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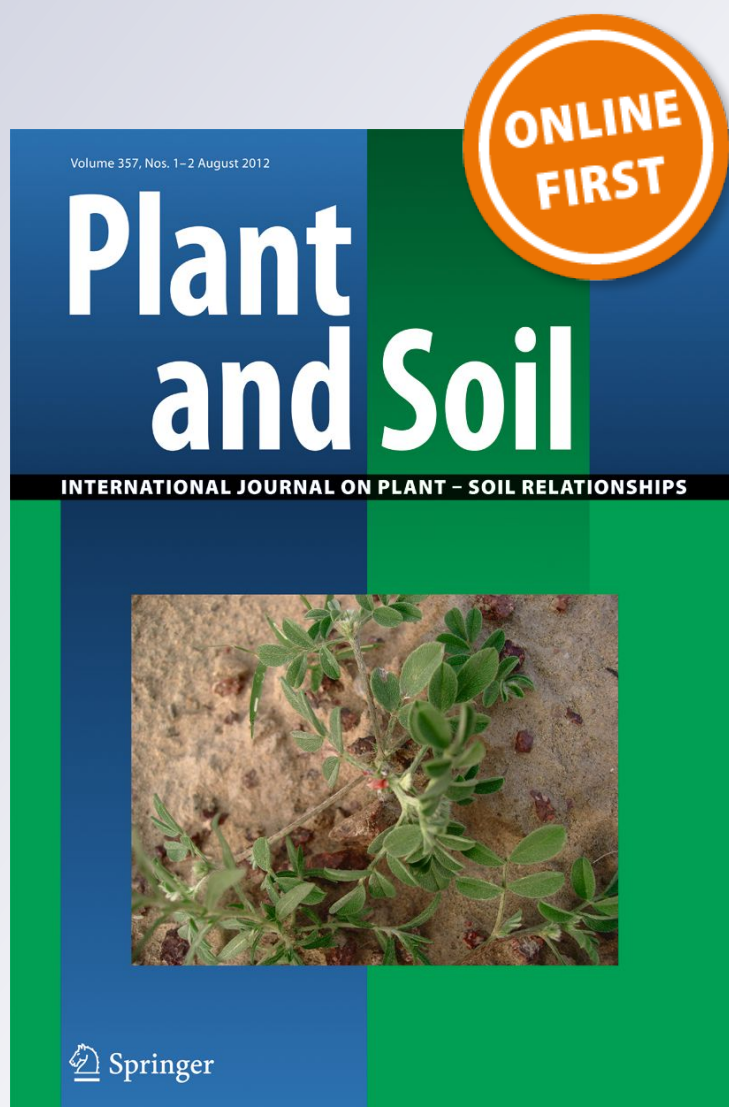
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# Absorption of $^{15}\text{NH}_3$ volatilized from urea by *Citrus* trees

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## Abstract

**Background and aims** Gaseous losses of ammonia ( $\text{NH}_3$ ) have been observed in citrus orchards when urea is surface-applied to the soils, and this loss might significantly limit the effectiveness of the nitrogen (N) fertilizer. However, a portion of the volatilized  $\text{NH}_3$  might be absorbed by the plants through the leaves. To quantify the contribution of the leaf absorption of  $^{15}\text{NH}_3$ , a study with sweet oranges was conducted in two field areas where trees were grown at standard (480 trees  $\text{ha}^{-1}$ ) and high densities (617 trees  $\text{ha}^{-1}$ ).

**Methods** Plastic trays were filled with soil, covered with mown grass to simulate field management conditions, fertilized with  $^{15}\text{N}$  labeled urea (12 atom % excess) and placed under each of three trees in the orchards. This experimental procedure prevented the uptake of N from the labeled urea by the roots. Two weeks after  $^{15}\text{N}$  fertilization, the trays were removed from the field, and the soil was homogenized and sampled for chemical analyses. The citrus trees under which the trays were placed were destructively harvested, and the total N concentrations and  $^{15}\text{N}/^{14}\text{N}$  ratios were determined.

**Results** After urea application, the  $\text{NH}_3$  losses peaked within three days and subsequently decreased to negligible amounts after 10 days. The total  $\text{NH}_3$  losses accounted for 55–82 % of the applied N. Although the  $\text{NH}_3$  absorption by the citrus leaves was proportional to the tree density in the field, only 3–7 % of the  $^{15}\text{NH}_3$  volatilized from the soil was recovered by the citrus trees, and the  $\text{NH}_3$  absorption was also influenced by the proximity of citrus trees to the site of urea application and the leaf areas of the trees.

**Conclusions** The citrus trees can absorb the  $\text{NH}_3$  volatilized from urea, even though, the amount recovered by the trees is small and does not represent a significant proportion of total gaseous N losses, what demonstrates the importance of enhanced N use efficiency practices in field to reduce losses of  $\text{NH}_3$  when urea is applied to soil surfaces.

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## Introduction

The response of citrus trees to N fertilization is likely influenced by tree nutrient reserves, rates and timing of N fertilizer applications, fertilizer sources and N soil processes, such as nitrification, denitrification, immobilization/mineralization, leaching and volatilization (Lea-Cox et al. 2001; Martinez et al. 2002; Cantarella et al. 2003; Mattos Jr. et al. 2003; Alva et al. 2008).

