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Full Length Research Paper

Inorganic Nitrogen Dynamics in Vertisols: Influence of Drying Method, Storage Time, and Carbon-to-Nitrogen Ratio

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In order to monitor spatial inorganic N concentrations in soils over time, collected soil samples are composited and thoroughly mixed. The sticky characteristic of Vertisol when wet makes it difficult to thoroughly mix wet samples. There is the need for appropriate drying of Vertisol samples to facilitate thorough mixing and storage to retain mineral N content reflective of field status. We determined the effects of drying method, storage period and C:N ratio on inorganic N contents of tropical Vertisols. The treatments comprised of three soils with different C:N ratios, five drying methods inclusive of a check (method 1), and five storage periods including a check (period 1). Storage temperature was -10°C. In method 1, field-fresh soil samples were kept at -10°C and later used for mineral N extraction using 0.5 M K_2SO_4. Period 1 involved storing soil samples for two days from sampling date before mineral N extraction. Ammonium-N and NO_3 -N were determined using colorimetric method. Method 1 x period 1, for each soil, resulted in NH_4^+-N and NO_3^--N contents significantly different from other treatments indicating that extraction for mineral N should be done on field moist soil not later than two days after sampling.

Key words: Drying method, mineral nitrogen, storage period, soil preparation, Vertisols.

INTRODUCTION

Nitrogen (N) is the plant nutrient element most limiting to crop performance in the majority of soils in the tropics (Bekunda et al., 2007). In tropical Vertisols, N is the most universally deficient plant nutrient (Coulombe et al., 1996), and it is the most limiting element to crop production in Vertisols in Kenya (Smaling and Bouma, 1992). Dynamics of N (that is, transformations and translocations) in soils play important roles in N cycling, ground water pollution, and N availability to crops (Stevenson, 1986; Aulakh et al., 1992). Most of soil N is in organic form ($-NH_2$) and is only made available to plants through mineralization to inorganic NH_4^+ and NO_3^- (Brady and Weil, 2002). Inorganic N concentrations in a particular soil layer at a point in time reflect the net effects of N inputs from mineralization, inorganic fertilizers and atmosphere on the one hand, and outputs through plant uptake, leaching, bypass flow, denitrification, volatilization and immobilization on the other hand (Wong and Nortcliff, 1995). Mineralization, immobilization and denitrification are biochemical processes which depend on soil moisture, temperature and carbon (Aulakh et al., 1992) and since these processes vary with time and space, the concentration of soil inorganic N inevitably varies temporally and spatially. Soil drying and rewetting increases the rate of inorganic N released from organic form (Birch, 1958; Wong and Nortcliff, 1995). The highest concentration of inorganic N in the top soil in the tropics is at the transition from the dry season to the wet season. Most of the mineralized N is lost if its release and plant demand for it are not synchronized. Synchrony requires that the rate of release of a nutrient into a plant-available form is closely related to the rate at which the nutrient is needed by the plants. Plant uptake of nitrogen depends on positional relationship between plant roots and available N forms (Wiesler and Horst, 1994). Thus, the N form available to plants at a given time and at a soil depth is dependent on N dynamics in the soil in relation to plant roots. Thorough understanding of N dynamics in the soil is, therefore, prerequisite in formulating strategies to

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Table 1. Physical and chemical properties of the experimental soils

Property	Soil sample		
	Soil 1	Soil 2	Soil 3
%Sand	24	26	27
%Silt	13	15	14
%Clay	63	59	59
Textural class	Clay	Clay	Clay
pH water	5.9	5.8	5.8
%N	0.17	0.14	0.18
%C	2.29	2.11	2.81
C:N ratio	13.5	15.1	15.6
K cmol (+) kg^{-1}	0.4	0.3	0.6
Mg cmol (+) kg^{-1}	11.3	12.3	10.8
Ca cmol (+) kg^{-1}	22.9	24.4	22.2
CEC	38	45	48

synchronize supply and crop requirement of N and protect the environment from unutilized N.

