Annual and residual urea nitrogen contribution to the nutrition of peach trees (Prunus persica L.) grown under subtropical climate

Annual and residual urea nitrogen contribution to the nutrition of peach trees (*Prunus persica* L.) grown under subtropical climate

Betania Vahl de Paula[a*], Beatriz Baticini Vitto[a], Paula Beatriz Sete[b], Talita Trapp[b], Jovani Zalamena[c], George Wellington Bastos de Melo[d] , Elena Baldi[e], Moreno Toselli[e], Danilo Eduardo Rozane[f], Gustavo Brunetto[a]

[a] Department of Soil Science, Center of Rural Science, Federal University of Santa Maria, Av. Roraima 1000, Camobi, Santa Maria, RS, 97105-900, Brazil. E-mail: behde-paula@hotmail.com; beabaticinivitto@gmail.com; brunetto.gustavo@gmail.com

[b] Department of Rural Engineering, Center of Agricultural Sciences, Federal University of Santa Catarina, Rod. Admar Gonzaga 1346, Florianópolis, SC, 88034-000, Brazil. E-mail: paulasete@gmail.com; talipptrali@yahoo.com.br

[c] Federal Institute of Education, Science and Technology of Rio Grande do Sul, Rua Alberto Hoffmann 285, Restinga, Porto Alegre, RS, 91791-508, Brazil. E-mail: jovanizalamena@yahoo.com.br

[d] Embrapa Uva e Vinho, Rua Livramento, 515, Centro, Bento Gonçalves, RS, 95701-008, Brazil. E-mail: wellington.melo@embrapa.br

[e] Department of Agricultural and Food Sciences, University of Bologna, Viale Fanin 44, Bologna, 40127, Italy. E-mail: elena.baldi7@unibo.it, moreno.toselli@unibo.it

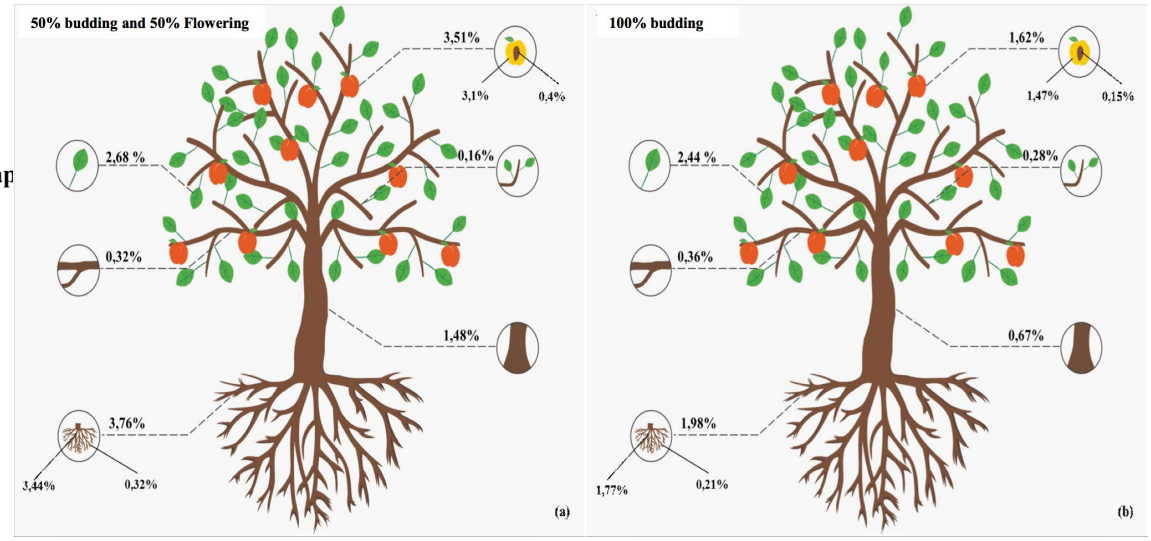
[f] São Paulo State University Julio de Mesquita Filho, Registro Campus, Rua Nelson Brihi Badur 430, Registro, SP, 13900-000, Brazil. E-mail: danilo.rozane@unesp.br

[*] Corresponding author. E-mail: behdepaula@hotmail.com

Highlights

- Residual urea nitrogen was found in the soil after two years.
- Higher N deriving from fertilizer in leaves and fruits when the dose is split.
- Most N found in trees is from sources other than nitrogen fertilizer.

Grap



Recovery of N deriving from fertilizer (%) in peach organs subjected to the application of 40 kg N ha^{-1} in its urea form at budding (100% of the dose at budding - 100B) and split into two doses (50% of the dose at budding and 50% at flowering - 50B + 50F).