Urea is a dry, soluble fertilizer that has a high N concentration, making it an important N source for crop production. However, the N losses that result from the volatilization of  $\text{NH}_3$  from soil surface-applied urea are a potential problem that decreases the effectiveness of fertilizer (Lightner et al. 1990). Studies have demonstrated that  $\text{NH}_3$  volatilization results in losses that range from 20 to 50 % of the urea applied to the soil surface (Terman 1979; Lightner et al. 1990; Cantarella et al. 2003; Mattos Jr. et al. 2003; Fenilli et al. 2007). These losses are influenced by the soil pH, temperature and moisture content and urease activity, the gradient of  $\text{NH}_3$  partial pressure between the atmosphere and superficial soil, the rate and method of fertilizer application, the soil texture and cation exchange capacity and mulching (Lightner et al. 1990; Mattos Jr. et al. 2003). Mulch management is increasingly used in fruit tree orchards because of its beneficial impacts on fruit tree cultivation (Fidalski et al. 2010). However, the accumulation of mulch in orchards from the mechanical mowing of weeds provides favorable environmental conditions for urea hydrolysis, which can contribute to the volatilization of  $\text{NH}_3$  and decreased effectiveness of N fertilizers.

Furthermore, plants can either lose nitrogenous compounds directly from their leaf tissue into the atmosphere or absorb significant  $\text{NH}_3$  quantities from the air. Even at low atmospheric concentrations, this loss can occur depending on the  $\text{NH}_3$  compensation point in the plant-atmosphere system, or this loss can occur after the compounds dissolve in the film of water on the leaf epidermis that arises in the stomatal cavities during dew formation (Hutchinson et al. 1972; Farquhar et al.

1980; Wetselaar and Farquhar 1980; Mattsson et al. 1998; Fenilli et al. 2007).

Evidence of  $\text{NH}_3$  absorption from the atmosphere and N assimilation by leaves was obtained by monitoring the  $\text{NH}_3$  decrease within a plant chamber or using the  $^{15}\text{N}$  tracer technique (Hutchinson et al. 1972; Porter et al. 1972; Janzen and Bruinsma 1989; Castro et al. 2006; Fenilli et al. 2007). These studies demonstrated that approximately 15–40 % of the N in grain crops, vegetables, pine tree seedlings and coffee was derived from the absorption of atmospheric  $\text{NH}_3$  by the leaves and represented N quantities amounting up to  $100 \text{ kg ha}^{-1}$ . In contrast, direct estimates of the gaseous  $\text{NH}_3$  absorbed by citrus tree leaves under field conditions have yet to be reported. Therefore, based on the importance of N management and the potential for  $\text{NH}_3$  losses from the urea fertilizers used in citrus orchards, the present work quantified the contributions of the leaf  $^{15}\text{NH}_3$  absorption of trees that were planted at two different densities (480 and 617 trees  $\text{ha}^{-1}$ ) to the N level of the whole plant.

## Methods

Two experiments were conducted in commercial citrus orchards to evaluate the citrus leaf absorption of  $\text{NH}_3$  that was volatilized from the soil surface-applied urea and its contribution to the whole plant N level. The first experiment was performed in an orchard that was located in Santa Cruz do Rio Pardo, São Paulo, Brazil ( $22^\circ 48' \text{ S}$  lat;  $49^\circ 23' \text{ W}$  long), with a high planting density (HPD; 617 trees  $\text{ha}^{-1}$  with  $6.0 \times 2.7 \text{ m}$  spacing) of 8-year-old trees and sandy loam soil [sand =  $840 \text{ g kg}^{-1}$ ; organic matter =  $22 \text{ g dm}^{-3}$ ; pH ( $0.01 \text{ mol L}^{-1} \text{ CaCl}_2$ ) = 5.8], where environmental conditions were characterized by daily mean air temperature =  $24^\circ \text{C}$ ; wind speed =  $0.4 \text{ ms}^{-1}$  (maximum  $7.6 \text{ ms}^{-1}$ ) and relative humidity varying from 50 to 80 %. The second experiment was performed in an orchard that was located in Descalvado, São Paulo, Brazil ( $21^\circ 57' \text{ S}$  lat;  $47^\circ 40' \text{ W}$  long), with a standard planting density (SPD; 408 trees  $\text{ha}^{-1}$  with  $7.0 \times 3.5 \text{ m}$  spacing), 4-year-old trees and clay loam soil [sand =  $233 \text{ g kg}^{-1}$ ; organic matter =  $37 \text{ g dm}^{-3}$ ; pH ( $0.01 \text{ mol L}^{-1} \text{ CaCl}_2$ ) = 5.5]. In this second site, predominant environment presented daily mean air temperature =  $21^\circ \text{C}$ ; wind speed =  $0.3 \text{ m s}^{-1}$  (maximum =  $6.2 \text{ ms}^{-1}$ ) and relative humidity from 45 to 80 %. Both sites were planted with 'Valencia'

sweet orange scions [*Citrus sinensis* (L.) Osbeck] that were grafted onto 'Swingle' citrumelo rootstock [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.].