In order to monitor the net effects of N dynamics (that is, inorganic N concentrations) in soils over time, composite soil sample is collected at time intervals, covering the entire period during which the N changes are to be determined. Inorganic N is invariably determined in field-fresh soil samples (Recous et al., 1988; Tripathi and Ladha, 1997) to reflect the position in the soil at the time of sampling (Anonymous, 1995). Soil sampling and sample preparation constitute the most important steps in any soil-testing program. Laboratory analysis does not compensate for poorly collected, poorly prepared or unrepresentative soil sample (Anderson and Ingram, 1993). The collection of a representative soil sample is critical because only small amount (~10 to 25 g) of moist soil is used in analysis. Soil sample, therefore, should be collected from many spots in an experimental plot, composited, thoroughly mixed, and then ~200 g sub-sampled by quartering method (Anonymous, 1995).

The sticky characteristic of Vertisol when wet makes it difficult to thoroughly mix wet composite samples to obtain a representative sample of an area, hence determination of inorganic N content of Vertisols over a period of time would require some adequate drying of samples to facilitate thorough mixing and storage, but both the drying temperature and the length of storage period have effects on soil extractable nutrients (Houba and Novozamsky, 1998; Magesan et al., 2002; Gray and Mclaren, 2003). Carbon:nitrogen ratio also influences mineralization and immobilization processes (Brady and Weil, 2002). Total soil carbon vary with ecosystem (Bernoux et al., 2002) and soil type (Sakin et al., 2011). The C:N ratio is also influenced by both ecosystem and soil type (Sakin, 2012). We determined the effects of drying method, storage period and C:N ratio on inorganic N contents of tropical Vertisols.

MATERIALS AND METHODS

Experimental soils

To characterize the experimental soils, samples were collected from three sites within Muhoroni Division, Kenya, located at 00° 10.06' S, 035° 13.50' E, and at an elevation of 1339.7 m above sea level. We selected on the basis of different C:N ratios. Soil samples were air dried and ground to pass through 2.0 mm sieve and analyzed for texture, pH, total N, organic carbon, exchangeable bases and cation exchange capacity (CEC). Soil texture was determined by the hydrometer method (Bouyoucos, 1962). Soil pH, total N, organic C, exchangeable K, Ca, Mg, and CEC were determined following methods described by Okalebo et al. (2002). The properties of the experimental soils are summarized in Table 1. Sigunga (1997) classified the soils of the experimental site as pellic-eutric Vertisols according to Anonymous (1997).

Experimental treatments and design

The treatments comprised of three soils with different C:N ratios, five storage periods, and five drying methods (Table 2). Soil samples awaiting inorganic N extraction were stored at-10°C in order to minimize biochemical changes during storage (Bremner, 1996). The five levels of drying methods were as follows: in drying method 1, control (method 1) field-fresh soil samples were kept at -10°C and later used for inorganic N extraction 2, 5, 10, 20, and 40 days after soil sampling date. Drying method 2 (method 2) involved air drying field-fresh soil samples at room temperature (ca. 20°C) for two days, then stored at -10°C and later used for inorganic N extraction on 0, 3, 8, 18 and 38 days after drying. The first sub-sample in this group was used for inorganic N extraction immediately after air drying (that is, 0 days after drying). In drying method 3 (method 3), field-fresh soil samples stored at -10°C were sub-sampled 0, 3, 8, 18 and 38 days after sampling date. The sub-samples were air dried at room temperature for two days, and then used for inorganic N extraction immediately. In drying method 4 (method 4), field-fresh soil samples were oven dried at 60°C for two days. The dried samples were cooled in a dessicator, then packed in polyethylene bags and stored at -10°C until inorganic N extraction on 0, 3, 8, 18, and 38 days after drying. Drying method 5 (method 5) involved oven drying field-fresh soil samples at 100°C for two days. The dried samples were cooled in a dessicator, then packed in polyethylene bags and stored at -10°C until inorganic N extraction on 0, 3, 8, 18, and 38 days after drying. The 75 treatments were combined in a factorial arrangement in a completely randomized design (CRD) replicated three times.

Data collection and statistical analysis

It is not feasible to obtain a composite representative fresh sample of wet Vertisol due to its stickiness that makes thorough mixing before sub-sampling impossible. To go round this problem and also to minimize the impact of spatial heterogeneity in soil properties both in horizontal and vertical directions, a small cylindrical block of soil was excavated using a PVC cylinder 20, 10, and 1. 5 cm in diameter, height and thickness, respectively. Excavated soil samples were immediately placed in polyethylene bags in cool box to avoid loss of water and to minimize biochemical changes during transportation to the laboratory.

