 ab	11.8 c	0.93 de	2.23 bc
25/20	Control	1.15 cd	0.06 ab	0.18 a	3.12 ef	0.28 f	0.46 de	4.1 e	0.48 f	0.58 i
	Urea	1.84 ab	0.10 ab	0.17 a	6.64 c	0.48 de	0.93 c	11.3 c	0.97 de	1.25 fg
	AP	1.11 cd	0.07 ab	0.10 a	9.51 a	0.94 a	1.37 a	16.5 a	1.77 a	2.30 ab
	BM	1.44 abc	0.08 ab	0.13 a	8.83 ab	0.72 bc	1.30 ab	16.6 a	1.55 b	1.95 cd
	CM	2.02 a	0.12 a	0.18 a	8.59 ab	0.74 b	1.34 a	14.2 b	1.50 b	2.62 a

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

Table 3.2.5. Temporal changes in NH_4^+- and NO_3^--N contents in soil treated with different organic N sources used to grow kale at different temperatures.[†]

		Days after transplanting											
		0			15			30			40		
				Inorganic			Inorganic			Inorganic			Inorganic
Temperature	N source	NH_4^+	NO_3^-	N	NH_4^+	NO_3^-	N	NH_4^+	NO_3^-	N	NH_4^+	NO_3^-	N
°C		mg N kg^{-1} soil											
15/10	Control	1 c	27 c	27 c	1 g	16 h	17 jk	1 b	2 e	4 e	1 a	1 c	3 c
	Urea	2 bc	27 c	29 c	99 a	85 cd	184 a	16 a	48 ab	64 ab	6 a	28 a	34 a
	AP	6 b	30 a	36 b	64 b	38 fg	102 f	4 b	21 c	25 c	3 a	2 c	5 c
	BM	1 c	27 c	28 c	58 bc	71 de	129 cd	9 ab	61 a	69 a	5 a	7 c	11 bc
	CM	41 a	29 b	70 a	7 ef	47 f	55 h	3 b	6 de	9 de	4 a	2 c	6 c
20/15	Control	--	--	--	1 g	4 i	5 k	1 b	3 de	4 de	1 a	1 c	3 c
	Urea	--	--	--	52 c	107 ab	159 b	7 b	42 b	48 b	4 a	22 ab	26 ab
	AP	--	--	--	16 de	68 e	83 g	3 ab	5 de	8 de	3 a	3 c	6 c
	BM	--	--	--	22 d	96 bc	118 de	5 b	14 cd	19 cd	4 a	4 c	8 c
	CM	--	--	--	4 fg	32 g	36 i	4 b	7 de	10 de	4 a	3 c	7 c
25/20	Control	--	--	--	2 fg	4 i	5 k	1 b	3 de	4 e	1 a	2 c	3 c
	Urea	--	--	--	25 d	112 a	137 c	4 b	44 b	47 b	3 a	26 a	30 a
	AP	--	--	--	6 fg	85 c	91 fg	2 b	4 de	6 de	3 a	3 c	6 c
	BM	--	--	--	11 ef	91 bc	102 ef	3 b	7 de	10 de	4 a	4 c	8 c
	CM	--	--	--	3 fg	20 h	23 ij	3 b	5 de	7 de	3 a	3 c	6 c

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

25/20°C (Table 3.2.4). At 30 DAT, with exception of the AP- and BM-treated plants at 15/10°C, the N-treated plants produced significantly greater leaf dry matter than the control (Table 3.2.4). Although the urea- and CM-treated plants produced significantly greater leaf dry matter than the AP- and BM-treated plants at 15/10°C, the plants treated with the natural organic materials produced greater leaf dry matter than the urea-treated plants at 20/15°C, with the difference magnified at 25/20°C. This was because leaf dry matter in the urea treatment did not increase beyond 20/15°C, whereas that in the other treatments increased in relation to temperature (Figure 3.2.1). At 40 DAT, the AP- and BM-treated plants produced significantly greater leaf dry matter than the BM- and CM-treated plants at 20/15 and 25/20°C (Table 3.2.4). Maximum dry matter production of leaves (16.5 and 16.6 g $plant^{-1}$) occurred in the AP- and BM-treated plants at 25/20°C. Among the N-treated plants the lowest dry matter production of leaves (7.2 g $plant^{-1}$) occurred in the BM-treated plants at 15/10°C. However, this was not significantly different than leaf dry matter in the other N-treated plants at same temperature level. The control, urea-, AP-, BM-, and CM-treated plants increased leaf dry matter by 61, 42, 94, 130, and 69%, respectively, as temperature increased from 15/10 to 25/20°C (Table 3.2.4).

The results show that less growth response to temperature occurred in the urea treatment compared to the other treatments. It was reasonable to assume that molybdenum (Mo) deficiency limited plant growth in the urea treatment for several reasons. Firstly, visual symptoms of Mo deficiency were observed in the urea-treated plants. At the late growth stage, the older leaves showed chlorosis, and the younger leaves showed crinkling and curling upward with chlorosis between veins (Figure 3.2.2a).