Abstract

Nitrogen (N) fertilizers such as urea are applied to peach orchards worldwide whenever the soil cannot meet plants' N demand. However, the actual nutritional contribution of different N supply modes applied to peach crops, or the contribution of residual N in the subsequent cycle, is yet to be fully known. The current study aims to assess the annual and residual urea N contribution to the nutrition of peach trees grown under subtropical climate. Forty kilograms of N per hectare supplied as enriched urea (3.0 at. % ^{15}N), were applied to peach in full production at a single rate (100% at budding) or split in two rates (50% at budding and 50% at flowering). Total ^{15}N and N concentrations in the application year and in the year after treatments were assessed in peach leaves throughout the cycle, in fruits (pulp and stone) at harvest and in stratified soil samples. Total ^{15}N, N concentrations and total dry mass were measured in annual and perennial tree organs in the year after treatment application. Peach trees evidenced higher N deriving from fertilizer (NDFF) in leaves and fruits (pulp and stone) in the year N was applied to the soil, as well as in the following year when N application was split into two rates. The highest NDFF amounts recorded in the year following N application were observed in leaves and fruits (annual organs) and thick roots (perennial organ), mainly when N was splitted. However, mainly in the year following its application, due to the small residual N, the N found in trees derived from sources other than N fertilizer; this justifies annual

N applications, whenever necessary. The cultivation of cover crops and the preservation of organic matter could help N peach nutrition that seems to take advantage more of residual N in soil than on fertilizers.

Keywords: Fruit farming, ^{15}N isotope, N distribution, N fertilization, orchard.

1. Introduction

Nitrogen (N) deriving from the mineralization of organic matter (OM) and decomposition of plant residues is not always able to satisfy the N demand of peach trees (*Prunus persica* L.). To avoid N deficiency, which can cause a decrease in crop yield and negatively affect fruit quality parameters (Damour et al., 2014; Jannoyer et al., 2011), N fertilizers are often applied to the soil surface. To establish the correct N rates to supply, soil OM concentration and plant nutritional status, as well as growth and yield parameters, should be evaluated (Brunetto et al., 2016b).

Urea is the N fertilizer most used in orchards thanks to its high N concentration and low cost per nutrient unit (Brunetto et al., 2016b). However, urea in the soil is rapidly hydrolyzed by extracellular ureases enzymes produced by microorganisms such as bacteria and fungi, and produce ammonium carbonate $(NH_4^+)_2CO_3$, which is not stable in the soil. Urea decomposes into bicarbonate ion (HCO_3^-), hydroxide ion (OH^-) and ammonium ion (NH_4^+) when it gets in contact with water. NH_4^+ can react to OH^- and stimulate ammonia (NH_3) loss due to volatilization; however, part of NH_4^+ in the soil can be transformed into nitrite (NO_2^-) due to biological oxidation and, later, into nitrate (NO_3^-) (Brunetto et al., 2016a), which is often the prevalent form of N found in drained soils. However, NO_3^- in the soil is extremely mobile; therefore, it can be easily leached and contaminate subsurface water in orchards (Baram et al., 2016; Nevison et al., 2016). Therefore, it is recommended to split N supply during the vegetative season, according to the phenological stage and plant needs focusing on the period of maximum requirement. Nitrogen supply at budding and spring can be able to increase root emission and longevity in the soil since this highly active organ accounts for the absorption of larger volumes of water and nutrients such as N (Jordan, 2015). In addition, intense cell division and elongation in shoot organs can be mainly observed just after flowering, a fact that leads to dry matter increase and increases plant demand for N (Ventura et al., 2010). However, peach trees planted in soil presenting clayey texture and average organic matter contents, which was subjected to the application of single or split low N doses such as 40 kg N ha^{-1} (CQFS-RS/SC, 2016), may absorb similar N amounts from the fertilizer. This outcome

would be the desirable one since it would decrease costs with split nitrogen fertilizer applications, as well as potential water-contamination rates and increase the amount of N (from the fertilizer) absorbed by peach trees.

Part of the N absorbed by roots is preferably transported to growing organs such as leaves, annual shoots and fruits (El-Jendoubi et al., 2013; Jordan, 2015). Moreover, part of the N accumulated in annual organs is redistributed, after harvesting, to storage organs such as stem, shoots older than one year and, mainly to the roots (Rivera et al., 2016; Zhang et al., 2012). If large amounts of N from the fertilizer accumulate inside peach trees in the fertilizer application year, it may not be necessary applying large N rates to the soil in the next crop to reduce soil dependence on the fertilizer (Jordan et al., 2012) or even on N, since this element often derives from organic matter mineralization and the decomposition of plant residues (Sabahi et al., 2016; TerAvest et al., 2010).

It is not clear whether the peach tree absorbs a greater amount of N, when the fertilizer rate is applied in a fractional way and if the highest concentration of N present in the plant is from the origin of the fertilizer or other sources. However, the N amount accumulated in plant organs in the fertilizer application year, and in the subsequent year when N different application modes (single or split N rates) are adopted are yet to be fully known and this information could be more reliable when ^{15}N is used as a tracer (Brunetto et al., 2014). The hypothesis of the study is that peach trees absorb and accumulate greater amounts of N of the fertilizer when the application is split and that the greater amount of N absorbed is derived from other N sources than not of the fertilizer.

The current study aimed to evaluate the annual and residual contribution of N derived from the urea at a single rate or split for the nutritional status of peach trees.