A randomized experimental design was used, and three replicates were performed. For each experiment, three uniform sets of orange trees (3 rows × 3 trees per row), which were separated by at least 50 m, were selected from each experimental site. To simulate the N fertilization in each orchard, four plastic trays (total area=1.74 m<sup>2</sup>) were filled with soil (covered with grass clippings), fertilized with <sup>15</sup>N-urea and placed under the tree canopy of the central tree in each plot at a distance of 0.5 m from the trunk. Prior to the simulations, soil samples were collected from the top layer (0–5 cm depth) of the experimental sites, sieved, air dried and homogenized. In each of the trays, the urea fertilizer was labeled with a 12.0 atom % excess of <sup>15</sup>N, as described below. This experimental procedure prevented the uptake of the labeled urea by the citrus tree roots.

The weeds, which were mainly grasses, that were located adjacent to the tree planting lines of each orchard were harvested, air dried and coarsely ground. The resulting mulch amounted to 3 tha<sup>-1</sup> of dry matter. To reduce the absorption of the <sup>15</sup>NH<sub>3</sub> volatilized from the urea by plants other than the citrus trees, the weeds under the trees were controlled with herbicide, and the weeds situated between the rows were mechanically mowed one week before the <sup>15</sup>N-urea was applied to the trays. Five days prior to the <sup>15</sup>N-urea application, the previously harvested grasses were moistened with deionized water in a 1:1.5 (w/v) proportion. One day prior to the <sup>15</sup>N-urea application, the soil was re-moistened to 50 % of the field capacity and covered with a layer (equivalent to 3 tha<sup>-1</sup>) of the ground grass material. To ensure that the water content of the soil was similar between the trays and the field, the soil in the trays was also re-moistened whenever necessary. Such procedures were used to enhance the urease activity in the soil trays and, thus, simulate the volatilization of NH<sub>3</sub>.

Dry, soluble, granular urea was applied over the mown grass in the trays at the rate of 133 g and 100 g N per tree of <sup>15</sup>N-urea in the HPD and SPD orchards, respectively, which corresponded to 1/3 of the annual rate recommended for citrus trees (400 g of N/tree in HPD and 300 g of N/tree in SPD) (Quaggio et al. 2010). The trays were removed from the field at the end of the evaluation period, and the soil was

homogenized and sampled for the total N concentration using steam-distillation and mass spectrometry to determine the N isotope ratios (i.e., <sup>15</sup>N/<sup>14</sup>N). The total N volatilized from the trays was estimated as the difference between the quantity of applied <sup>15</sup>N and the quantity remaining in the soil.

In a separate set of trees in the same orchards, semi-open trapping systems of H<sub>3</sub>PO<sub>4</sub> + glycerol-soaked plastic foam discs were used to estimate the daily losses of gaseous NH<sub>3</sub> from non-labeled urea fertilizer following the model of Nõmmik (1973) and adapted by Cantarella et al. (2003). During the <sup>15</sup>N-urea application, a system of eight trapping devices was installed on the soil surface along the tree rows of each experimental plot. Five of these devices received equivalent quantities of mown grass and urea that were used in the trays for a surface-area basis; the other three devices served as control treatments without urea. The NH<sub>3</sub>-trapping devices were composed of the following: 1) a top portion constructed of PVC tubes that were 0.20 m in diameter and 0.45 m in height and 2) 10 bases of PVC tubes that were 0.19 m in diameter and 0.15 m in height. The bases were inserted into the soil, and the top part of the trapping device was mounted on the top of the first base. The foam discs were placed inside the devices to trap NH<sub>3</sub> and were replaced every 2 to 4 days for 14 days. To expose the fertilizer applications to ambient conditions, the top portion of the chamber was moved to another base after each of the trapping foam discs was replaced. No rain fell during the experiments.

One week after the soil was collected from the trays, the central tree of each plot was destructively harvested and separated into the following samples: the new branches (<1.5 cm Ø) and leaves; old branches (>1.5 cm Ø) and leaves; tree trunk; fibrous roots; woody roots and fruits, which were mature in the HPD and 5.5 cm in diameter in the SPD. These tree components were weighed in the field and sampled for the determination of the tissue dry mass, total N concentration and isotopic ratios. The branches, leaves and fruits of eight neighboring trees that surrounded the destructively harvested tree were sampled. In the same site, the branches, leaves and fruits of trees that were at least 100 m from the experimental plots were also sampled and used as controls for the calculations of the natural abundance of <sup>15</sup>N atoms.

The samples were dried at 65 °C and ground to a fine powder (250 µm) prior to analyzing the total N

and  $^{15}\text{N}$  abundance using an automated mass spectrometer that was coupled to a N analyzer (model ANCA-GSL, from Sercon Co., UK). The total N concentrations and  $^{15}\text{N}/^{14}\text{N}$  ratios were determined according to Barrie and Prosser (1996). For the tree components, the percentages of N derived from fertilizer (Ndff) were calculated using the isotopic dilution equation described by Hauck and Bremner (1976), as follows:

$$N_{\text{dff}} = \left[ \frac{(AT\%^{15}\text{N}_{\text{samp}} - AT\%^{15}\text{N}_{\text{uft}})}{(AT\%^{15}\text{N}_{\text{fert}} - AT\%^{15}\text{N}_{\text{uft}})} \right] * 100, \quad (1)$$

where  $\% N_{\text{dff}}$  = the percentage of N derived from fertilizer;  $AT\%^{15}\text{N}_{\text{samp}}$  = the atom % of  $^{15}\text{N}$  in the sample;  $AT\%^{15}\text{N}_{\text{uft}}$  = the atom % of  $^{15}\text{N}$  in unfertilized tissues and  $AT\%^{15}\text{N}_{\text{fert}}$  = the atom % of  $^{15}\text{N}$  in the fertilizer.

