Nitrogen Volatilization And Mineralization In A Sandy Entisol Of Florida Under Citrus

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Nitrogen Volatilization and Mineralization in a Sandy Entisol of Florida Under Citrus[#]

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ABSTRACT

Increasingly nitrate nitrogen (NO_3-N) is found in excess of maximum contaminant limit (MCL) in groundwater adjacent to citrus production areas of central Florida. Understanding of fate of N in the environment following the application of fertilizers is critical to improve nutrient

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uptake efficiency, minimize nutrient losses and reduce the adverse effects on the groundwater. A study evaluated ammonia volatilization (NH_3) and effects of ammonium nitrate (AN) and urea (UR) on N budget of citrus trees on a sandy Entisol. Dry granular N fertilizers were surface applied to irrigated 'Hamlin' orange trees. Ammonia volatilization was evaluated using a semi-open NH_3 trapping system with and without additional air circulation, while net N mineralization and leaching losses were evaluated using *in situ* incubations with polyvinyl chloride (PVC) columns. Significant N losses by NH_3 volatilization were observed from applied N fertilizers, which accounted for up to 13% of applied N as AN, and 33% as UR with additional air circulation in the collection chamber. These values were significantly greater than those observed with no additional air circulation. Maximum rates of NH_3 volatilization occurred within 5 d after fertilizer application and were greater during the day than during the evening. This study demonstrated that fertilizer use efficiency is reduced due to increased gaseous losses of NH_3 when urea is used, and that N fertilization affects soil N mineralization in a short time period after fertilizer application. Fertilization decreased N mineralization and increased the amount of soil microbial biomass N during incubation. Leaching of N accounted to 13% of applied N during a 90-d period.

Key Words: Ammonia; Ammonium nitrate; Gaseous loss; Microbial biomass N; N leaching; Urea.

INTRODUCTION

Nitrogen (N) is a major nutrient element for citrus production.[1] About 75% of the total U.S. citrus production come from Florida. About 28% of Florida citrus acreage is on sandy entisols along the central Florida ridge. In sandy soils, application of N in excess of tree requirements, inadequate placement and timing of N application, and irrigation scheduling can result leaching of nitrate (NO_3^-) below the root zone.[2]

Studies have been conducted under careful irrigation scheduling to evaluate the effects of a wide range of N rates and sources on fruit production, as well as leaching of nitrate-N below the roots on bearing orange trees.[3–5] This research was part of an ongoing project aimed to provide information to develop best management practices (BMP) of N fertilization and irrigation for nonbearing and bearing citrus trees in Florida. Investigations on nutrient management strategies and their impact on all components of citrus N budget in the field, including mineralization, N_2 fixation, plant uptake and various losses of plant available N in the soil, which can result in adverse environmental impacts should be addressed in a comprehensive manner.

Urea is the most common N fertilizer used for various crops around the world. This is due to high content of N, which favors the economics of manufacturing, handling, storage, transportation, and spreading in the field. However, N uptake efficiency from urea is quite low if it is not incorporated, due to gaseous loss of N in the form of ammonia (NH_3), a process called as volatilization. Volatilization of NH_3 from other forms of N fertilizer is less significant than that from urea in non-alkaline soil.[6] The general process of N volatilization from applied urea is related to a localized increase in soil pH after dissolution and hydrolysis of the fertilizer.

Marshall and Debell[7] reported four techniques to measure gaseous losses of NH_3 from soils. Three techniques use acid sorbers for trapping ammonia but differ in the mechanism by which NH_3 and other gases may move from the soil to the sorber: (i) closed-static system, which restricts exchange with the atmosphere, thereby creating a closed NH_3 sink; (ii) semi-open system, which permits exchange with the atmosphere via diffusion, and (iii) closed-dynamic systems in which NH_3 and other gases are removed from the soil surface by air bubbled through a NH_3 trap. The fourth technique uses ^{15}N-enriched urea, where the amount of N that cannot be accounted for in all measurable pools is considered as the gaseous loss, presumed to be mainly NH_3. Variations of the semi-open system have been tested in the field, which allowed continuous measurement of NH_3 volatilization over a broad range of environmental conditions and with a number of different treatments.[8–11] Estimations obtained by those authors indicated that up to 47% of applied N may volatilize. Environmental conditions, such as wind and vapor pressure, influence the rates of gaseous losses.

Field evaluation of NH_3 losses can contribute to an accurate estimation of reduction of efficiency of the applied N fertilizer, which is important for developing an accurate N budget for a given cropping system.

The mineralization of N from soil organic matter, crop residues, composts, and animal manure contributes significantly to the soil mineral-N reserve. The amount of net N mineralized (N-min) in soil for various crops ranges from 50 to $150 \, kg \, N \, ha^{-1} \, yr^{-1}$.[12] Dou et al.[13] reported that mineralization of N from leaf residues under the citrus tree canopy contributed 40 to $150 \, kg \, N \, ha^{-1} \, yr^{-1}$ depending on the soil type and tree age. Dou and Alva[14] found a rapid increase in NH_4-N concentration in soil amended with citrus leaves during a short period of incubation in a pot experiment with fine sand.