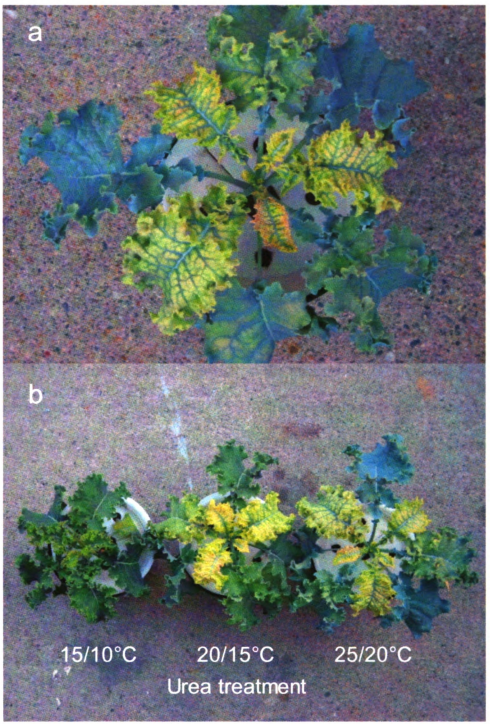

Figure 3.2.2. Kale plants treated with urea at 40 DAT. Upper (a): larger view of the urea-treated plant grown at 25/20°C. Lower (b): the urea-treated plants grown at indicated temperatures (b).

This was more apparent at higher temperatures (Figure 3.2.2b). Secondly, the urea-treated plants had a higher demand for Mo. Because Mo is a component of the enzyme nitrate reductase, Mo requirement depends on NO_3^- supply (Havlin et al., 1999). At 15 DAT, the greatest NO_3^--N content (85-112 mg kg^{-1}) was recovered in the urea-treated soil at all temperature levels (Table 3.2.5). Thirdly, the urea-treated soil had a lower Mo availability. Soil pH is the major factor affecting Mo availability. Vlek and Lindsay (1976) found a 10-fold decrease in Mo availability per unit decrease in pH in four different soils. Since the soils used in this study was strongly acid (Table 3.2.1), Mo availability was presumably very low. In addition, urea application lowered soil pH below the initial value, whereas the other N sources increased soil pH (Figure 3.2.3). Leaf and soil NO_3^--N test also provided evidence for Mo deficiency. Accumulation of NO_3^- in leaves at 40 DAT was significantly greater in the urea treatment compared to the other treatments (Figure 3.2.4), indicating the lack of nitrate reductase in the urea-treated plants. Similarly, residual soil NO_3^- at 40 DAT was significantly greater in the urea treatment

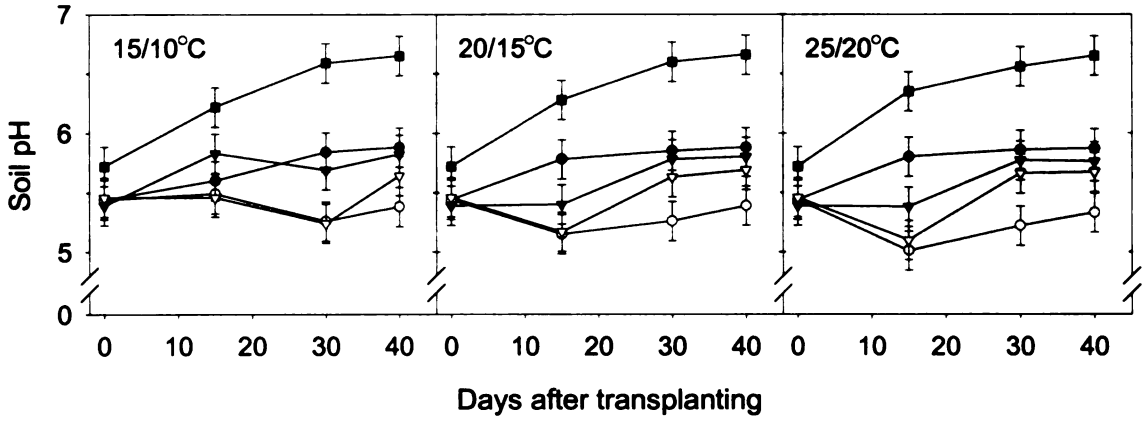

Figure 3.2.3. Temporal changes in pH of soils treated with different organic N sources used to grown kale at different temperatures. The symbols correspond to (●) control; (○) urea; (▼) AP; (▽) BM; (■) CM. Error bars represent 1 SE.