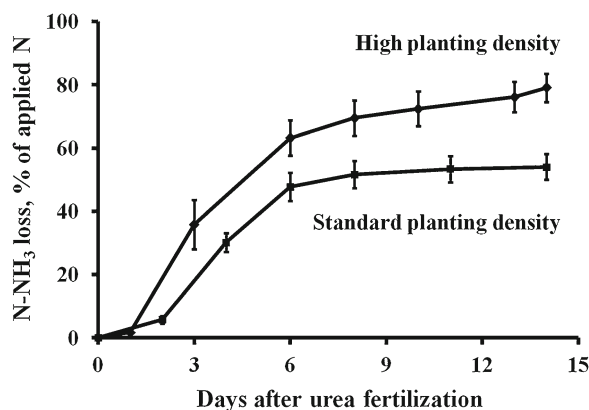
The total amount of N recovered from the labelled fertilized by citrus trees was calculated using the determinations of dry mass, total N and  $\%N_{\text{dff}}$  of the central and neighboring tree components. The dry mass of the central and neighboring trees were assumed to be similar.

For each time point after the application of  $^{15}\text{N}$ -urea to the soil, standard deviations were calculated using descriptive statistics for the mean values of the  $\% \text{NH}_3$  loss, dry mass yield of the tree components, total N concentration and Ndff.

## Results

The losses of N-urea through  $\text{NH}_3$  volatilization were 82 % and 55 % of the fertilizer applied in the HPD and SPD orchards, respectively. During the first day after the fertilizer application, the  $\text{NH}_3$  losses were low and most likely occurred in response to limited urea hydrolysis (Black et al. 1985). Losses then rapidly increased and peaked between the third and sixth days, and subsequently decreased to negligible values by the tenth day after fertilization (Fig. 1).

Total N concentrations in tissues of the new tree components were higher than in the old components. The relationships between the N concentrations of the different tree components were as follows: new leaves > old leaves; new branches > fruit > old branches > tree trunk and fibrous roots > woody roots. The leaves represented 34 % (SPD) and 37 % (HPD) of the total



**Fig. 1** Gaseous losses of  $\text{NH}_3$  volatilized from soil surface-applied urea. The values were estimated using a semi-open trapping system. The vertical bars indicate the standard errors of the means ( $n=5$ )

N content and 14 % of the total dry mass of trees (Table 1).

The Ndff values of the HPD and SPD trees were approximately 2000 mg and 650 mg per tree, respectively. Furthermore, the Ndff values of the leaves and fibrous roots were higher than the total N contents of these tissues, results that are explained by the demand for N during the synthesis of new tissues. In contrast, the Ndff values were lower than the corresponding N contents of the well-developed fruits and woody tissues (trunk, branches and woody roots) (Table 1). For the control treatment, the samples were collected from trees not fertilized with  $^{15}\text{N}$ -urea, and these samples did not show differences in the natural abundance of  $^{15}\text{N}$  atoms ( $n=3$  for each tree component); the values were 0.367 in the leaves, 0.366 in the branches and 0.365 in the fruit. The abundance of  $^{15}\text{N}$  atoms in the trees fertilized with the labeled urea varied from 0.378 to 0.413 in the leaves, 0.373 to 0.398 in the branches and 0.369 to 0.381 in the fruit. The lowest values were found in the neighboring trees, whereas the highest values were found in the central trees.

After 20 days of urea application, the  $^{15}\text{NH}_3$  absorbed by the trees in both orchards was recovered in the leaves (51 %), roots (23 %), branches (20 %) and fruits (6 %) (Table 1). The leaf absorption of  $^{15}\text{NH}_3$  was 7 % and 3 % of the  $^{15}\text{NH}_3$  volatilized in the HPD and SPD orchards, respectively; based on the total N applied in the experimental trays, these percentages represent N-use efficiencies of 6.1 % and 1.7 %, respectively (Table 2). In comparison to the SPD, the greatest absorption of  $^{15}\text{NH}_3$  in the HPD orchard

corresponded to the greatest estimated leaf area (LA) per tree. The specific leaf weights of the citrus trees were 145 and 157 g of dry mass per m<sup>2</sup> of leaf area, respectively, for the new and old leaves; in HPD= 62.8 m<sup>-2</sup> per tree (38,800 m<sup>2</sup> ha<sup>-1</sup>) and SPD= 41.1 m<sup>-2</sup> per tree (16,800 m<sup>2</sup> ha<sup>-1</sup>).