The current interest in nutrient cycling in both managed and natural ecosystems has led to renewed attempts to develop reliable methods for measuring N mineralization using either laboratory or field techniques.[15] The evaluation of soil-N mineralization has emphasized indexes of potentially

mineralizable N (N_0) as laboratory-based methods. Keeney[16] discussed chemical extraction procedures, based on an empirical assessment of organic-N pools. *In situ* methods using soil cores are expected to give more reliable estimates of soil-N mineralization than laboratory methods, because soil-N turnover is strongly affected by microenvironmental conditions. Soil disturbance during sample preparation markedly affects N mineralization.[17] In the absence of plant uptake, denitrification, and leaching of N from soil cores, the difference in inorganic N concentrations between the soil at the beginning and end of the incubation is a direct measure of net mineralization or immobilization.[18] Adams et al.[19] proposed the use of perforated columns to allow moisture equilibrium across the interface between the soil inside the columns and the bulk soil. The minimal soil disturbance, ease of column insertion and removal from the soil are some of the advantages of this technique. The incubation column is generally capped to avoid application of excess water, by irrigation and rainfall, during the incubation, which can result in leaching of a portion of mineralized N below the depth of column. Therefore, comparisons of the amount of N mineralized during a given duration of incubation between the capped and open incubation columns provides an estimate of leaching of soluble N.

The soil microbial biomass is another important component of the soil organic matter that regulates the transformation and storage of nutrients. Observation that K_2SO_4-extractable NH_4-N increased after soil fumigation with chloroform ($CHCl_3$) suggested that a procedure developed for evaluation of biomass C could be used to measure biomass N. Assuming that soil fumigation does not affect the mineralization rate of non-microbial biomass, then the quantity of mineral N provides a measure of the amount of biomass N in a soil.[20] Therefore, the objectives of this study were to evaluate: (i) soil net N-mineralization; (ii) the relationship between N mineralization and soil microbial biomass N; (iii) N leaching losses; and (iv) NH_3 volatilization from surface applied NH_4NO_3 or urea to a sandy entisol with 6-yr-old citrus trees.

MATERIALS AND METHODS

Experimental Area

The experiment was conducted in an experimental grove of 6-yr-old 'Hamlin' orange trees [*Citrus sinensis* (L.) Osb.] on Swingle citrumelo rootstock [*Poncirus trifoliata* (L.) Raf. x *C. paradisi* Macf.] planted in a Candler fine sand (hyperthermic, uncoated Typic Quartzipsaments) in Florida. The trees were planted in September 1993 at 7.6 by 4.6 m spacing.

Table 1. Selected characteristics of Candler fine sand at the experimental site.

Soil characteristics	0–15 cm	15–30 cm
pH[a]	7.03	6.98
Organic carbon, $g\,kg^{-1}$[b]	6.1	5.1
Total nitrogen, $g\,kg^{-1}$[b]	0.4	0.2
P, $mg\,kg^{-1}$[c]	53	42
Bulk density, $g\,cm^{-3}$	1.47	1.51
Sand, $g\,kg^{-1}$	967	972
Silt, $g\,kg^{-1}$	8	5
Clay, $g\,kg^{-1}$	25	23

[a] Water/soil ratio, 2:1 (v/w).
[b] CNS Analyzer, NA-1500, Carlo Erba, Haak-Buchler Instruments, Saddlebrook, NJ.
[c] Mehlich 1 extraction.

The Candler soil is a deep, well-drained sand with no confining soil horizons. This soil is characterized by a low organic matter content, low water holding capacity, and thus needs frequent irrigation. Selected soil physical and chemical characteristics of Candler fine sand are presented in Table 1.

Treatments and Irrigation Management

The experiment was conducted using a randomized design with three replications. Treatments included: unfertilized trees, and trees fertilized with urea or ammonium nitrate. Single tree plots were used in this experiment. Fertilizer was applied uniformly as dry granules on the soil surface in a circular area (1.10 m radius) under the tree canopy. Twenty-five percent of the annual recommended rate of 230 g N per tree[21] was applied on February 15, 1999. Fertilizer application was followed by 7 mm of irrigation water using micro-sprinklers to promote fertilizer dissolution and shallow incorporation into the soil. Trees were irrigated using one emitter under the tree canopy with a delivery rate of $50\,L\,h^{-1}$ per tree covering about $7\,m^2$ area. Irrigation was scheduled when available soil moisture depletion attained 33% in the top 40-cm soil depth. Soil moisture was continuously monitored using capacitance probes (EnviroSCAN®, Sentek PTY Ltd., South Australia) placed at the drip line of citrus trees at various depths.[22] The area under the tree canopy was maintained free of weeds by herbicide use and hand weeding.

The amount of irrigation water applied to the orange trees and the weather information (rainfall, air temperature, soil temperature) collected from the FAWN data set (Florida Automatic Weather Network) for the Citrus Research and Education Center in Lake Alfred, FL were recorded from February to May 1999 and presented in Figure 1.