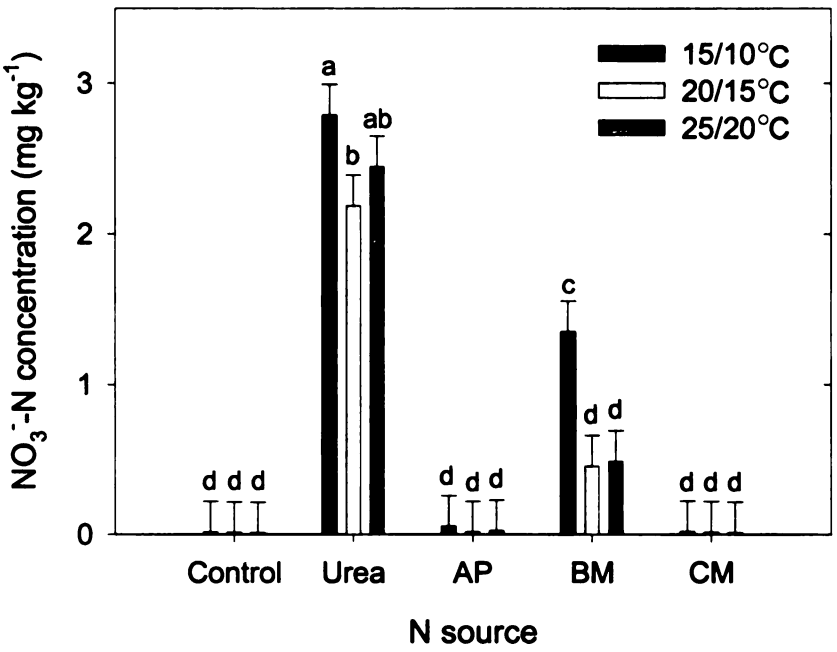

Figure 3.2.4. Nitrate N concentration in leaves of kale treated with different organic N sources grown at different temperatures at 40 DAT. Error bars represent 1 SE. Bars with the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

compared to the other treatments (Table 3.2.5). It was likely that the urea-treated plants could not effectively utilize the soil NO_3^- due to Mo deficiency.

Stem dry matter increased with time in a similar manner to leaf dry matter. Relative differences among treatments were similar between leaf and stem dry matter at all growth stages (Table 3.2.4). As plants matured, the percentage of dry matter partitioned in stem increased slightly, with the increase magnified at higher temperatures.

Root dry matter also increased with time in a similar manner to leaf dry matter; however, root growth was influenced by N source and temperature differently from shoot growth. At 15 DAT, the control produced root dry matter comparable to the urea- and

CM-treated plants, though it produced smaller shoot dry matter (Table 3.2.4). At 30 DAT, increasing temperature did not affect root dry matter in the control, though it significantly increased shoot dry matter. With these exceptions, relative differences among treatments were similar between leaf and root dry matter at 15 and 30 DAT (Table 3.2.4). At 40 DAT, the CM-treated plants at 25/20°C produced the greatest root dry matter (2.64 g $plant^{-1}$), though it produced significantly smaller leaf dry matter than the AP- and BM-treated plants. The AP-treated plants produced significantly greater root dry matter than the BM-treated plants at 20/15 and 25/20°C, though there was no difference in leaf dry matter between the AP- and BM-treated plants. Increasing temperature did not enhance root growth as much as shoot growth. Root dry matter in the CM treatment increased in relation to temperature, whereas that in the other treatments did not increase beyond 20/15°C (Table 3.2.4).

The ratio of shoot to root dry matter was determined as an indicator of dry matter partitioning in roots. The shoot/root ratio at 15/10°C declined with time regardless of N source (data not shown). The shoot/root ratio at 20/15 and 25/20°C declined with time in the AP- and CM-treated plants but remained relatively constant in the control, urea-, and BM-treated plants throughout the growing period (data not shown). At 40 DAT, the highest shoot/root ratio was obtained in the urea-treated plants at all temperature levels (Figure 3.2.5). This was due to less root production rather than to more shoot production. The lowest shoot/root ratio among the N-treated plants was obtained in the CM-treated plants at all temperature levels. This was due to more root production rather than to less shoot production. In all treatments increasing temperature enhanced shoot production to a greater degree than root production, thereby increasing shoot/root ratio (Figure 3.2.5).

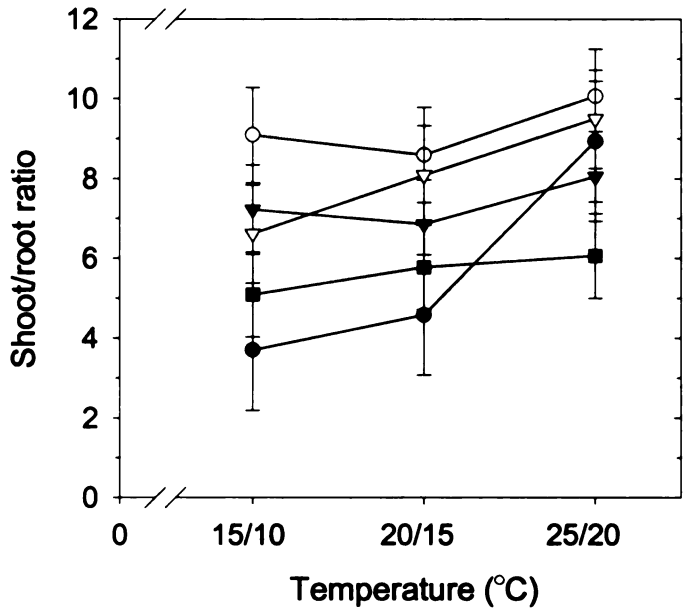

Figure 3.2.5. Effects of temperature on shoot/root ratio of kale treated with different organic N sources at 40 DAT. The symbols correspond to (●) control; (○) urea; (▼) AP; (▽) BM; (■) CM. Error bars represent 1 SE.