2. Materials and methods

2.1. Experimental Site and Treatments

The experiment was conducted in July 2016, in Bento Gonçalves County, Rio Grande do Sul State, Southern Brazil (latitude 29°9'54.50"S; longitude 51°32´3.87"W) on a peach orchard of the cultivar 'Chimarrita' grafted on 'Capdeboscq' rootstock . Trees, trained as in "Epsilons" system were planted in 2009 at density of 1,666 plants ha^{-1} (1.5 m between plants and 4 m between rows). The orchard was planted in Cambisol Humic (Sibcs, 2013) and Typic Hapludalf soil (Soil Survey Staff, 2014) and presented, before planting, in the 0-0.2 m layer, the following characteristics: clay (310 g kg^{-1}), silt (468 g kg^{-1}) and sand (280 g kg^{-1}); OM (26.5 g kg^{-1}); pH in water 5.7 (1:1 ratio); exchangeable

Al (0.0 $cmol_c\ dm^{-3}$), Ca (7.4 $cmol_c\ dm^{-3}$) and Mg (2.3 $cmol_c\ dm^{-3}$) extracted through 1mol KCl L^{-1}; available P (8.6 mg dm^{-3}) and K (207 mg dm^{-3}) - both extracted through Mehlich-1; and total N (2.60 g kg^{-1}). Climate in the region is subtropical (type Cfa); the mean annual rainfall is 1,736 mm (Table S1).

The following N application strategies were compared as in completely randomized block design with three replicates: 100% N rate supplied at budding (100B) and N splitted as 50% of total rate and at budding + 50% of N at the end of flowering (50B + 50F). Each replicate comprised five plants; the three central plants were subjected to N application and evaluated. Nitrogen was applied at a rate of 40 kg N ha^{-1}, which is equivalent to 54.5 g N $plant^{-1}$; this N rate is the quantity recommended for soils presenting 2.6% to 5.0% OM (CQFS-RS/SC, 2016) and was supplied as enriched urea (3.0 at. % ^{15}N) in 2016. The fertilizer was applied on the soil surface of a 1-m^{-2} area considering the tree stem in the center of the area. Ground cover plants found in the urea application area were manually removed at treatment application time. Cover plants found in the treatment application region were desiccated with non-residual herbicide (glyphosate was the active ingredient) every 30 days, throughout the peach tree cycle.

2.2. Assessment and analyses

Ten full expanded leaves per plant were collected at budding, flowering, fruit growth, harvest and senescence in 2016 and 2017. Leaves were then washed, dried, weighted, milled and analyzed for N and ^{15}N concentration determined with mass spectrometry (Finnigan MAT mass spectrometer, Delta Plus model), according to Brunetto (2014).

At harvest of both years, the yield was recorded and fruits were counted; in addition, a sample of 10 fruits was collected, fruit pulp was manually separated from the stone; organs were dried, weighted and analyzed as described for leaves. In 2017, plants were uprooted with the aid of a tractor and separated into leaves, annual shoots, branches older than one year and stem. Roots were manually separated from the soil and divided into thin (diameter ≤ 2 mm) and thick (diameter > 2 mm) roots (Hendrick and Pregitzer, 1992). They were washed with running water and, subsequently, with distilled water. All organs were weighted to determine fresh weight; a subsample of each organ was then collected and fresh and dry weight was determined. All organs were then ground and analyzed as described before. Soon after fruit harvest in 2016 and 2017, soil samples were collected at the depth of 0.0-0.025, 0.026-0.05, 0.051-0.10 and 0.11-0.20 m in the crown projection

area where treatments were applied. The soil was air-dried, subjected to 2-mm-mesh sieve and stored for total N and ^{15}N analysis.

2.3. Calculations and statistical analysis

Excess ^{15}N atoms was calculated according to the following equation (Equation 1):

Excess ^{15}N atoms in the sample (%) = % ^{15}N atoms in the sample – 0.3663% Equation 1

The percentage of excess ^{15}N atoms in the sample, total N amount and the percentage of ^{15}N in the fertilizer applied to the soil were used to calculate N deriving from the fertilizer (NDFF) (Equations 2 and 3):

NDFF (%)=(% excess ^{15}N atoms in the sample/% excess ^{15}N atoms in the fertilizer) x100 Equation 2

NDFF (g)=Total N in the sample (g)x(% excess ^{15}N atoms in the sample/% excess ^{15}N atoms in the fertilizer) Equation 3

Afterwards, results of the aforementioned equations were used to calculate N deriving from soil (NDFS) (Equation 4):

NDFS (%) = 100-NDFF (%) Equation 4

Recovery of N deriving from the fertilizer by plants (R) was calculated according equation 5:

R (%) = NDFF / Amount N fertilizer applied to the soil (mg) x 100 Equation 5

Total N, NDFF and NDFS content in each organ for calculated by multiplying N concentration for organ dry weight.