In the extraction of inorganic N, about 10 g of soil sample was mixed with 100 ml of 0.5 M K_2SO_4 in a plastic bottle and shaken for 1 h at 150 reciprocations per minute. The soil extract was filtered using Whatman No. 42 filter paper. The filtrate was stored at -10°C and later analyzed for NH_4^+-N and NO_3 -N. Both NH_4^+-N and NO_3 -N were determined as described by Okalebo et al. (2002). Inorganic N content of the experimental soils was corrected to oven dry weight basis.

Table 2. Details of treatment factors and levels.

Factor	Level designation	Level description/characteristic
Soil	Soil 1	C:N = 13.5; %N = 0.17
	Soil 2	C:N = 15.1; %N = 0.14
	Soil 3	C:N = 15.6; %N = 0.18
Drying method	Method 1	Control. Field-fresh soil samples stored at -10°C and later used for inorganic N extraction.
	Method 2	Field-fresh soil samples were air dried at room temperature for 2 days then stored at -10^{0}C and later used for inorganic N extraction.
	Method 3	Field-fresh soil samples stored at -10°C were sub-sampled 0, 3, 8, 18 and 38 days after collection. The sub-samples were air dried at room temperature for two days and then used for inorganic N extraction immediately.
	Method 4	Field-fresh soil samples were oven dried at 60°C for two days. The dried samples were cooled in a dessicator and stored at -10°C until inorganic N extraction.
	Method 5	Field-fresh soil samples were oven dried at 100°C for two days. The dried samples were cooled in a dessicator and stored at -10°C until inorganic N extraction
Storage period	Period 1	The soil samples were stored for 2 days from sampling date and then used for Mineral N extraction
	Period 2	The soil samples were stored for 5 days from sampling date and then used for Mineral N extraction
	Period 3	The soil samples were stored for 10 days from sampling date and then used for Mineral N extraction
	Period 4	The soil samples were stored for 20 days from sampling date and then used for Mineral N extraction
	Period 5	The soil samples were stored for 40 days from sampling date and then used for Mineral N extraction

Table 3. Analysis of variance table of the effects of soils, drying methods and storage periods on mineral nitrogen content (mg N kg^{-1} oven-dry soil) of the experimental soils.

Source of variation	Degrees of freedom	F value	
		NH_4^+	NO_3^-
[1]Soils	2	11.85*	224.76*
[2]Drying methods	4	285.05*	65.39*
Soils x drying methods	8	4.88*	15.56*
[3]Storage periods	4	5.71*	14.21*
Soils x storage periods	8	5.12*	10.69*
Drying methods x storage periods	16	5.55*	3.21*
Soils x drying methods x storage periods	32	2.47*	3.14*
Error	150	-	-

*Indicates significant difference at 0.05 level. [1]Soils, [2]drying methods and [3]storage periods are as explained in Table 2.

Inorganic N obtained from the extracts was statistically analyzed using analysis of variance (ANOVA), and separation of means due to treatments was done using least significant difference (LSD) with the help of GENSTAT computer software package (Anonymous, 2010).

RESULTS AND DISCUSSION

Influence of drying methods on mineral N content of Vertisols

Drying methods significantly (p=0.05) influenced the mean NH_4^+-N and NO_3^--N contents of the soils (Table 3). Drying method 1 (Method 1), the control, resulted in significantly (p=0.05) the lowest while Method 5 produced the highest mean NH_4^+-N content of the soils, respectively (Table 4). The order was method 5 > method 4 > method 3 = method 2 > method 1. Nitrate-N content of the soils was also significantly influenced by the drying methods with method 1 resulting in the lowest while methods 2 and 3 resulted in the highest NO_3^--N contents (Table 4). The order was method 3 = method 2 > method 5 = method 4 > method 1.