Nitrogen concentration and partitioning among leaves, stem, and roots

Nitrogen concentration in leaves, stem, and roots was significantly affected by N source, temperature, and growth stage based on ANOVA ($P < 0.05$, data not shown). Partitioning patterns of N concentration among the tissue types differed between growth stages.

Figure 3.2.6 shows N concentration plotted versus available soil N. The relationship between N concentration and available soil N level was analyzed for each tissue type. At 15 DAT, leaf N concentration was positively correlated with available soil N (Figure 3.2.6). Addition of the organic N sources increased available soil N (Table 3.2.5), resulting in increased leaf N concentration. In fact, an unusually high level of N

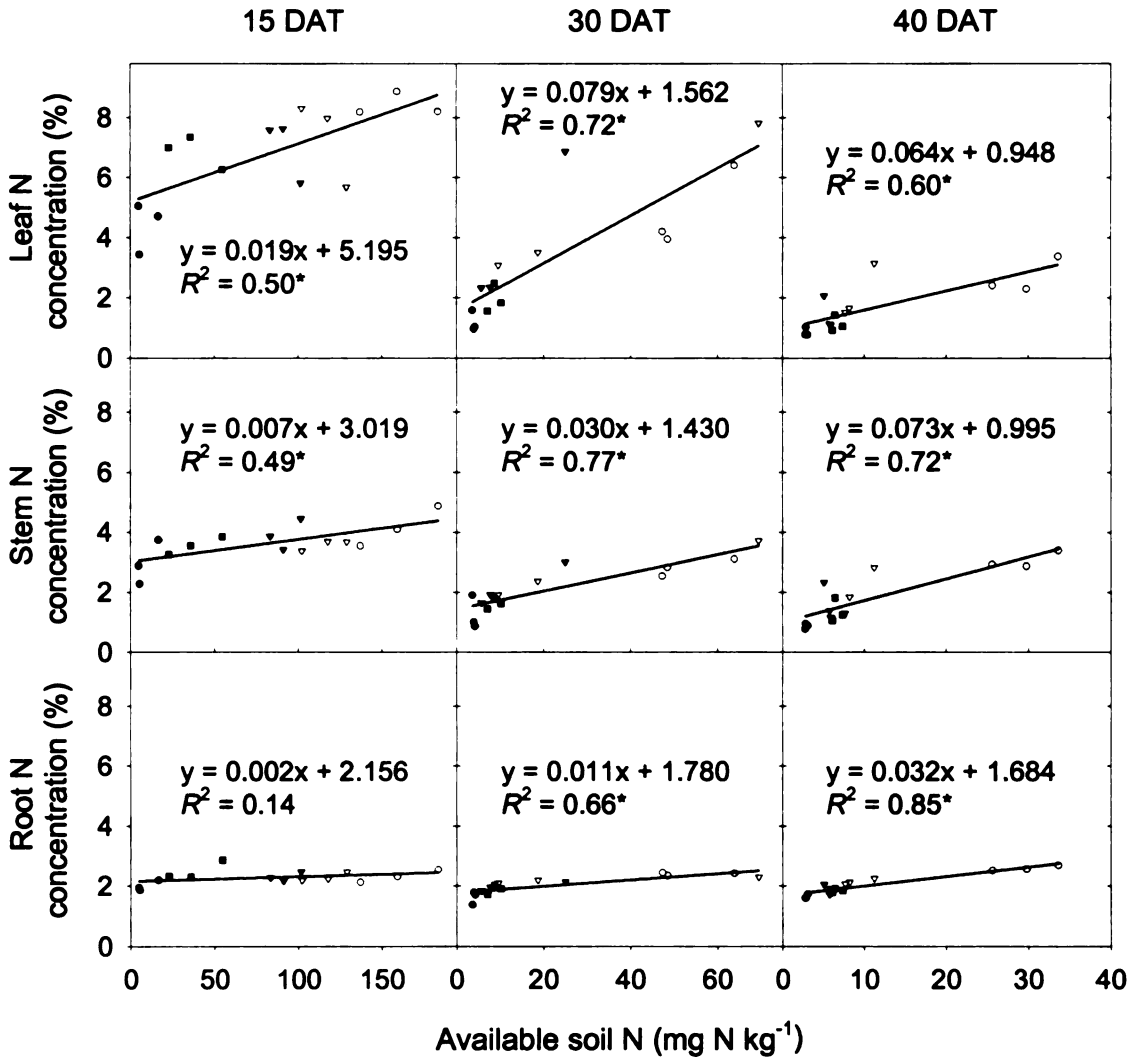

Figure 3.2.4. Relationship between tissue N concentration and available soil N level at 15, 30, and 45 DAT. The symbols correspond to (●) control; (○) urea; (▼) AP; (▽) BM; (■) CM. An asterisk (*) indicates that coefficient of determination (R^2) is statistically significant at $\alpha = 0.05$.