Results were subjected to the D'Agostino-Pearson normality test. Data of total N, excess ^{15}N and NDFF in 2016 and 2017 in leaves, were analyzed as in a factorial experimental design with application mode (2 levels: 100B and 50B+50F) and sampling time (4 levels: budding, end of flowering, fruit growth, fruit harvest) as main factors. Data of total N, excess ^{15}N NDFF in 2016 in fruits, were analyzed as in a factorial experimental design with application mode (2 levels: 100B and 50B+50F) and organ (2 levels: pulp and stone) as main factors. Total N and ^{15}N in soil were analyzed as in a factorial experimental design with application mode (2 levels: 100B and 50B+50F) and sampling depth (4 levels: 0-0.025, 0.026-0.05, 0.051-0.01, 0.011-0.02) as main factors. When analysis of variance showed a statistical effect of treatments ($P \leq 0.05$), means were separated by Student Newman Keuls test. When the interaction between factors was significant, 2 times standard error of means (2SEM) was used as the minimum difference between two means statistically different for $P \leq 0.05$.

3. Results

Interaction between treatment and sampling time was not significant for total N, excess ^{15}N and NDFF in leaves in 2016 and 2017, consequently Tables 1 and 2 report the effects of main factors. Total N was higher in 100B than in 50B+50F in 2016 (Table 1) and 2017 (Table 2); excess ^{15}N and NDFF were higher in 50B+50F than in 100B both in 2016 (Table 1) and 2017 (Table 2). The highest total N concentrations were observed in leaves collected at budding and end of flowering, followed by those sampled at fruit harvest, senescence and fruit growth (Table 1). Excess ^{15}N atoms and NDFF rates were higher in leaves collected at end of flowering and fruit harvest than those sampled at fruit growth, senescence and budding (Table 1). Leaves sampled at fruit growth showed higher ^{15}N excess and NDFF than those at budding and senescence that showed similar values (Table 1).

In 2017, total N concentration, as well as excess ^{15}N atoms and NDFF, were higher in leaves collected at the fruit growth than all other sampling data (Table 2). Excess ^{15}N and NDFF showed similar values between all other sampling data. Leaves total N was similar at budding and end of flowering and higher than the values measured at fruit harvest and senescence (Table 2).

Interaction between treatment and sampling time was not significant for total N, excess ^{15}N and NDFF in fruits in 2016, consequently table 3 only reports the effects of main factors. Total N concentration, as well as excess ^{15}N atoms and NDFF, have observed fruits of trees subjected to 50B + 50F than those supplied with a single treatment (Table 3). All values were higher in pulp than in stone (Table 3).

Fruits (pulp and stone), leaves, shoot and stem dry weight was higher in 50B+50F than in 100B; the opposite was observed for thick roots; no significant differences were observed for the 1-year shoot and fine roots (Table 4).

Total N content in leaves, fruit pulp and stone and thick roots was higher in trees subjected to 50B+50F applications than 100B; the opposite was observed for the shoot (Table 5). No significant difference was observed for other organs (Table 5).

N deriving from fertilizer in fruit stone and pulp and thick roots was higher in trees subjected to 50B+50F applications in comparison to 100B; no significant differences were observed between treatments for other organs (Table 6).

N deriving from soil in fruits (pulp and stone), leaves, shoot and thick roots was higher in 50B+50F than 100B; no differences were observed for other treatments (Table 7).

Total N content in the soil profile between 0 and 0.10 m was higher when N was split in comparison to the entire rate in 2016; while at 0.011-0.02 m of depth the values of total N were higher for 100B than for 50B+50F (Table 8). In 2017 total N was higher in soil 50B+50F than in 100B (Table 8). Total N decreased with depth both in 2016 and 2017 (Table 8).

The excess ^{15}N atoms were higher, in 2016 and 2017, in all layers of soil subjected to 100B applications (Table 9) and, for both treatments, the values decreased with depth (Table 9).

Soil NDFF was higher in 50B+50F than 100B along with the entire soil profile both in 2016 and 2017; moreover, the values decreased with depth for all treatments and in all years (Table 10).

4. Discussion

The split of N rates in two different phenological phase enhanced leaves ^{15}N atoms excess and NDFF in both years probably because this application method improved the synchronism between the availability of mineral N in the soil and N uptake by the root system (Radicetti et al., 2017; Sabahi et al., 2016). According to some authors (Brunetto et al., 2016a; Neto et al., 2008; Roccuzzo et al., 2012) at flowering peach trees start producing new roots that enlarged the surface area and increased the volume of soil explored by the root system, enhancing, consequently, the uptake of water and nutrients. In the period immediately after flowering, there is also intense vegetative activity due to the formation of new shoots that become a sink for nutrients, mainly N (Brunetto et al., 2016a; Carranca et al., 2018).

In 2017, the year after the application of enriched urea, leaves showed lower ^{15}N atoms and NDFF in both N application techniques (100B and 50B + 50F) probably because plants allocated part of the ^{15}N assimilated to fruits as also evidenced previously (Muhammad et al., 2015). According to our results, we evidenced that N was mainly allocated to the pulp and, to a lesser extent, to stones with more evident results in 2016 than in 2017. Fruits from plants fertilized with a split mode (50B+50F) evidenced, in both years, higher ^{15}N values, reinforcing the hypothesis of a greater synchronism between N applications and absorption. Moreover, peach pulp recorded higher excess ^{15}N atoms and NDFF than stone as also demonstrated previously (Kuo et al., 2016; Pescie et al., 2018). However, excess N allocation to the pulp can lead to worse fruit quality and an increase of fungal diseases in the field or during storage (Brunetto et al., 2015; Bush et al., 2018).