The total amount of NH<sub>3</sub> recovered by the central tree, under which the trays were placed, was 1.5 times greater in the HPD (1.8 %) compared to the SPD (1.2 %), these values were proportional to the tree sizes and dry mass of leaves (HPD=9.2 kg and SPD=6.1 kg per tree) (Table 1). And in neighboring citrus trees the amount absorbed was almost three

times higher in HPD (5.5 %) than in SPD (2.0 %) (Table 2). It was also verified that the recovery of <sup>15</sup>NH<sub>3</sub> from the neighboring trees was influenced by the proximity of these trees to the trays (Fig. 2).

## Discussion

The gaseous losses of NH<sub>3</sub> volatilized from soil surface-applied urea were greater than the 47 % field loss estimated by Cantarella et al. (2003) and Mattos Jr. et al. (2003) in citrus orchards (Fig. 1), because the biochemical reactions that led to the release of NH<sub>3</sub>

**Table 1** Total N concentration and content in plant organs and the N absorbed from the NH<sub>3</sub> derived from the <sup>15</sup>N-urea fertilizer placed under citrus trees in two orchards planted at different densities

Tree	Standard planting density				
component	Dry mass	N	N content	Ndff <sup>a</sup>	
	kg/tree <sup>a</sup>	g kg <sup>-1</sup>	g/tree (%) <sup>b</sup>	%	mg/tree (%) <sup>c</sup>
Fruits	13.1±0.9 <sup>f</sup>	10.8±0.5	140±5 (26.8)	0.024±0.004	34±3 (5.2)
New leaves <sup>d</sup>	4.2±0.4	29.7±0.7	125±10 (23.9)	0.194±0.026	244±19 (37.7)
Old leaves <sup>e</sup>	1.9±0.5	26.5±1.5	53±11 (10.1)	0.155±0.043	87±11 (13.4)
New branches	1.5±0.7	14.1±1.8	21±5 (4.0)	0.112±0.026	23±2 (3.5)
Old branches	3.9±0.7	9.8±3.0	36±5 (6.9)	0.082±0.016	30±3 (4.6)
Trunk	10.8±0.8	5.5±0.5	59±6 (11.3)	0.079±0.009	47±5 (7.3)
Fibrous roots	1.2±0.3	16.1±1.8	20±2 (3.8)	0.631±0.249	123±19(19.0)
Woody roots	9.8±0.2	6.9±0.4	68±4 (13.0)	0.088±0.017	60±8 (9.3)
TOTAL	46.4±1.9		523 (100.0)		648 (100.0)
	High planting density				
	Dry mass	N	N content	Ndff	
	kg/tree	g kg <sup>-1</sup>	g/tree (%)	%	mg/tree (%)
Fruits	9.7±0.7	12.1±1.0	118±18 (15.4)	0.097±0.018	116±16 (5.8)
New leaves	8.0±0.8	31.2±1.5	251±37 (32.7)	0.382±0.011	955±113 (47.5)
Old leaves	1.2±0.3	27.8±0.8	33±6 (4.3)	0.257±0.032	86±22 (4.3)
New branches	3.3±0.6	17.0±1.2	56±6 (7.3)	0.234±0.025	133±21 (6.6)
Old branches	15.4±3.0	7.6±0.8	118±27 (15.4)	0.210±0.017	252±29 (12.5)
Trunk	14.0±1.5	5.0±0.1	71±7 (9.3)	0.146±0.023	105±13 (5.2)
Fibrous roots	2.9±0.4	17.0±0.7	49±7 (6.4)	0.458±0.017	224±30 (11.1)
Woody roots	9.6±0.9	7.4±0.2	71±11 (9.3)	0.199±0.161	140±10 (7.0)
TOTAL	64.2±6.8		767 (100.0)		2010 (100.0)

<sup>a</sup> Ndff is the quantity of N derived from urea fertilizer that was absorbed by the central tree of each experimental plot

<sup>b</sup> The percentage of the total N in each tree component

<sup>c</sup> The percentage of the Ndff in each tree component

<sup>d</sup> New leaves were from branches that were <1.5 cm in Ø

<sup>e</sup> Old leaves were from branches that were >1.5 cm in Ø

<sup>f</sup> Mean values ± SE (n=3)

**Table 2** Nitrogen recovered in orange trees that were planted at two different densities and sites. The N was recovered from  $\text{NH}_3$  that was volatilized from labeled urea fertilizer placed under the tree canopies

N recovery	Standard planting density	High planting density
	% $^{15}\text{NH}_3$ recovered from N volatilized	
In the central tree	1.2±0.1 <sup>a</sup>	1.8±0.3
In 8 neighboring trees	2.0±0.1	5.5±0.6
In all 9 trees	3.2	7.3
	% of total urea applied	
Total	1.7±0.1	6.1±0.3
	kg ha <sup>-1</sup>	
Total	0.7±0.05	4.9±0.4