Volatilization of Ammonia

Ammonia volatilized from dry-granular AN and UR fertilizers applied on the soil surface was evaluated using a semi-open static system of ammonia sorbers.[8] A modification of the Nômmik's apparatus was made in order to assess NH_3 losses under forced air flux (dynamic system) using a cooling fan (Brusheless Fan, 12 VDC, 7.6 cm diameter; Radioshack City) set inside the collection chamber and hooked up to a rheostat. The fan speed was calibrated in the laboratory using a portable anemometer, and manually set to $0.7\,\mathrm{m\,s^{-1}}$. The collection chamber consisted of a PVC-cylinder, 35 cm long and 30 cm in diameter. The cylinder was driven 5 cm into the soil. Two discs of
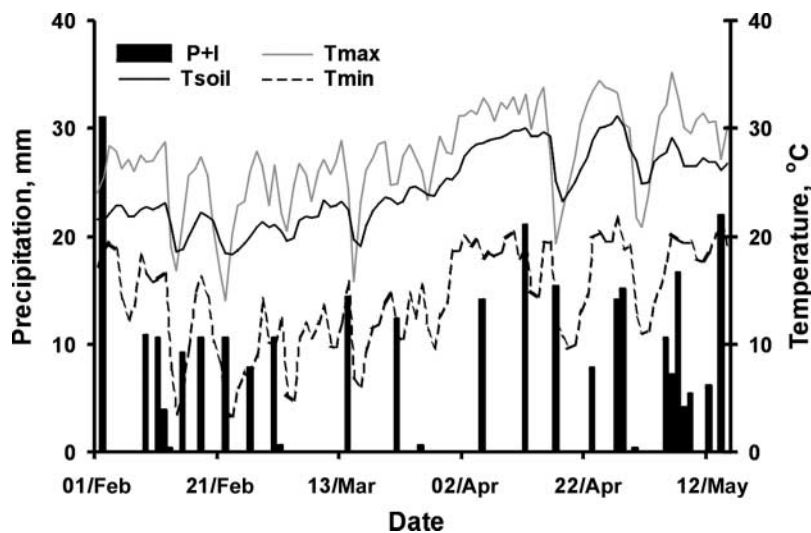

Figure 1. Weather data for the experiment site. Legend: P + I = rainfall plus irrigation water; Tmax and Tmin = daily maximum and minimum average air temperature, respectively; Tsoil = average soil temperature (10 cm depth).

polyurethane plastic foam (density $= 0.03\,g\,cm^{-3}$; thickness $= 2\,cm$; and circular area $= 723\,cm^2$) soaked in $2.25\,mol\,L^{-1}$ H_3PO_4 plus glycerol 4.0% (v/v) solution were placed inside the PVC-cylinder at 10 and 20 cm from the top. Each disc retained 120 to 150 mL of the acid–glycerol solution. The lower disc traps the ammonia volatilized from the soil, while top disc serves to trap any atmospheric NH_3, which could contribute to overestimation of NH_3 volatilized from the soil.

Chambers were placed on the soil surface within the fertilized area under the tree canopy immediately following application of the fertilizer and initial irrigation. After each period of exposure, the NH_3 sorber discs were removed and replaced by fresh sorber discs. The duration of exposure was 9 to 15 h during the first week after fertilization. Subsequent measurements were made on day 8, 10, 14, 18, 24, 29, 34, 39, and 44 after application. Discs collected in the field were placed in sealed plastic bags, and stored in a cold room at 4°C until extracted. Ammonia trapped by the foam discs placed immediately above the soil inside the PVC-cylinder was extracted in the laboratory by placing the foam in $1\,mol\,L^{-1}\,KCl$, and squeezing the excess solution. This procedure was repeated and volumes of solution were adjusted to 500 or 1000 mL. Extracts were analyzed for NH_4-N colorimetrically.[23] Soil temperature was recorded daily at 8 AM and 5 PM within a 10 cm depth layer. Wind speed was also monitored daily using a 3-cup anemometer (Weather Instruments, Princeton, NJ; from Science Associates, Inc.) installed at 0.5 m above the soil level. Soil pH (water:soil ratio $= 2$:1 v/w) was measured using a glass electrode on samples collected from the 0 to 15 cm soil depth layer during the course of the experiment.

In-Situ Soil Column Study

Soil-N leaching and mineralization were measured using an *in situ* soil column technique.[13] Polyvinyl chloride (PVC) columns (35-cm high; 5-cm in diam.) were driven 30 cm into the soil under the tree canopy area, about 80-cm apart from the irrigation micro-sprinkler emitter. At each location of measurement two columns were used. One column was kept open at the top, while the other was closed with a PVC cap to prevent application of water through irrigation and rainfall, which could leach the mineralized N below the depth of column. The difference in the amount of N mineralization measured between the two columns, provides an estimate of soluble N leached from the depth of sampling. The cap was loosely fitted to the PVC column to allow air exchange above the soil with the outside environment. Columns had 8 holes (diam. $= 1\,cm$) on

the walls in order to promote moisture equilibrium between the soil inside the column and the bulk soil outside.[13]

Columns were driven into the soil after fertilizer application and thereafter during a 75-d period starting in February 15, 1999, for a total of six 2-wk-incubation periods. At each installation of incubation columns soil samples were also collected adjacent to the column at 0 to 15 and 15 to 30 cm depths to estimate the status of available N forms at time zero (initial concentration). At the end of the incubation period, columns were excavated from the soil and divided into 0 to 15 and 15 to 30 cm sections to measure the soil moisture content and concentrations of NH_4-N and NO_3-N (final concentration). Ten gram of field-moist soil was weighed into 250 mL centrifuge flasks, 100 mL of 2 mol L^{-1} KCl solution was added. Flasks were agitated on an orbital shaker for 30 min. at 120 rpm. The suspension was centrifuged (1500 g force; 10 min) and filtered through Whatman No. 42 filter paper. Extracts were stored in a cold room (4°C) and then analyzed colorimetrically for inorganic NH_4-N[23] on a Technicon AutoAnalyzer II (Technicon Industrial Systems, Tarrytown, NY) and for NO_3-N[24] on a rapid flow analyzer (ALPKEM Corporation, Clackamas, OR).