The significantly (p = 0.05) lower NH_4^+-N contents due to method 1 (control) compared to the other drying methods underscores the effect of drying on NH_4^+-N changes in soils. Nelson and Bremner (1972) found that both air drying in the laboratory for 72 h and oven drying at 55°C for 16 h produced increase in exchangeable NH_4^+-N. Ammonia absorption from the air could also contribute to the

Table 4. Mean effects of main treatments on mineral nitrogen content (mg N kg^{-1} oven-dry soil) of the experimental soils.

Main treatment	NH_4^+-N	NO_3^--N
***Soils (n = 75)**		
Soil 1	25.8	0.8
Soil 2	27.5	1.1
Soil 3	28.0	1.3
$LSD_{0.05}$	0.96	0.25
***Drying methods (n = 45)**		
Method 1	18.2	0.5
Method 2	23.3	2.6
Method 3	24.2	2.5
Method 4	33.8	1.0
Method 5	35.9	1.1
$LSD_{0.05}$	1.24	0.33
***Storage periods (n = 45)**		
Period 1	28.4	1.1
Period 2	26.3	1.2
Period 3	27.0	1.5
Period 4	26.9	1.9
Period 5	27.2	2.2
$LSD_{0.05}$	1.24	0.33

*Soils, drying methods, and storage periods are as explained in Table 2.

moderate increase in NH_4^+-N content when soils were air-dried (methods 2 and 3). Selmer-Olsen et al. (1971) reported that drying soils in open boxes produced more ammonium than drying in trays at 20°C. The pronounced increase NH_4^+-N content of soils due to oven drying methods (methods 4 and 5) was ascribed to organic and other inorganic sources such as ammonium phosphate complexes that were decomposed during heating and released ammonia which was later retained by cation exchange sites of the soil colloids. Frye and Hutcheson (1981) reported that oven drying increases NH_4^+ -N released from soils and that oven drying at 110°C released greater amount of NH_4^+-N.

The significantly (p = 0.05) lower NO_3^--N contents due to methods 5 and 4 compared to methods 3 and 2 (Table 4) was attributed to high oven drying temperatures (70 and 100°C, respectively) that could have reduced the activity of nitrifying organisms. The high oven drying temperatures reduced the soil moisture levels and, inevitably, reduced microbial activity. Linn and Doran (1984) reported low microorganism activity at soil moisture contents below 10% water filled pore space. The high NO_3^--N levels in methods 2 and 3 could be attributed to enhanced mineralization process during air drying of soil samples at room temperature (20°C). Selmer-Olsen et al. (1971) reported that NO_3^--N increased with time at 20°C. The significantly (p=0.05) higher NO_3^--N contents of the experimental soils due to methods 3 and 2 than method 1 (control), although all the three treatments involved air drying and storage at - 10°C, indicates that mineralization continued at the low storage temperature. Magesan et al. (2002) also observed that nitrification proceeded during low temperature storage. Much lower storage temperature would, possibly, be necessary if nitrification were to be stopped completely. Selmner-Olsen et al. (1971) reported that there were no changes in mineral-N with time if soil samples were stored at -23°C. The storage temperature used in the current experiment was -10°C. The significantly lower NH_4^+ -N and NO_3^--N due to method 1 than due to any other drying method indicate that extraction for mineral N should be done on field moist soil sample.

Effects of storage period on mineral nitrogen contents

Storage periods had significant (p=0.05) effect on both NH_4^+-N and NO_3^--N contents of the soils (Table 3). Storage period 1 (Period 1) resulted in significantly (p=0.5) highest NH_4^+-N contents of the experimental soils while there were no differences due to other storage periods (Table 4). There is no clear pattern of relationship between NH_4^+-N content of the soils with storage period. Periods 1 and 2 lead to similar but significantly lowest, while periods 4 and 5 gave significantly highest mean NO_3^--N contents of the soils (Table 4).