Peach trees subjected to split N applications (50B + 50F) accumulated more N than those subjected to a single application mainly in leaves and pulp, which are annual organs that present intense growth and increased dry mass throughout the phenological stages (Pescie et al., 2018; Roccuzzo et al., 2017) being the main N sinks during the vegetative season.

The N plant content in the second year was derived mainly from sources other than the fertilizer, probably from organic matter mineralization and from waste decomposition, since the NDFF rate in most organs did not exceed 2.5%, except for the thick roots, which recorded values close to 3.5%.

Decreased total N, excess ^{15}N atoms and NDFF values in leaves at senescence, or even at fruit growth, are probably due to protein degradation and remobilization of N forms from leaves to reserve organs such as stems, branches older than one year, or roots (Brunetto et al., 2016a; Roccuzzo et al., 2017). Part of the N accumulated in storage organs may be redistributed to annual organs growing in the subsequent cycle, a fact that can decrease fruit tree dependence on N applied in the year (Carranca et al., 2018; Roccuzzo et al., 2017).

It is well known that in spring, peach trees use N stored in perennial organs and it was estimated that the majority of N remobilization occurs before root uptake starts (Rufat and DeJong 2001. The remobilization process is little affected by the amount of soil N (Tagliavini and Millard, 2005) but the duration depends on the amount of stored N, being longer in trees with large storage pools (Grassi et al., 2003). Once remobilization finishes, root uptake provides the remainder of the N used for growth; consequently, from this stage until the end of the season it is important to maintain adequate N in the soil.

As a consequence of soil OM mineralization in the soil there is an increase of N availability that not derives from enriched urea and determines an increase of the amount of N deriving from the soil in the plant (García-Orenes et al., 2016). This partly justifies the low leaf NDFF rates in the N application year (2016) and in the following year (2017) which did not exceed 17% and 3% respectively, throughout leaf collection times in both N supply techniques. Moreover, it is also evidenced by the higher NDFS content that NDFF showed in plants at the end of the experiment.

The higher excess ^{15}N atoms and NDFF values observed in topsoil layers, mainly in soil subjected to split N application (50B+50F), can be attributed to the complexation/adsorption of part of the N applied to organic compounds of organic matter on the soil (Zhang et al., 2015), a fact that was observed in orchards subjected to different N

rates and application times (Brunetto et al., 2016a; Roccuzzo et al., 2017). Thus, the split mode should induce greater soil mineral N availability in the most superficial layers, those more explored by roots, explaining the greater incidence of applied N in split mode (50B+50F) on leaves and fruits N concentration.

The lower values measured in 2017 could be due to loss of the N supplied in 2016 due to volatilization (Carranca et al., 2018; Dominghetti et al., 2016; Pescie et al., 2018), denitrification (Nevison et al., 2016), leaching (Sparks, 2018; Lynch and Wojciechowski, 2015) and runoff (Baram et al., 2016; Dominghetti et al., 2016; Pescie et al., 2018).

Data from this experiment evidence that N should be applied yearly and possibly in split mode to reduce as much as possible loss of N in the environment and at the same time, meet plant needs. Moreover, the maintenance of high soil OM levels with different techniques such as minimum soil tillage, organic fertilization, ground cover plant cultivation and maintenance of plant residues on the soil (Baldi et al., 2016; Brunetto et al., 2014; Radicetti et al., 2017) could help to reduce N loss in the environment and improve plant nutritional status.

5. Conclusion

Peach trees allocated more N derived from urea in leaves and fruits than in other organs, to a higher extent in the year of fertilizer application more than in the following year, probably due to N loss in the environment or absorbed by plants. The best results in terms of N concentration were observed as a consequence of the split mode showing that with this technique it is easier to meet plants' need.

For the purpose of recommendation, it is important to make it clear that although the plants show better results with the fractionation of N rates; however, at the time of application it should be checked whether the costs for fertilizer supply (which includes double use of machinery and labor) offset the productivity gain and guarantee profits for the producer.

Author Contributions

Betania Vahl de Paula carried out the assembly and experimental collection, analysis and interpretation of data and co-wrote the paper. Beatriz Baticini Vitto, Paula Beatriz Sete, Talita Trapp and Jovani Zalamena collected the data, laboratory analysis and statistical analysis. Gustavo Brunetto and Elena Baldi analyzed the data and co-wrote the paper. George Wellington Bastos de Melo, Moreno Toselli, Danilo Eduardo Rozane revised the paper.

Funding

We acknowledge the financial support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) and Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA).

Conflicts of Interest

The authors have declared that no competing interests exist.