The difference in the concentrations of available N forms between the closed and open columns at the end of incubation periods and within a given treatment provided estimates of N leached from the top 30 cm of soil. Estimates of net-N mineralization was only possible starting at the second incubation period (from March 1 to March 15), which were calculated by the increase in the concentration of NH_4-N or NO_3-N in the final as compared to that in the initial soil sampling of each incubation period.

Microbial Biomass Nitrogen

Microbial biomass N (MBN) was determined by the chloroform fumigation–extraction (CFE) technique[25] in soil samples collected outside the PVC columns starting on March 1, 1999. Twenty-five grams of field moist soil were transferred to 50-mL glass beakers placed in an amber-glass vacuum desiccator with an additional beaker containing 60 mL of chloroform and several boiling chips. The desiccator was evacuated until 2 min after the chloroform began to boil. This procedure was repeated three times; air was allowed back into the desiccator by means of a screw control valve on the lid after the first two evacuations. After the third evacuation, the desiccator was sealed under vacuum for 24 h. Fumigated and nonfumigated (paired) samples

were immediately extracted with 100 mL of $0.5 \, mol \, L^{-1} \, K_2SO_4$ for 1 h on a orbital shaker (180 rpm) and filtered through Whatman No. 42 filter paper.

Extracts were stored in a refrigerator at 2°C until analyzed. Aliquots of K_2SO_4 extracts (25 mL) were subjected to Kjeldahl-N digestion.[26] Recovery of organic N was checked by performing digestions using a solution prepared with primary standard of tris (hydroxymethyl) aminomethane (tradename, THAM®).[27] Samples were brought to 25 mL with deionized-water. Extracts were analyzed for NH_4-N colorimetrically.[23] Microbial biomass N was determined by subtracting the total N extracted in the K_2SO_4 solution of non-fumigated from that of fumigated samples.[25] The efficiency of organic microbial N (k_N) for CFE 1d incubation used to calculate the MBN was 0.54.[25]

Statistical Analysis

Data were tested for significant differences among treatments for a randomized complete block ANOVA with three replications using the GLM procedure of the SAS® system.[28] Regression analysis was performed with data collected for NH_3 volatilization from fertilized plots using the SAS REG procedure. The model was of the type $y = a + b/x$; where $y = NH_3$-N loss (% of applied N), and $x =$ time interval after fertilization (day). Rates of NH_3 evolution during the day and evening were tested using the PROC MIX procedure.

RESULTS AND DISCUSSION

Volatilization of Ammonia

Cumulative NH_3 losses from applied AN or UR fertilizers during 44 d of field evaluation are presented in Figure 2. The response model fitted for each of the fertilizer source showed R^2 values of 0.76 to 0.78 ($P < 0.001$). Volatilization losses were much greater for UR as compared to that for AN. Volatilization of ammonia increased rapidly during the initial 5 d after fertilizer N application. However, in the case of UR, volatilization continued at low rates until 10 d after application.

Differences in NH_3 volatilization from AN and UR source were also related to changes in soil pH following fertilizer application. Average soil pH with AN application was 6.9 ± 0.1, which was similar to that of unfertilized soil. In the case of UR amended soil, the pH initially increased to 8.4 ± 0.4, and took about 32 d to return to the original pH. Volatilization of NH_3 depends
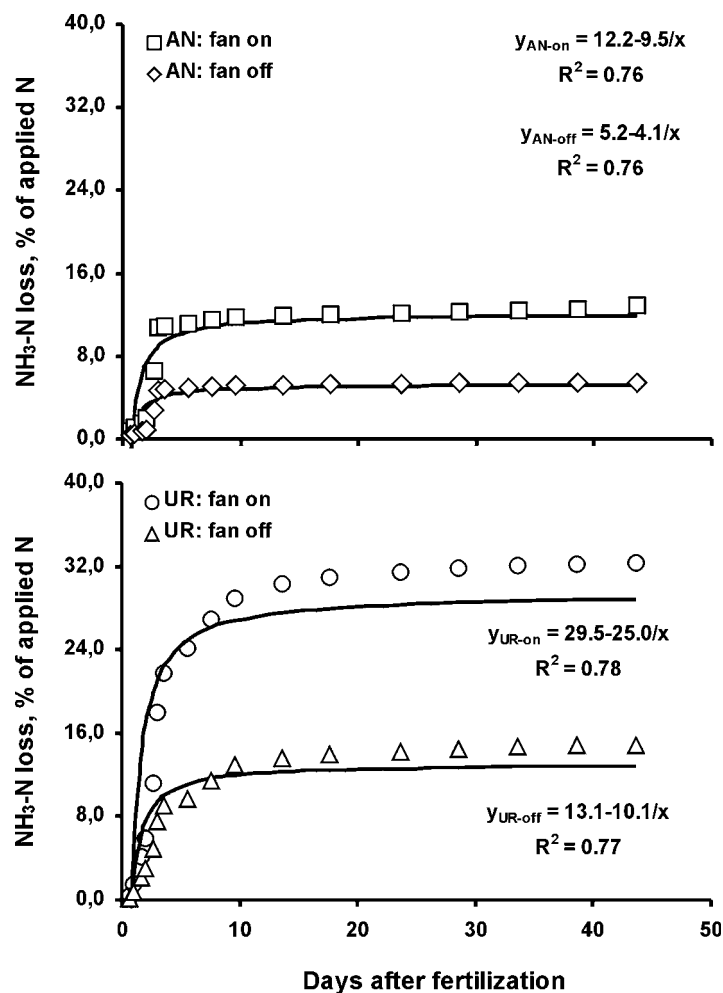
Figure 2. Ammonia losses as measured by a semi-open collector system from field fertilized with ammonium nitrate (AN) or urea (UR). Ammonia collector system set with (fan on) and without (fan off) additional air circulation.