was detected, particularly at high temperature (Table 3.2.6). Rapid N supply in a rate exceeding N demands by kale likely contributed to this luxury N uptake. Stem N concentration was also positively correlated with available soil N, but the positive effect of available soil N on stem N concentration was less noticeable than that on leaf N concentration (Figure 3.2.6). Stem N concentration decreased significantly with

Table 3.2.6. Temporal changes in N concentration in leaves, stem, and roots of kale treated with different organic N sources grown at different temperatures.[†]

		Days after transplanting								
		15			30			40		
Temperature	N source	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
°C		% N								
15/10	Control	4.7 g	3.7 cde	2.2 ef	1.6 fg	1.9 ef	1.4 h	1.0 cd	0.9 gh	1.6 hi
	Urea	8.2 abc	4.9 a	2.5 b	6.4 b	3.1 b	2.4 a	3.4 a	3.4 a	2.7 a
	AP	5.8 efg	4.5 ab	2.5 bc	6.9 ab	3.0 bc	2.1 bcd	2.1 bc	2.3 cd	2.1 cdef
	BM	5.7 fg	3.7 cde	2.5 bcd	7.8 a	3.7 a	2.3 ab	3.2 a	2.8 bc	2.3 bc
	CM	6.3 def	3.8 cd	2.9 a	2.5 def	1.8 f	2.0 cde	1.4 bcd	1.8 de	1.9 defg
20/15	Control	5.0 g	2.9 f	1.9 fg	1.0 g	0.9 h	1.7 g	0.8 d	0.8 h	1.6 i
	Urea	8.9 a	4.1 bc	2.3 bcde	3.9 c	2.8 bcd	2.3 ab	2.4 ab	2.9 ab	2.5 ab
	AP	7.6 bc	3.9 cd	2.3 bcde	2.3 def	1.9 ef	2.0 cdef	1.1 cd	1.4 efg	1.7 ghi
	BM	8.0 abc	3.7 cde	2.2 cde	3.5 cd	2.4 de	2.2 abc	1.7 bcd	1.8 de	2.1 cd
	CM	7.3 bcd	3.6 cde	2.3 bcde	1.8 efg	1.6 f	1.9 defg	1.1 cd	1.2 gh	1.9 efgh
25/20	Control	3.4 h	2.3 g	1.9 g	1.0 g	1.0 gh	1.8 efg	0.8 d	0.9 gh	1.7 ghi
	Urea	8.2 abc	3.5 de	2.1 efg	4.2 c	2.5 cd	2.4 a	2.3 ab	2.9 abc	2.6 a
	AP	7.6 bc	3.4 def	2.2 ef	2.3 def	1.6 f	1.8 efg	1.1 cd	1.2 gh	1.9 defg
	BM	8.3 ab	3.4 def	2.2 def	3.1 cde	1.9 ef	2.1 bcd	1.5 bcd	1.3 fgh	2.1 cde
	CM	7.0 cde	3.3 ef	2.3 bcde	1.5 fg	1.4 fg	1.7 fg	0.9 d	1.0 gh	1.8 fghi

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

increasing temperature (Table 3.2.6), due to reduced available soil N resulting from increased growth rate (Table 3.2.5). No significant correlation between root N concentration and available soil N was observed (Figure 3.2.6). This was related to small variation in root N concentration among treatments (Table 3.2.6).

At 30 DAT, leaf and stem N concentrations decreased markedly below 20 mg available N kg^{-1} (Figure 3.2.6). As temperature increased, leaf and stem N concentrations decreased in all treatments, probably because increased growth rate resulted in rapid soil N depletion (Table 3.2.5 and 3.2.6). Root N concentration decreased slightly below 20 mg available N kg^{-1}. Root N concentration was relatively constant across temperature levels. Leaf N concentration depended on available soil N level more than did stem and root N concentrations, illustrated in Figure 3.2.6.

At 40 DAT, leaf and stem N concentrations decreased markedly below 10 mg available N kg^{-1} (Figure 3.2.6). Leaf and stem N concentrations were highest in the urea-treated plants at all temperature levels (Table 3.1.5). Chlorosis on the lower leaves, indicative of N deficiency, appeared in the control and CM-treated plants (Figure 3.2.7). Root N concentration decreased slightly below 10 mg available N kg^{-1}. Leaf and stem N concentrations depended on available soil N level more than did root N concentration, illustrated in Figure 3.2.6.