References

Baldi, E., Marcolini, G., Quartieri, M., Sorrenti, G., Muzzi, E., Toselli, M., 2016. Organic fertilization in nectarine (*Prunus persica* var. nucipersica) orchard combines nutrient management and pollution impact Nutr. Cycl. Agroecosyst. 106, 129–130. doi:10.1007/s10705-016-9785-y

Baram, S., Couvreur, V., Harter, T., Read, M., Brown, P.H., Kandelous, M., Smart, D.R., Hopmans, J.W., 2016. Estimating nitrate leaching to groundwater from orchards: comparing crop nitrogen excess, Deep Vadose Zone Data-Driven Estimates, and HYDRUS Modeling. Vadose Zo. J. 15, 0. doi:10.2136/vzj2016.07.0061

Brunetto, G., Ceretta, C.A., Bastos de Melo, G.W., Kaminski, J., Trentin, G., Girotto, E., Ferreira, P.A.A., Miotto, A., Trivelin, P.C.O., 2014. Contribution of nitrogen from agricultural residues of rye to 'Niagara Rosada' grape nutrition. Sci. Hortic. 169, 66–70. doi:10.1016/J.SCIENTA.2014.02.019

Brunetto, G., Ceretta, C.A., de Melo, G.W.B., Girotto, E., Ferreira, P.A.A., Lourenzi, C.R., da Rosa Couto, R., Tassinaria, A., Hammerschmitt, R.K., da Silva, L.O.S., Lazzaretti, B.P., de Souza Kulmann, M.S., Carranca, C., 2016a. Contribution of nitrogen from urea applied at different rates and times on grapevine nutrition. Sci. Hortic. 207, 1–6. doi:10.1016/j.scienta.2016.05.002

Brunetto, G., Melo, G.W.B. De, Toselli, M., Quartieri, M., Tagliavini, M., 2015. The role

of mineral nutrition on yields and fruit quality in grapevine, pear, and apple. Rev. Bras. Frutic. 37, 1089–1104. doi:10.1590/0100-2945-103/15

Brunetto, G., Oliveira, B.S., Ambrosini, V.G., Couto, R. da R., Sete, P.B., Santos Junior, E. dos, Loss, A., Silva, L.O.S. da, Gatiboni, L.C., Brunetto, G., Oliveira, B.S., Ambrosini, V.G., Couto, R. da R., Sete, P.B., Santos Junior, E. dos, Loss, A., Silva, L.O.S. da, Gatiboni, L.C., 2018. Nitrogen availability in an apple orchard with weed management. Ciência Rural 48, 1-5. doi:10.1590/0103-8478cr20160895

Brunetto, G., Rozane, D.E., Melo, G.W.B. De, Zalamena, J., Girotto, E., Lourenzi, C.R., Couto, R.R., Tiecher, T., Kaminski, J., 2016b. Manejo da fertilidade de solos em pomares de frutíferas de clima temperado, in: Tiecher, T. (Ed.), Manejo e Conservação Do Solo e Da Água Em Pequenas Propriedades Rurais No Sul Do Brasil: Práticas Alternativas de Manejo Visando a Conservação Do Solo e Da Água. UFRGS, Porto Alegre, cap. IX, p. 141–158.

Bush, E.A., Yoder, K.S., Smith, A.H., 2018. Brown Rot on Peach and Other Stone Fruits, in: Virginia Cooperative Extension. Virginia, pp. 450–721.

Carranca, C., Brunetto, G., Tagliavini, M., 2018. Nitrogen Nutrition of Fruit Trees to Reconcile Productivity and Environmental Concerns. Plants 7, 4. doi:10.3390/plants7010004

CQFS-RS/SC, 2016. Manual de Calagem e Adubação do RS/SC, 11th ed. Porto Alegre.

Damour, G., Dorel, M., Quoc, H.T., Meynard, C., Risède, J.M., 2014. A trait-based characterization of cover plants to assess their potential to provide a set of ecological services in banana cropping systems. Eur. J. Agron. 52, 218–228. doi:10.1016/j.eja.2013.09.004

Dominghetti, A.W., Guelfi, D.R., Guimarães, R.J., Caputo, A.L.C., Spehar, C.R., Faquin, V., Dominghetti, A.W., Guelfi, D.R., Guimarães, R.J., Caputo, A.L.C., Spehar, C.R., Faquin, V., 2016. Nitrogen loss by volatilization of nitrogen fertilizers applied to coffee orchard. Ciênc. agrotec. 40, 173–183. doi:10.1590/1413-70542016402029615

El-Jendoubi, H., Abadía, J., Abadía, A., 2013. Assessment of nutrient removal in bearing peach trees (*Prunus persica* L. Batsch) based on whole tree analysis. Plant Soil 369, 421–437. doi:10.1007/s11104-012-1556-1

García-Orenes, F., Roldán, A., Morugán-Coronado, A., Linares, C., Cerdà, A., Caravaca, F., 2016. Organic fertilization in traditional Mediterranean grapevine orchards mediates changes in soil microbial community structure and enhances soil fertility. L. Degrad. Dev. 27, 1622–1628. doi:10.1002/ldr.2496

Hendrick, R.L., Pregitzer, K.S., 1992. The Demography of Fine Roots in a Northern Hardwood Forest. Ecology 73, 1094–1104. https://doi.org/10.2307/1940183