on the quantity and equilibrium of NH_3 and NH_4 forms in the soil solution, which are highly dependent on soil pH. The relative concentration of NH_3 (pKa = 9.3) increases 10% and 50% at pH 8 and 9.3, respectively.[6] For AN, the overall soil pH is important to determine NH_3 volatilization losses whereas the hydrolysis of urea causes a marked increase in the soil pH close to

the fertilizer particles. The soil pH in the immediate vicinity of the urea granules may reach values of up to 9,[6] thus, demonstrating the importance of chemical reactions in soil microsites affecting N transformation. Ammonia volatilization is favored in sandy soils with low buffering capacity, since the ability of NH_4 to form electrostatic bonds with clay minerals and organic colloids is low to impair losses of soil and fertilizer N.

The NH_3 volatilization losses from both N sources were greater with additional air circulation inside the collection chamber to simulate the ambient air movement as compared to that with no additional air circulation. About 5.5 and 12.8% of N applied as AN ($16\,kg\,N\,ha^{-1}$) were lost by NH_3 volatilization without or with additional air circulation inside the chambers, respectively. The corresponding data for urea were 14.9 and 32.3%. Average wind speed measured on the citrus grove was $0.7\,m\,s^{-1}$ (range of 0.4 to $1.3\,m\,s^{-1}$) during the initial 7 d of fertilizer application. A similar condition to the initial week occurred from 7 to 44 d after fertilization, when average wind speed was $0.6\,m\,s^{-1}$ (range of 0.4 to $0.9\,m\,s^{-1}$). Environmental conditions such as aeration, temperature, and soil moisture can have marked influence on volatilization measurements made in the field. Wind speed controls NH_3 volatilization through its effect on the rate of transport of NH_3 away from the soil–air interface. Ammonia volatilization is driven by the difference in NH_3 partial pressure between the atmosphere and that in equilibrium with the moist soil. Marshall and Debell[7] reported that lowest NH_3 losses from UR fertilizer were estimated in field experiments by a close-static (13%) and a semi-open (17%) methods, with restricted airflow as compared to a closed-dynamic method (22–26%). Artificial conditions created in a closed environment can hinder NH_3 diffusion from the soil surface to the sorbers and may result in NH_3 re-adsorption by the soil.

The NH_3 evolution rates during the first week after N fertilizer application to the soil surface is shown in Table 2. The NH_3 volatilization losses were significantly greater during the day as compared to that during the evening measurements. The values measured in the current study were substantially lower than those measured following urea application to a ryegrass/white clover pasture (40 to $110\,mg\,NH_3\text{-}N\,m^{-2}\,h^{-1}$).[29] Such patterns are closely related to soil temperature differences since deviations for NH_3 losses were observed for measurements made by the semi-open system using the fan-off or the fan-on, irrespective to the period evaluated. Soil temperature measured in the 10 cm depth layer was $18.5 \pm 3.3°C$ at 5 PM, and $14.3 \pm 2.4°C$ at 8 AM, which represents a difference greater than 4°C. Ferguson et al.[10] reported that maximum rates of NH_3 loss from UR solution applied to the soil were observed near midday, when water content at the soil surface was beginning to decline and the surface temperature was rapidly rising. Similarly,

Table 2. Ammonia volatilization rates from either ammonium nitrate (AN) or urea (UR) from a Candler fine sand during the first week of fertilizer application.

N source	Fan condition	Day[a] $(mg\,NH_3\text{-}N\,m^{-2}\,h^{-1})$	Evening[a] $(mg\,NH_3\text{-}N\,m^{-2}\,h^{-1})$	$P < F$
AN	Off	5.2	2.7	0.1208
	On	12.0	6.0	0.0062
UR	Off	9.4	5.3	0.0044
	On	22.9	12.7	0.0407

[a] Mean value ($n = 4$).

Lightener et al.[30] reported that the peak of urea-N losses through NH_3 volatilization occurred between midmorning and early afternoon. This corresponded to periods of increasing air temperature and moisture flux toward the soil surface. Volatilization increases with increasing temperature, which is related to higher reaction rates and urease activity.

Lara-Cabezas et al.[31] estimated the volatilization chamber efficiency factor (E) for a semi-open static system similar to that used by Nômmik[8] as 1 to 50%, by estimating the ratio of volatilized NH_3 retained in the chamber with ^{15}N mass balance method. The authors found models that would correct the lower efficiency of the semi-open system. Then, estimated losses in the present study could be lower than the real condition, especially for the system that had no fan installed in the chamber.

Nitrogen Leaching

The differences in concentrations of NH_4-N and NO_3-N measured between the open and closed columns at each incubation interval represent N losses primarily due to leaching below the depth of soil column. Nitrogen loss through denitrification varies greatly and is important mainly under conditions of poor drainage (anaerobiosis) and abundant supply of readily available organic carbon for denitrifying microorganisms.[32] The soil used in this study was well drained and irrigation was carefully scheduled only to replenish the deficit soil moisture. Therefore, the experimental conditions were not conducive to maintain the soil under anaerobic condition. Thus, denitrification loss was quite likely to be insignificant under the conditions of this study. On the other hand, NH_3 volatilization could be an important component of N losses for surface applied urea. Thus, estimation of N leaching losses by mass balance without including the volatilization losses would overestimate

Table 3. Inorganic N losses at 0 to 15 and 15 to 30 cm soil depth under the canopy of 6-yr-old 'Hamlin' orange tress during incubation using the *in situ* incubation PVC columns.