In general, leaf and stem N concentrations decreased with time, but root N concentration remained constant throughout the growing period (Table 3.2.6). Although N concentration at 15 DAT was highest in the order: leaves > stem > roots in all treatments, that at 40 DAT was higher in roots than in leaves in almost all treatments. The explanation for the contrasting responses to available soil N level among the three tissue

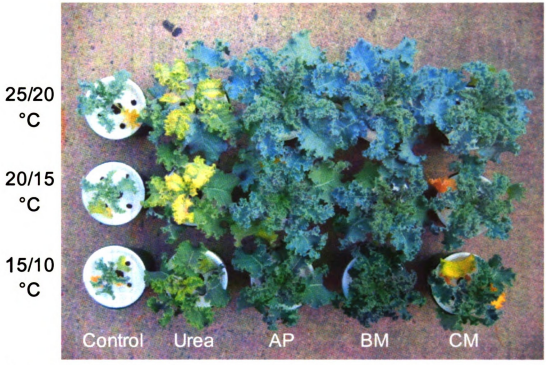

Figure 3.2.7. Kale plants treated with different organic N sources grown at different temperatures at 40 DAT.

types may be that N was translocated from leaves into stem and roots under N deficiency (Gastal and Lemaire, 2002).

Nitrogen accumulation and partitioning among leaves, stem, and roots

Accumulation of N in leaves, stem, and roots, calculated as the product of dry matter and N concentration, is shown in Table 3.2.7. In all tissue types N accumulation was significantly affected by N source, temperature, and growth stage based on ANOVA ($P < 0.05$, data not shown). Partitioning patterns of N accumulated among the tissue types were also influenced by N source, temperature, and growth stage.

At 15 DAT, even though there were significant differences in N concentration among treatments (Table 3.2.6), N accumulation was more closely associated with dry

Table 3.2.7. Temporal changes in N accumulation in leaves, stem, and roots of kale treated with different organic N sources grown at different temperatures.[†]

		Days after transplanting								
		15			30			40		
Temperature	N source	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
°C		mg N								
15/10	Control	18 g	0.7 hi	1.2 a	32 g	2.0 gh	6.7 e	25 e	1.9 i	11.8 g
	Urea	41 ef	1.2 g	1.4 a	256 ab	6.4 de	12.2 de	266 a	14.3 de	25.5 f
	AP	15 g	0.6 i	0.6 a	191 de	4.4 ef	7.2 e	172 c	11.6 ef	27.4 ef
	BM	19 fg	0.6 i	0.9 a	168 e	4.5 efg	7.5 e	222 b	10.9 fg	26.8 ef
	CM	35 fg	1.2 g	1.8 a	122 f	4.7 e	16.1 cd	118 d	9.5 g	34.0 cde
20/15	Control	40 fg	1.1 g	2.9 a	27 g	1.7 h	8.8 e	25 e	2.6 i	12.9 g
	Urea	105 bc	2.6 cd	2.9 a	252 abc	12.1 bc	18.4 bcd	261 a	23.2 b	36.6 bcd
	AP	50 ef	1.2 g	1.6 a	192 de	11.2 bc	21.7 abc	160 c	15.6 d	40.0 abc
	BM	57 de	1.0 gh	2.4 a	234 bc	11.8 bc	22.0 abc	235 ab	18.6 c	40.8 abc
	CM	101 c	2.8 c	3.3 a	131 f	7.9 d	24.7 ab	122 d	11.4 f	40.9 abc
25/20	Control	37 fg	1.2 f	3.3 a	30 g	3.0 fgh	8.2 e	31 e	4.3 h	10.0 g
	Urea	150 a	3.4 b	3.5 a	271 a	12.0 bc	22.7 abc	257 a	27.6 a	31.9 def
	AP	85 cd	2.3 e	2.2 a	221 cd	15.4 a	25.1 ab	179 c	20.4 bc	43.9 ab
	BM	119 ab	2.5 d	2.8 a	269 a	13.7 ab	27.3 a	250 ab	19.9 b	40.3 abc
	CM	141 a	3.8 a	4.2 a	133 f	10.6 c	23.2 ab	130 cd	15.6 d	46.8 a

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

matter production in all tissue types (Table 3.2.4 and 3.2.7). This was due to greater variation among treatments in dry matter than in N concentration. Since N application increased N concentration, the positive effect of N application on N accumulation was more noticeable than that on dry matter production (Table 3.2.4 and 3.2.7).

From 15 to 30 DAT, leaf N accumulation increased in the urea-, AP-, and BM-treated plants at all temperature levels, but it decreased in the control at 20/15 and 25/20°C and the CM-treated plants at 25/20°C (Table 3.2.7). Stem and root N accumulation increased in all treatments. The N depletion in leaves can be explained by translocation of N from leaves into stem and roots under N deficiency. At 30 DAT, greater leaf N accumulation did not consistently correlate with greater dry matter production. The positive effect of temperature on leaf N accumulation was less noticeable than that on leaf dry matter (Table 3.2.4 and 3.2.7). Among N sources, leaf N accumulation was closely associated with N concentration rather than with dry matter production. It was likely that increased growth rate resulted in decreased N concentration and consequent N depletion to a greater degree. In stem and roots, with relatively small variation in N concentration, N accumulation was more closely associated with dry matter production.