Jannoyer, M.L., Le Bellec, F., Lavigne, C., Achard, R., Malézieux, E., 2011. Choosing cover crops to enhance ecological services in orchards: A multiple criteria and systemic approach applied to tropical areas. Procedia Environ. Sci. 9, 104–112. doi:10.1016/j.proenv.2011.11.017

Jordan, M.-O., 2015. C depletion and tree dieback in young peach trees: a possible consequence of N shortage? Ann. For. Sci. 72, 529–537. doi:10.1007/s13595-015-0466-9

Jordan, M.O., Wendler, R., Millard, P., 2012. Autumnal N storage determines the spring growth, N uptake and N internal cycling of young peach trees. Trees - Struct. Funct. 26, 393–404. doi:10.1007/s00468-011-0600-8

Kuo, D., XueMei, W., BoLang, C., ZhongPing, C., XueFeng, W., 2016. Characteristics on nitrogen absorption and accumulation of Korla fragrant pear tree. Southwest China J. Agric. Sci. 29, 847–851.

Lynch, J.P., Wojciechowski, T., 2015. Opportunities and challenges in the subsoil: pathways to deeper rooted crops. J. Exp. Bot. 66, 2199–2210. doi:10.1093/jxb/eru508

Melo, G.W.B. de, Sete, P.B., Ambrosini, V.G., Freitas, R.F., Basso, A., Brunetto, G., 2016. Nutritional status yield and composition of peach fruit subjected to the application of organic compost. Acta Sci. Agron. 38, 103. doi:10.4025/actasciagron.v38i1.25638

Muhammad, S., Sanden, B.L., Lampinen, B.D., Saa, S., Siddiqui, M.I., Smart, D.R., Olivos, A., Shackel, K.A., DeJong, T., Brown, P.H., 2015. Seasonal changes in nutrient content and concentrations in a mature deciduous tree species: Studies in almond (*Prunus dulcis* (Mill.) D. A. Webb). Eur. J. Agron. 65, 52–68. doi:10.1016/J.EJA.2015.01.004

Neto, C., Carranca, C., Clemente, J., de Varennes, A., 2008. Nitrogen distribution, remobilization and re-cycling in young orchard of non-bearing "Rocha" pear trees. Sci. Hortic. 118, 299–307. doi:10.1016/j.scienta.2008.06.023

Nevison, C., Hess, P., Riddick, S., Ward, D., 2016. Denitrification, leaching, and river nitrogen export in the Community Earth System Model. J. Adv. Model. Earth Syst. 8, 272–291. doi:10.1002/2015MS000573

Pescie, M.A., Borda, M.P., Ortiz, D.P., Landriscini, M.R., Lavado, R.S., 2018. Absorption, distribution and accumulation of nitrogen applied at different phenological stages in southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrid). Sci. Hortic. 230, 11–17. doi:10.1016/j.scienta.2017.11.005

Radicetti, E., Campiglia, E., Marucci, A., Mancinelli, R., 2017. How winter cover crops and tillage intensities affect nitrogen availability in eggplant. Nutr. Cycl. Agroecosys. 108, 177–194. doi:10.1007/s10705-017-9849-7

Rivera, R., Bañados, P., Ayala, M., 2016. Distribution of 15N applied to the soil in the 'Bing'/'Gisela®6' sweet cherry (*Prunus avium* L.) combination. Sci. Hortic. 210, 242–249. doi:10.1016/j.scienta.2016.06.035

Roccuzzo, G., Scandellari, F., Allegra, M., Torrisi, B., Stagno, F., Mimmo, T., Zanotelli, D., Gioacchini, P., Millard, P., Tagliavini, M., 2017. Seasonal dynamics of root uptake and spring remobilisation of nitrogen in field grown orange trees. Sci. Hortic. 226, 223–230. doi:10.1016/j.scienta.2017.08.010

Roccuzzo, G., Zanotelli, D., Allegra, M., Giuffrida, A., Torrisi, B.F., Leonardi, A., Quiñones, A., Intrigliolo, F., Tagliavini, M., 2012. Assessing nutrient uptake by field-grown orange trees. Eur. J. Agron. 41, 73–80. doi:10.1016/j.eja.2012.03.011

Sabahi, H., Jamian, S.S., Ghashghaee, F., 2016. Assessment of urea coated with pomegranate fruit powder as N slow-release fertilizer in maize. J. Plant Nutr. 39, 2092–2099. doi:10.1080/01904167.2016.1193607

Soil Survey Staff, 2014. Soil Survey Staff - Keys to Soil Taxonomy, Twelfth Ed. ed. Washignton D.C.