<sup>a</sup> Mean values ± SE ( $n=3$ )

into the atmosphere found in the present study were favored due to the weed residue, adequate soil moisture and possible increases in localized urease activity in the soil surface (Denmead et al. 2008). During the five days after the  $^{15}\text{N}$  fertilization, the differences between the %  $\text{NH}_3$  losses in the HPD and SPD orchards occurred in response to differences in the N rates (133 g per tree in the HPD and 100 gN per tree in the SPD) because the dissolution of urea within the overlapping areas of adjacent granules would occur at the highest rate, thus the pH increase would likely be greater in the soil microsites of HPD (in which the rate of N application was 33 % higher than in SPD). The observed loss was also likely due to the environmental conditions, as the daily air temperature was higher in the HPD orchard (24 °C) than in the SPD orchard (21 °C). The  $\text{NH}_3$  losses peaked within three days after urea application; more than 80 % of the volatilization losses occurred in the 6 d following fertilizer application (Fig. 1), these losses were proportional to the expected quantity and equilibrium of  $\text{NH}_3$  and  $\text{NH}_4^+$  in the soil solution (Lara Cabezas et al. 1999; Cantarella et al. 2003; Mattos Jr. et al. 2003).

The relative biomass yield and N content in the tree components were similar for the trees within the same size class of the HPD and SPD orchards as reported by Morgan et al. (2006). Leaves are the most important N reserve in mature citrus trees, containing from 33 % to 42 % of the total N in the tree (Legaz et al. 1995). In our study, the leaves represented approximately 35 % of the total N content and more than 51 % of Ndff (Table 1).

The leaf absorption of  $^{15}\text{NH}_3$  likely occurred because the vapor pressure of  $\text{NH}_3$  at the canopy level after N fertilization was above the leaf compensation point (Table 1). Such exchanges between the canopy and atmosphere are affected by the timing of fertilizer application, environmental conditions, crop growth characteristics and N nutrition status of the plants (Sutton et al. 2008); these factors were expected to be favorable in our study. In all the tree components the Ndff values in the HPD was higher in the SPD orchards, which were correlated with the tree sizes and dry masses of the leaves (Table 1). Furthermore, the Ndff values of the leaves and fibrous roots were higher than the total N contents of these tissues, results that are explained by the demand for N during the synthesis of new tissues (Lea-Cox et al. 2001; Mattos Jr. et al. 2003).

In both orchards, almost 50 % of the  $^{15}\text{NH}_3$  absorbed by the leaves was remobilized to other tree components (roots=23 %, branches=20 % and fruits=6 %) after 20 days of urea application (Table 1). The  $\text{NH}_3$  is quickly assimilated into organic compounds in the shoot to avoid the physiological damage caused by toxic endogenous levels of free ammonium in the leaf cells (Pearson and Stewart 1993; Tamaki and Mercier 2001) and is then redistributed to the growing organs and reserves (Yoneyama et al. 2003).

The leaf absorption of  $^{15}\text{NH}_3$  volatilized from fertilizer was higher in the HPD (7 %) than in the SPD (3 %) (Table 2). Large LA values in the HPD (38,800 m<sup>2</sup> ha<sup>-1</sup>) compared to SPD (16,800 m<sup>2</sup> ha<sup>-1</sup>) increase the absorption area for  $\text{NH}_3$  in the canopy, mainly in the neighboring trees, and also attenuate the local wind speed in the orchard, which consequently affects the rate of gas transport away from the soil-air interface (Mattos Jr. et al. 2003). These changes provide favorable conditions for the plant absorption of  $\text{NH}_3$ . A similar study reported the absorption of  $^{15}\text{NH}_3$  volatilized from labeled urea by coffee trees of 43 % (Fenilli et al. 2007), what was much higher than our findings and others reported for wheat [(3.3 %) Sommer et al. 1993; (11 %) Ping et al. 2000]. However, in that experiment, the coffee trees were protected from the wind in the field by PVC sheets (2 m height), which provided greater residence time of volatilized  $\text{NH}_3$  in the air on the vicinity of plant foliage. Furthermore, the coffee trees were closely planted what determined increased LA and probably reduced wind speed between rows. Therefore,

differences in the tree architecture and canopy density might explain variations of  $^{15}\text{N}$  recovery observed from volatilized  $\text{NH}_3$ .

Observations led Denmead et al. (2008) to suggest that losses in volatilized  $\text{NH}_3$  were reduced by delaying the application of urea until a substantial LA had developed in a sugarcane field. The levels of  $^{15}\text{N}$  that are recovered after soil applications using different fertilizer sources, plant ages, and growing conditions of citrus vary from 20 to 50 % (Mattos Jr. et al. 2007). In our study the N-use efficiencies of the total N applied, which considered only the leaf absorption of the  $^{15}\text{NH}_3$  volatilized, was 6.1 % and 1.7 % in the HPD and SPD orchards, respectively (Table 2).