From 30 to 40 DAT, N depletion in leaves was observed in all N-treated plants at 25/20°C, and this was most apparent in the AP-treated plants (Table 3.2.7). Stem and root N accumulation increased continuously in all treatments. At 40 DAT, greater N accumulation in leaves and stem did not consistently correlate with dry matter production. Although increasing temperature significantly increased leaf dry matter, there was no significant difference in N accumulated in leaves among temperature levels due to greater

N depletion at higher temperatures (Table 3.2.7). Among N sources, N accumulation in leaves and stem was closely associated with N concentration rather than with dry matter production. In roots, variation in N concentration remained small and N accumulation was more closely associated with dry matter production.

In general, the percentage of N partitioned in leaves was highest (> 90%) at 15 DAT and decreased with time (Table 3.2.7). At 40 DAT, the greatest N partitioning (87%) in leaves was obtained in the urea-treated plants at 15/10°C (Table 3.2.7). The lowest N partitioning (62%) in leaves was obtained in the control at 20/15°C. Greater N partitioning in leaves consistently correlated with greater leaf N concentration. Therefore, it was likely that N translocation from leaves into stem and roots decreased N partitioning in leaves.

Net N uptake

Net N uptake, calculated as the sum of N accumulated in leaves, stem, and roots, is shown in Table 3.2.8. Net N uptake was significantly affected by N source, temperature, growth stage, and all interactions based on ANOVA ($P < 0.05$, data not shown). The sum of N recovered as available soil N and net N uptake is also shown in Table 3.2.8. Greater N recovery may indicate greater N released from an applied N source. At all temperature levels and growth stages, N recovery was greatest in the treatment order: urea > BM > AP > CM > control.

At 15 DAT, greater net N uptake did not consistently correlate with greater N recovery. Even though considerable N was released from AP and BM, it did not enhance N uptake at 15/10°C (Table 3.2.8). The CM-treated plants accumulated greater N than the

Table 3.2.8. Temporal changes in net N uptake by kale treated with different organic N sources grown at different temperatures.[†]

		Days after transplanting						
		0	15		30		40	
Temperature	N source	soil N	N uptake	N uptake + soil N	N uptake	N uptake + soil N	N uptake	N uptake + soil N
°C		mg pot^{-1}						
15/10	Control	52 c	20 g	52 h	40 h	47 f	39 g	45 h
	Urea	54 c	44 fg	393 b	274 bc	396 a	306 a	370 a
	AP	68 b	16 g	209 e	203 de	251 cd	211 d	220 e
	BM	53 c	20 g	266 d	180 ef	312 b	260 b	281 c
	CM	134 a	38 fg	142 g	143 g	159 e	162 f	174 g
20/15	Control	--	44 fg	53 h	37 h	46 f	40 g	46 h
	Urea	--	111 cd	414 ab	282 abc	375 a	321 a	370 a
	AP	--	53 f	212 e	225 d	240 d	215 cd	226 e
	BM	--	61 ef	284 d	268 c	304 b	294 a	310 b
	CM	--	108 cd	175 f	163 fg	183 e	174 ef	188 fg
25/20	Control	--	42 fg	52 h	41 h	49 f	45 g	51 h
	Urea	--	157 a	418 a	306 ab	396 a	317 a	373 a
	AP	--	89 de	262 d	262 c	273 c	244 bc	255 d
	BM	--	124 bc	318 c	310 a	328 b	310 a	324 b
	CM	--	149 ab	192 ef	167 fg	180 e	193 de	204 ef

[†] Means in a column followed by the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

AP- and BM-treated plants at all temperature levels, though CM released less N. This indicates that the CM-treated plants effectively utilized inorganic N initially contained in CM (Table 3.2.8). The urea-treated plants accumulated N nearly equal to the CM-treated plants at all temperature levels (Table 3.2.8). In the urea treatment, rapid N release likely enhanced N uptake. Increasing temperature significantly increased net N uptake by the N-treated plants (Table 3.2.8). Nitrogen recovery data shows that N release from the organic N sources was enhanced with increasing temperature, which may partly account for the increases in net N uptake. In addition, movement of N in soil to roots was likely enhanced at higher temperatures for two reasons. Firstly, mass flow of N to roots accelerated by increased plant growth rate and transpiration (Havlin et al., 1999). Secondly, root interception of soil N increased by extensive root growth (Havlin et al., 1999).

From 15 to 30 DAT, the control increased net N uptake only at 15/10°C, whereas the N-treated plants increased net N uptake at all temperature levels (Table 3.2.8). More N was recovered in the AP, BM, and CM treatments, indicating continuous N release from the natural organic materials. At 30 DAT, net N uptake was positively correlated with N recovery (Table 3.2.8). Increasing temperature significantly increased net N uptake by the AP- and BM-treated plants (Table 3.2.8). Nitrogen recovery data shows that nearly equal amounts of N were released at 15/10 and 20/15°C, but more N was released at 25/20°C (Table 3.2.8). Thus, the increased net N uptake at 20/15°C can be attributed to enhanced soil N movement, whereas that at 25/20°C can be attributed to greater N released from AP and BM. In the urea treatment, there was no significant difference in net N uptake among temperature levels despite sufficient N supply. As

discussed earlier, N uptake by the urea-treated plants was likely inhibited under Mo deficiency.