The lack of effect of storage periods longer than two days after soil sampling (period 1) on NH_4^+-N content is in contrast to some reports but in agreement with some. Green et al. (1994) and Houba and Novozamsky (1998) reported that extractable NH_4^+-N contents of soils increased with storage time while Gray and Mclaren (2003) reported that extractable NH_4^+-N content of soils did not increase with storage period. However, Selmer-Olsen et al. (1971) observed that soil NH_4^+-N increased in one day then decreased as storage period became longer. The authors also observed that storage of soil samples at 4°C in closed polyethylene bags decreased the content of NH_4^+-N. The apparent lack of clear relationship between storage period and NH_4^+-N contents of the soils is attributed to the complex interactions of soil characteristics, fixed NH_4^+-N, drying temperature, and mineralization and immobilization rates (Aduayi, 1981; Sanchez et al., 2001). The significant ($p=0.05$) increase of NO_3^--N contents of the soils with storage period is consistent with previous findings. Selmer-Olsen et al. (1971) reported that soil nitrate-N increased with storage period. Gray and Mclaren (2003) also report that concentrations of Ca^{2+}, Mg^{2+}, K^+, Na^+, NO_3^-, Cl^- and SO_4^{2-} in soil solution increased with storage time. In the current study, however, the increase in NO_3^- was significant only after period 3 indicating low nitrification rate. These results show that all the storage periods used in the current experiment lead to significantly lower NH_4^+-N than did period 1 (control) indicating that stored soil samples did not reflect the situation of field moist soil.

Differences in soils with respect to mineral nitrogen content

The soils had significant ($p = 0.05$) differences in terms of mean NH_4^+-N and NO_3^--N contents (Table 3). Soil 1 had significantly ($p = 0.05$) lowest NH_4^+-N and NO_3^--N contents, but there was no difference between soils 2 and 3 (Table 4). The magnitude of NH_4^+-N released from a soil is influenced by the soil's initial %C (Sertsu and Sanchez, 1978), %N (Sanchez et al., 2001), and C:N ratio (Brady and Weil, 2002). The C:N ratio of each of the three soils was less than 20, a situation that allowed net mineralization to occur (Wong and Nortcliff, 1995). The authors report that decomposition rate is sometimes better related to the %N than C:N ratio and a critical level of 0.17 %N was suggested for mineralization. Soil 3 with relatively high C:N ratio and %N of 15.6 and 0.18, respectively gave the highest NH_4^+-N and NO_3^--N, while, soil 1 with C:N ratio and %N of 13.5 and 0.17, respectively had the lowest NH_4^+-N and NO_3^--N contents. This observation is in agreement with the reports by Schomberg et al. (1994), Wong and Nortcliff (1995) and Sanchez et al. (2001). The significant difference in NH_4^+-N and NO_3^--N between soils 1 and 3 could be attributed to the differences in %N and C:N ratios, but the differences between soils 1 and 2 could not be explained in terms of the differences in %N and C:N ratios between the two soils. This suggests that there were other sources, besides organic matter, that contributed to the NH_4^+-N and NO_3^--N released from the soils. Frye and Hutcheson (1981) reported that both organic and inorganic exchangeable N contributed to the NH_4^+-N released from soils during oven drying. Green et al. (1994) also observed rapid (45%) release of fixed NH_4^+-N during nitrification in 15 days. The released NH_4^+-N would then be nitrified to NO_3^--N. These results indicate that other factors than organic C and N contributed to both NH_4^+-N and NO_3^--N released from the soils during drying and storage.

Interactive effects of treatments on mineral N contents of the soils

Interactions between the main treatments showed significant differences (Table 3). Soil 1 consistently gave significantly ($p=0.5$) lowest NH_4^+-N across methods 1, 2 and 3 and periods 1, 2 and 3 combinations (Table 5). There were no differences amongst the soils across methods 4 and 5, and periods 4 and 5 combinations. For each soil x method combination, NH_4^+-N tended to decrease with storage period, but the relationship was not consistent. Method 1 x period 1 (check) resulted in significantly ($p=0.05$) lowest NH_4^+-N content for each soil compared to the other drying method x storage period combinations (Table 5) indicating that none of the other combinations reflected status of the field moist soil. For each soil and each storage period, method 1 resulted in significantly ($p=0.05$) lowest, while methods 2 and 3 resulted in the highest NO_3^--N contents (Table 5). Soil 3 had significantly ($p=0.05$) highest NO_3^--N content across the drying methods and storage periods compared to the other soils. The consistency of results relating to the experimental soils across the drying methods and storage periods indicate that the cylindrical block of soil used sufficiently controlled heterogeneity. However, since the check (method 1 x period 1) resulted in both NH_4^+-N and NO_3^--N contents significantly different from those due to the other drying methods x storage period combinations, there is still no method that allows for compositing and mixing Vertisols samples to obtain a representative sample from field moist collections.