Most of the  $\text{NH}_3$  released into the atmosphere is quickly deposited near the emission source (Pearson and Stewart 1993) and is regulated by the local wind speed and direction.  $\text{NH}_3$  gas is highly reactive and can form solutions and aerosols that are deposited on the leaf surface of plants. Of the total amount of  $\text{NH}_3$  that was derived from the fertilizer and recovered by the citrus trees in the orchards, 25–30 % was absorbed by the central tree in the experimental plot under which the trays were placed (Fig. 2). The remainder (70–75 %) was absorbed by the neighboring citrus trees. The recovery of  $^{15}\text{NH}_3$  by the neighboring trees was correlated by the proximity of these trees to the trays (Fig. 2): the neighboring trees located closer to the trays absorbed higher quantities of  $\text{NH}_3$ . However, for those trees that were equidistant from the trays, variations in the absorption of  $^{15}\text{NH}_3$  were observed, which might occur in response to the predominant

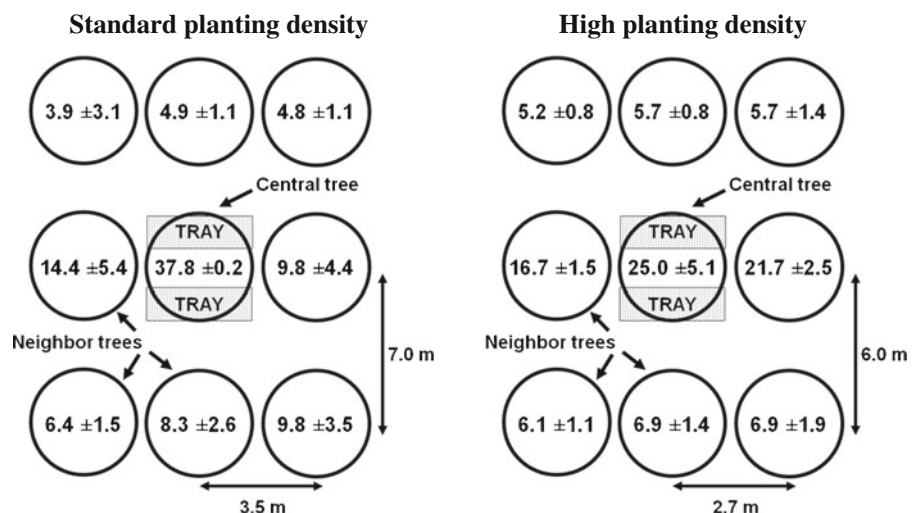
wind direction in the orchard. The pattern of  $\text{NH}_3$  absorption by the trees in this study was similar to the pattern reported for a wheat field by Ping et al. (2000) in which the foliar uptake of  $\text{NH}_3$  by wheat decreased with increasing distance from the site of urea application, and most of the  $\text{NH}_3$  (90 %) was recovered by the plants within the three rows adjacent to the fertilizer application site.

Our study demonstrates that the canopy of citrus trees can absorb the  $\text{NH}_3$  volatilized from urea fertilizer, although the total amount of N absorbed by the trees is small ( $<5.0 \text{ kg ha}^{-1}$  - Table 2) and does not represent a significant proportion of the N fertilizer used in the orchard. This study also shows that the  $\text{NH}_3$  assimilation in the orchard depends on the leaf area of the trees, planting density and distance from the fertilizer source.

## Conclusion

The highest rates of  $\text{NH}_3$  volatilization were observed three to six days after the application of dry urea fertilizer to the soil surface, and only small quantities (3–7 %) of  $^{15}\text{NH}_3$  volatilized from dry, granular, soil surface-applied urea were absorbed by the citrus leaves. This absorption increased with increases in the tree-planting density or the proximity of the trees to the site of urea application in the field. Although this study demonstrated that citrus trees absorbed  $\text{NH}_3$  from the air, the quantities recovered were limited. These results demonstrate the importance of

**Fig. 2** Schematic of the two orchards and the distribution of  $^{15}\text{N}$  absorption of  $\text{NH}_3$  that was volatilized from urea fertilizer by citrus trees. Each ellipse represents one tree; the trays were fertilized with urea labeled with  $^{15}\text{N}$  and placed under the central tree. The values shown are the mean percentages of the total absorbed N  $\pm$  the standard error of the means ( $n=3$ )



improving N-use efficiency and reducing the loss of  $\text{NH}_3$  volatilized from urea fertilizers.