From 30 to 40 DAT, the urea-, BM-, and CM-treated plants increased net N uptake, but the AP-treated plants decreased net N uptake at 20/15 and 25/20°C due to senescent leaf-fall (Table 3.2.8). At 40 DAT, maximum net N uptake (321 mg plant^{-1}) occurred in the urea-treated plants at 20/15°C. However, this was not significantly different than net N uptake by the urea-treated plants at 15/10 and 25/20°C and the BM-treated plants at 20/15 and 25/20°C. Among the N-treated plants the least net N uptake (162 mg plant^{-1}) occurred in the CM-treated plants at 15/10°C. The control, urea-, AP-, BM-, and CM-treated plants increased net N uptake by 16, 3, 16, 19, and 19%, respectively, as temperature increased from 15/10 to 25/20°C (Table 3.2.8).

Apparent N use efficiency (ANUE)

According to ANUE determined at 40 DAT, the proportion of applied N that was utilized by kale was greatest in the order: urea (63-67%) > BM (36-43%) > AP (26-30%) > CM (12-15%), regardless of temperature (Figure 3.2.8).

Using data from the previous soil incubation study, N availability from the organic N sources was estimated and compared with ANUE (Table 3.2.3). Apparently, greater ANUE was related to greater N availability. The urea-, AP-, BM-, and CM-treated plants increased ANUE by 2, 16, 20, and 20%, respectively, as temperature increased from 15/10 to 25/20°C (Figure 3.2.8). The estimated N availability reasonably predicted differential N availability among temperature levels. In the AP, BM, and CM treatments, increasing temperature enhanced N release, so that kale was able to utilize

more N.

Low ANUE relative to the estimated available N may be explained by N immobilization (Beauchamp, 1986; Martín-Olmedo et al., 1999; Burger and Jackson, 2003) or gaseous losses of N through denitrification (Paul and Beauchamp, 1993; Mulvaney et al., 1997; Khaili et al., 2002) and volatilization (Craig and Wollum II, 1982; Manjula and Malzer, 1994; Sullivan et al., 2003). Nitrate leaching likely did not occur, as soil moisture was maintained below field capacity throughout the growing period. No attempt was made to identify the unaccounted portion of applied N in this study.

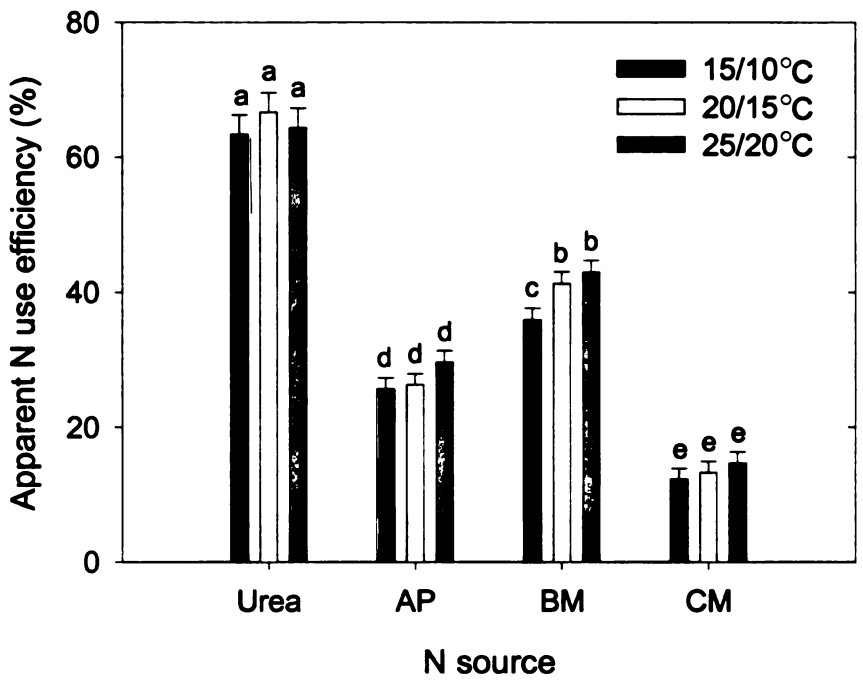

Figure 3.2.8. Effects of temperature on apparent N use efficiency by kale treated with different organic N sources at 40 DAT. Error bars represent 1 SE. Bars with the same letter are not significantly different according to paired *t* test at $\alpha = 0.05$.

CONCLUSIONS

Shoot production and N uptake by kale is affected directly by the level of temperature, as well as indirectly by the effect of temperature on N availability from an applied N source. The former temperature effect is consistent among N sources, whereas the latter temperature effect varies among N sources. Root production by kale is affected directly by the level of temperature. Root growth appears to be less dependent on N availability than shoot growth.

In strongly acidic soils, high rates of urea application may cause Mo deficiency and limit crop production. Increasing temperature will stimulate Mo deficiency by accelerating nitrification, which consequently increases Mo demands by plants and decreases Mo availability in soil.

Soil incubation data will be useful for predicting variations in N availability among N sources and temperatures on a field scale. When using natural organic materials, increasing temperature improves N availability, thereby contributing to better crop production.

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