Date of sampling	Incubation (d)	Non-fertilized			AN			UR			LSD[b] ($mg\,kg^{-1}$)
		NH_4-N ($mg\,kg^{-1}$)	NO_3-N ($mg\,kg^{-1}$)	Inorg-N[a] ($mg\,kg^{-1}$)	NH_4-N ($mg\,kg^{-1}$)	NO_3-N ($mg\,kg^{-1}$)	Inorg-N ($mg\,kg^{-1}$)	NH_4-N ($mg\,kg^{-1}$)	NO_3-N ($mg\,kg^{-1}$)	Inorg-N ($mg\,kg^{-1}$)	
				0–15 cm							
1 Mar.	15	0.2	0.6	0.8	9.9	7.4	17.3	24.2	2.3	26.5	6.85
15 Mar.	15	0.2	0.7	0.9	0.7	1.9	2.6	4.4	4.0	8.4	2.67
29 Mar.	15	0.2	0.4	0.6	0.8	1.3	2.1	0.2	1.8	2.0	1.94
12 Apr.	15	0.1	0.4	0.5	0.1	0.5	0.6	0.1	0.4	0.5	1.90
27 Apr.	16	0.1	− 0.1[c]	0	0.1	0.3	0.4	0	0.7	0.7	2.11
11 May	15	0.4	0.4	0.8	0.2	0.5	0.7	0.1	0.3	0.4	1.52
				15–30 cm							
1 Mar.	15	0.2	− 0.3	− 0.1	0.1	− 6.1	− 6.0	0.7	− 1.6	− 0.9	2.11
15 Mar.	15	0.1	0.3	0.4	0.9	6.6	7.5	0	3.1	3.1	2.28
29 Mar.	15	0.2	0.1	0.3	0.6	0.8	1.4	0.2	1.1	1.3	1.54
12 Apr.	15	− 0.2	0.1	− 0.1	0.4	0.9	1.3	0	0.3	0.3	1.17
27 Apr.	16	0	0.2	0.2	0	0.2	0.2	0.1	− 0.3	− 0.2	2.07
11 May	15	0.2	0.3	0.5	− 0.1	0.3	0.2	0	0.1	0.1	2.11

[a] Inorg.-N = NH_4-N + NO_3-N.
[b] The least significance difference ($P = 0.05$) for Inorg-N within fertilization treatments.
[c] Negative numbers indicate accumulation of N.

the leaching losses. Table 3 shows the variation of inorganic N forms in the soil during the incubation periods from March 1 to May 11. A great variation of inorganic N was observed during the incubation period ending on March 1, in which 17.3 and 26.5 mg N kg^{-1} were lost at the 0 to 15 cm soil depth for AN and UR treatments, respectively.

Since NH_3 volatilization was greater for the urea treatment, we can infer that the differences between fertilizer sources were due to gaseous loss rather than leaching losses. An increase in N concentration observed at the 15 to 30 cm soil depth layer during the incubation period which ended on March 1, 1999, for the AN treatment (-6.0 mg N kg^{-1}) could be due to NO_3^- leaching from the 0 to 15 cm depth soil (Table 3). This effect was not evident in the UR treatment, due to the lag time required in the process of urea nitrogen to be transformed into ammonium form and subsequently into nitrate form.[33] In the case of the urea treatment, the transformation of urea into other N forms would have been completed by March 15, 1999. Therefore, the losses observed in the subsequent sampling times would indicate the losses due to leaching only, since volatilization was quite unlikely during the latter period of incubation. Leaching was prominent until March 29 for both soil layers. After this period, the N leaching from the columns decreased substantially (< 1.0 mg N kg^{-1}) (Table 3). Total rainfall during April 30 through May 9 was 33 mm. There was a single rain event of 14 mm on March 14 (Fig. 1), which could have contributed to greater N losses from the 15 to 30 cm depth soil recorded for the period ending on March 15 (7.6 and 3.1 mg N kg^{-1} for AN and UR, respectively). The irrigation in the experimental site was scheduled to replenish the soil moisture content within the rooting depth back to field capacity level when moisture content was depleted below recommended levels.