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## References

- Alva AK, Mattos D Jr, Quaggio JA (2008) Advances in nitrogen fertigation of citrus. *J Crop Improv* 22:121–146
- Barrie A, Prosser SJ (1996) Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In: Boutton TW, Yamasaki S (eds) *Mass spectrometry of soils*. Marcel Dekker, New York, USA, pp 1–46
- Black AS, Sherlock RR, Cameron KC, Smith KM, Goh KM (1985) Comparison of three methods for measuring ammonia volatilization from urea granules broadcast on to pasture. *J Soil Sci* 36:271–280
- Cantarella H, Mattos D Jr, Quaggio JA, Rigolin AT (2003) Fruit yield of Valencia sweet orange fertilized with different N sources and the loss of applied N. *Nutr Cycl Agroecosyst* 67:215–223
- Castro A, Stulen I, Posthumus FS, de Kok LJ (2006) Changes in growth and nutrient uptake in *Brassica oleracea* exposed to atmospheric ammonia. *Ann Bot* 97:121–131
- Denmead OT, Freney JR, Dunin FX (2008) Gas exchange between plant canopies and the atmosphere: case-studies for ammonia. *Atm Environ* 42:3394–3406
- Farquhar GD, Firth PM, Wetselaar R, Weir B (1980) On the gaseous exchange of ammonia between leaves and the environment: determination of the ammonia compensation point. *Plant Physiol* 66:710–714
- Fenilli TAB, Reichardt K, Trivelin PCO, Favarin JL (2007) Volatilization of ammonia derived from fertilizer and its re-absorption by coffee plants. *Comm Soil Sci Plant Anal* 38:1741–1751
- Fidalski J, Auler PAM, Beraldo JMG, Marur CJ, Faria RT, Barbosa GMC (2010) Availability of soil water under tillage systems, mulch management and citrus rootstocks. *Rev Bras Ciênc Solo* 34(3):917–924
- Hauck RD, Bremner JM (1976) Use of tracer for soil and fertilizer nitrogen research. *Agron J* 20:219–266
- Hutchinson GL, Millington RJ, Peters DB (1972) Atmospheric ammonia: absorption by plant leaves. *Science* 175:771–772
- Janzen HH, Bruinsma Y (1989) Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoots to  $^{15}\text{N}$ -labeled ammonia. *Soil Biol Biochem* 21:189–196
- Lara Cabezas WAR, Trivelin PCO, Bendassolli JA, Santana DG, Gascho GJ (1999) Calibration of a semi-open static collector for determination of ammonia volatilization from nitrogen fertilizers. *Comm Soil Sci Plant Anal* 30(3–4):389–406
- Lea-Cox JD, Syvertsen JP, Graetz DA (2001) Springtime  $^{15}\text{N}$ -nitrogen uptake, partitioning, and leaching losses from young bearing citrus trees of differing nitrogen status. *J Amer Soc Hort Sci* 126:242–251
- Legaz F, Serna MD, Primo-Millo E (1995) Mobilization of the reserve N in citrus. *Plant Soil* 173:205–210
- Lightner JW, Mengel DB, Rhykerd CL (1990) Ammonia volatilization from nitrogen fertilizer surface applied to orchard grass sod. *Soil Sci Soc Amer J* 54:1478–1482
- Martinez JM, Bañuls J, Quiñones A, Martín B, Primo-Milo E, Legaz F (2002) Fate and transformation of  $^{15}\text{N}$  labeled nitrogen applied in spring to citrus trees. *J Hortic Sci Biotech* 77(3):361–367
- Mattos D Jr, Alva AK, Graetz DA, Paramasivam S (2003) Nitrogen volatilization and mineralization in a sandy soil of Florida under citrus. *Comm Soil Sci Plant Anal* 34(13–14):1803–1824
- Mattos D Jr, Quaggio JA, Cantarella H, Boaretto AE (2007) Nitrogênio e enxofre na cultura dos citros. In: Yamada T, Abdalla SRS, Vitti GC (eds) *Nitrogênio e enxofre na agricultura brasileira*. International Plant Nutrition Institute, Piracicaba, Brazil, pp 413–443
- Mattsson M, Husted S, Schjoerring JK (1998) Influence of nitrogen nutrition and metabolism on ammonia volatilization in plants. *Nutr Cycl Agroecosyst* 5(1):35–40
- Morgan KT, Scholberg JMS, Obreza TA, Wheaton TA (2006) Size, biomass, and nitrogen relationships with sweet orange tree growth. *J Am Soc Hort Sci* 131(1):149–156
- Nômmik H (1973) The effect of pellet size on the ammonia loss from urea applied to forest soil. *Plant Soil* 39:308–318
- Pearson J, Stewart GR (1993) The deposition of atmospheric ammonia and its effects on plants. *New Phytol* 125:283–305
- Ping J, Bremer E, Janzen HH (2000) Foliar uptake of volatilized ammonia from surface-applied urea by spring wheat. *Comm Soil Sci Plant Anal* 31(1–2):165–172
- Porter LK, Viets FG Jr, Hutchinson GL (1972) Air containing nitrogen-15 ammonia: Foliar absorption by corn seedlings. *Science* 175:759–761
- Quaggio JA, Mattos D. Jr, Boaretto RM (2010) Citros. In: Prochnow, L.I.; Casarin, V.; Stipp, S.R. (Org.). *Boas práticas para uso eficiente de fertilizantes*. 1 ed. Piracicaba: International Plant Nutrition Institute, v. 3, p. 371–409
- Sommer SG, Jensen ES, Schjoerring JK (1993) Leaf absorption of atmospheric ammonia emitted from pig slurry applied beneath the canopy of winter wheat. *Acta Agriculturae Scandinavica. Section B. Soil Plant Sci* 43:21–24
- Sutton MA, Erisman JW, Dentener F, Möller D (2008) Ammonia in the environment: from ancient times to the present. *Environ Pollut* 156:583–604
- Tamaki V, Mercier H (2001) Effects of different ammoniacal nitrogen sources on N-metabolism of the atmospheric bromeliad *Tillandsia pohliana* Mez. *Rev Brasil Bot* 24(4):407–413
- Terman GL (1979) Volatilization losses of nitrogen as ammonia from surface-applied fertilizers, organic amendments, and crop residues. *Adv Agron* 31:189–223
- Wetselaar R, Farquhar GD (1980) Nitrogen losses from tops of plants. *Adv Agron* 33:263–302
- Yoneyama T, Ito O, Engelaar WMHG (2003) Uptake, metabolism and distribution of nitrogen in crop plants traced by enriched and natural: Progress over the last 30 years. *Phytochem Rev* 2:121–132