Conclusions

Drying of soils, whether in air or in oven, consistently resulted in significant increase in NH_4^+-N released from each soil under each storage period. The increase in NH_4^+-N released is higher with oven drying. The higher the drying temperature, the higher is the amount of NH_4^+-N released from the soils. On the other hand the higher the drying temperature, the lower the amount of NO_3^--N released from the soils indicating that the higher temperatures (70 and 100°C) employed in the oven drying in the current

Table 5. Interactive effects of soils, drying methods, and storage periods on mineral nitrogen contents (mg N kg^{-1} oven-dry soil) of the experimental soils.

***Soil**	***Drying method**	***Storage period**									
		Period 1		**Period 2**		**Period 3**		**Period 4**		**Period 5**	
		NH_4^+	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**
Soil 1	Method 1	17.2	0.1	16.4	0.4	17.1	0.3	23.1	0.3	14.7	0.4
	Method 2	22.5	0.7	19.2	0.7	18.2	0.7	24.9	0.7	19.2	0.7
	Method 3	22.9	0.7	18.2	0.7	19.6	0.9	24.1	0.6	20.9	0.8
	Method 4	33.5	0.2	32.3	0.3	29.7	0.2	33.6	0.4	33.4	0.2
	Method 5	30.5	0.3	32.5	0.3	34.1	0.2	39.2	0.3	39.5	0.1
Soil 2	Method 1	21.7	0.2	20.8	0.2	23.4	0.3	19.6	0.3	12.6	0.6
	Method 2	27.6	0.5	24.6	1.7	27.4	0.7	25.4	1.3	20.9	1.0
	Method 3	28.3	0.6	28.9	2.0	28.0	0.8	21.2	0.8	28.3	1.2
	Method 4	36.3	0.2	30.4	0.3	32.4	0.4	35.7	0.7	34.0	0.8
	Method 5	33.6	0.2	28.4	0.3	33.3	0.4	35.0	0.6	41.1	0.7
Soil 3	Method 1	21.5	0.5	22.0	0.6	21.8	1.1	15.5	0.9	12.5	1.8
	Method 2	26.7	2.4	22.4	2.8	21.4	2.5	27.8	2.6	23.8	2.4
	Method 3	26.9	2.0	23.2	2.2	26.9	2.3	22.1	2.5	26.1	2.1
	Method 4	35.5	1.6	36.5	1.9	36.4	1.7	33.7	1.3	28.7	1.5
	Method 5	37.5	1.2	41.2	2.1	41.4	1.3	36.6	1.3	36.1	1.4

The values are means of three observations. *****Soils, drying methods and storage periods are as explained in Table 2. LSD $_{0.05}$ for NH_4^+ and NO_3^- are 3.9 and 0.3, respectively.

study depressed the activities of nitrifying organisms. Since method 1 resulted in significantly lower NH_4^+-N and NO_3^--N than did the other drying methods, it was concluded that none of the tested drying methods was suitable substitute for the use of field fresh soil sample for the determination of mineral N contents of Vertisols.

The relationship between NH_4^+-N content of the soils and the storage period was not clear suggesting complex interactions involving soil characteristics, fixed NH_4^+-N, drying temperature and mineralization and immobilization rates. The significant increase in NO_3^--N with storage period only after period 3 indicated that storage temperature (-10°C) depressed but did not eliminate nitrification. The consistency of NH_4^+-N and NO_3^--N contents in respect of each soil across the drying methods and storage periods suggested that the cylindrical block of soil used in the current experiment sufficiently checked heterogeneity of the soils.

Since method 1 x period 1, for each soil, resulted in NH_4^+-N and NO_3^--N contents significantly different from those due of the other drying method x storage period combinations, it was concluded that extraction for mineral N should be done on field moist soil not later than two days after sampling. Further research should be conducted to determine drying method that would facilitate mixing of Vertisols samples and storage temperature that would retain mineral N content reflective of field moist soil status.

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