Nitrogen Mineralization and MBN

Net N mineralization was estimated for incubation periods of about 15 d (Figs. 3 and 4). An initial net immobilization of N was followed by a period of net mineralization at the 0 to 15-cm soil depth (Fig. 3A). Part of the fertilizer N applied to the soils may be converted into organic N as a component of the microbial biomass.[34] Accumulation of decomposed tissue increases the potential for N immobilization since more organic C is available to the soil microbes thus promoting increased microbial activity. Soil organic-C content was relatively greater at the surface as compared to that of 15 to 30 cm depth (Table 1). Work with ^{15}N suggests that 10 to 40% of fertilizer-N may be incorporated in soil organic matter.[35]
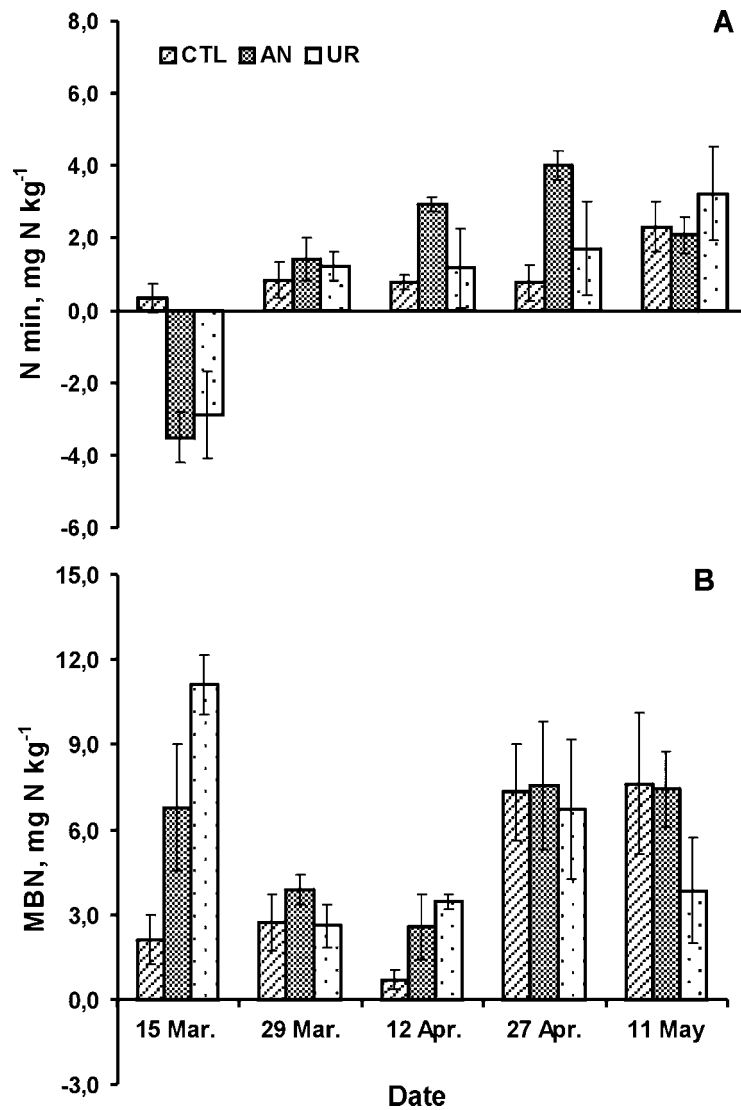
Figure 3. Net nitrogen mineralization (N-min) and microbial biomass N (MBN) in a sandy Florida Entisol under young citrus trees at 0 to 15 cm depth. Fertilizer was applied on February 15. Legend: CTL = unfertilized; UR = urea treated; and AN = ammonium nitrate treated. Vertical bars indicate the standard error of the mean (n = 3). a) Nitrogen mineralization vs. time; b) Microbial biomass N vs. time.

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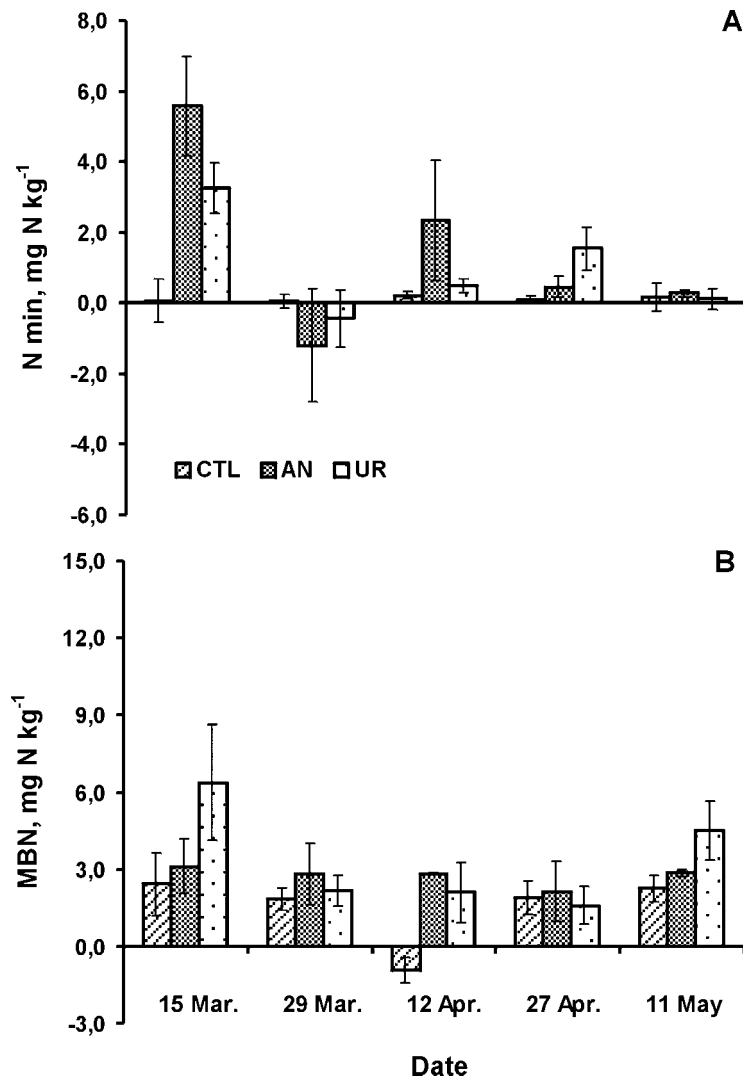

Figure 4. Net nitrogen mineralization (N-min) and microbial biomass N (MBN) in a sandy Florida Entisol under young citrus trees at 15 to 30 cm depth. Fertilizer was applied on February 15. Legend: CTL = unfertilized; UR = urea treated; and AN = ammonium nitrate treated. Vertical bars indicate the standard error of the mean (n = 3). a) Nitrogen mineralization vs. time; b) Microbial biomass N vs. time.

Figure 3B shows increased microbial biomass N for fertilized soil on March 15 as compared to the unfertilized treatment. On the other hand, fertilization promoted a significant increase in net N mineralization at the 15 to 30 cm soil depth with minor variation on the microbial biomass N following fertilizer application (Fig. 4A and B). Nitrogen mineralization was very low at the subsequent evaluation periods at the 15 to 30 cm soil depth. Bauhus[36] reported for an acid Cambisol covered by a forest floor, values of MBN of about $475\,mg\,N\,kg^{-1}$ for an organic horizon and about $30\,mg\,N\,kg^{-1}$ for a mineral horizon (5 to 10 cm depth). Microbial populations could be significant since different species are well adapted to such condition associated with an undisturbed environment regardless of the high acidity presented by the Cambisol. The MBN values in the current study were up to $10\,mg\,N\,kg^{-1}$ at the surface soil layer (Fig. 3B). Lovell and Hatch[37] using the chloroform fumigation and extraction method reported mean MBN value of $160\,mg\,N\,kg^{-1}$ dry soil with high clay content. The MBN values were unchanged during a short-term period after addition of N to a soil. However, the specific respiration, as an indicator of metabolic activity of the biomass, decreased immediately after the application of N and completely stopped following 5 wk of fertilization, when it was no longer significantly different from the initial value $(5\,\mu g\,CO_2\text{-}C\,mg^{-1}$ biomass-$C\,g^{-1}\,h^{-1})$. This work suggests that additions of fertilizer-N could influence soil microbial biomass activity without its size being measurably affected.

Roots injured during insertion of PVC columns into the soil could influence estimations of net N-min due to decomposition of tissue or by altering the input of root derived carbon (root exudates) and consequently microbial population activity. Bauhus[36] found no evidence to support the hypothesis that abscission of fine roots could induce N immobilization in a beech forest soil by microbial biomass. Incubation periods <30 to 60 d are recommended to minimize interference of severed roots on N-min measurements.[36]

Rates of net N-min for the unfertilized treatment were $<0.1\,mg\,N\,day^{-1}\,kg^{-1}$ soil at the 0 to 15 cm soil depth. The above rates are comparable to those reported by Dou et al.[13] based on the long term measurements conducted under the canopy of 4-yr-old orange trees on a fine sand. Nitrogen mineralization was greater at the 0 to 15-cm depth compared to that at the 15 to 30 cm depth (Figs. 3A and 4A). This is due to greater amount of organic residues in the 0–15 cm as compared to 15–30 cm depth soil, which originated from shed leaves, petals, and fruit as well as feeder roots.

The N-min quantities measured during April the 12 to 27 incubation period were greater that those for the other incubation periods (Fig. 3A).

The N-min for the unfertilized treatment was about $1 \, mg \, N \, kg^{-1}$ as compared to 1 to $5 \, mg \, N \, kg^{-1}$ for fertilized treatments during the above incubation period (Fig. 3A). The priming effect of N fertilizers on plant uptake of soil N has been reported.[34] Raison et al.[38] found that, when N mineralization was measured *in situ* on undisturbed forest soils, N fertilization increased the mineralization process 4-fold, since production of NO_3-N on fertilized soil was dominant in comparison to the unfertilized treatment where most N accumulated as NH_4-N. The major cause of the increased availability of N-min, as measured by ^{15}N-plant uptake, is a result of the interchange of fertilizer N for the native humus N.[32]

After March 15, net N-min occurred at all incubation periods (Fig. 3A). This corresponded to an increase in microbial biomass N over the entire duration of this study (Fig. 4B). Daily average soil temperature at 10 cm depth was 21°C during February and March, compared to about 28°C during April and May (Fig. 2). Temperature greatly influences microbial activity and mineralization of soil organic residues, with optimum range for N-min between 25 to 30°C.[39] The temperature coefficient, Q_{10}, is 2 over the range 5 to 35°C.[40] Thus, a 2-fold change in mineralization rate is associated with a shift of 10°C within this range of temperature. Dou et al.[13] also found a positive correlation between the quantity of net N-min and daily average temperature in citrus groves in two locations.

SUMMARY AND CONCLUSION

Gaseous losses of applied N fertilizer reduce fertilizer use efficiency, since volatilization accounted for 5.5 and 12.8% of applied N as AN or UR, respectively, without additional air circulation inside the collection chamber. The losses increased to 33.3% with additional air circulation of the mean natural wind speed, what showed the remarkable effect of environmental conditions on NH_3 volatilization. Leaching losses of N from the 15 to 30 cm soil depth layer were $< 9 \, mg \, N \, kg^{-1}$. This very low leaching loss could be attributed to the carefully managed irrigation scheduling using a continuous monitoring of soil moisture by capacitance probe. Further, the PVC column technique used for N leaching estimates was influenced by NH_3 losses of added N from the soil. Nitrogen fertilization affected mineralization/immobilization turnover irrespective of N-form applied as compared to that in the unfertilized treatment, and consequently could interfere with estimates of soil available N for tree growth and fruit yield. There was a correspondence between soil net-N immobilization and increased microbial biomass N (0–15 cm depth).

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