Sparks, D.L., 2018. Kinetics and mechanisms of chemical reactions at the soil mineral/water interface, in: Soil Physical Chemistry. CRC Press, pp. 135–192. doi:10.1201/9780203739280-4

TerAvest, D., Smith, J.L., Carpenter-Boggs, L., Hoagland, L., Granatstein, D., Reganold, J.P., 2010. Influence of orchard floor management and compost application timing on nitrogen partitioning in apple trees. Hortsci 45, 637–642. doi:10.21273/HORTSCI.45.4.637

Ventura, M., Scandellari, F., Bonora, E., Tagliavini, M., 2010. Nutrient release during decomposition of leaf litter in a peach (*Prunus persica* L.) orchard. Nutr. Cycl. Agroecosys. 87, 115–125. doi:10.1007/s10705-009-9317-0

Zhang, L., Han, M., Zhao, C., Liu, C., Zhang, F., Zhang, L., Li, B., 2012. Nitrogen study on absorption, distribution and utilization of nitrogen applied in early summer in red fuji apple. J. Plant Nutr. 35, 1557–1571. doi:10.1080/01904167.2012.689914

Zhang, Y., Zhang, J., Zhu, T., Müller, C., Cai, Z., 2015. Effect of orchard age on soil nitrogen transformation in subtropical China and implications. J. Environ. Sci. 34, 10–19. doi:10.1016/J.JES.2015.03.005

Tagliavini, M., Millard, P. 2005. Fluxes of nitrogen within deciduous fruit trees. Acta Sci. Pol., Hortorum Cultus, 4, 21-30.

Grassi G., Millard P., Giacchini P., Tagliavini M., 2003. Recycling of nitrogen in the xylem of *Prunus avium* trees starts when spring remobilization of internal reserves declines. Tree Physiol. 23, 1061–1068. doi:10.1093/treephys/23.15.1061

Rufat J., DeJong T. M., 2001. Estimating seasonal nitrogen dynamics in peach trees in response to nitrogen availability. Tree Physiol. 21, 1133–1140. doi:10.1093/treephys/21.15.1133

Table 1. Effect of urea application and sampling time on total N, excess ^{15}N atoms and N deriving from fertilizers (NDFF) on peach leaves in 2016.

Treatment	**Total N (% DW)**	**Atom ^{15}N excess (% DW)**	**NDFF (% DW)**
50B+50F	2.92	0.326	10.9
100B	3.08	0.242	8.08
Significance	*	***	***
Sampling Time			
Budding	3.55 a	0.120 c	4.00 c
End of flowering	3.51 a	0.403 a	13.4 a
Fruit growth	2.20 d	0.313 b	10.4 b
Fruit harvest	3.32 b	0.401 a	13.4 a
Senescence	2.42 c	0.184 c	6.14 c
Significance	***	***	***
Treatment×Time	*n.s.*	*n.s.*	*n.s.*

Values followed by the same letter are not statistically different according to Student Neuman Keul test (P≤0.05). n.s., *, ***: effect not significant, significant at P≤0.05 and P≤0.001, respectively.

476

477

478

Table 2. Effect of urea application and sampling time on total N, excess ^{15}N atoms and N deriving from fertilizers (NDFF) on peach leaves in 2017.

Treatment	**Total N (% DW)**	**Atom ^{15}N excess (% DW)**	**NDFF (% DW)**
50B+50F	3.15	0.048	1.61
100B	3.30	0.039	1.30
Significance	***	***	***
Sampling Time			
Budding	3.42 b	0.0388 b	1.29 b
End of flowering	3.45 b	0.0345 b	1.15 b
Fruit growth	3.74 a	0.0745 a	2.49 a
Fruit harvest	2.76 c	0.0341b	1.14 b
Senescence	2.76 c	0.0357 b	1.19 b
Significance	***	***	***
Treatment×Time	*n.s.*	*n.s.*	*n.s.*

Values followed by the same letter are not statistically different according to Student Neuman Keul test (P≤0.05). n.s., *, ***: effect not significant, significant at P≤0.001, respectively.

479
480

481

Table 3. Effect of urea application and organ on total N, excess ^{15}N atoms and N deriving from fertilizers (NDFF) on peach fruit in 2016.

Treatment	**Total N (% DW)**	**Atom ^{15}N excess (% DW)**	**NDFF (% DW)**
50B+50F	0.738	0.234	7.88
100B	0.580	0.182	5.68
Significance	**	*	***
Organ			
Pulp	0.893	0.242	8.17
Stone	0.424	0.174	5.40
Significance	***	*	***
Treatment×Organ	*n.s.*	*n.s.*	**

n.s., *, **, ***: effect not significant, significant at P≤0.05, P≤0.01 and P≤0.001, respectively.

482
483

484

Table 4. Effect of urea application on organ biomass (g pt^{-1}) at the end of the experiment.

Treatment	Stone	Pulp	Leaves	Branches	Shoot	Stem	Fine roots	Thick roots
50B+50F	929	6447	3142	698	1335	8966	305	4480
100B	712	4699	2554	518	1230	5836	349	4961
Significance	*	*	*	*	*n.s.*	*	*n.s.*	*

n.s., *: effect not significant and significant at $P \leq 0.05$, respectively.

485
486

487

Table 5. Effect of urea application on organ total N content (g pt^{-1}) at the end of the experiment.

Treatment	Stone	Pulp	Leaves	Shoot	Shoot (1 year old)	Stem	Fine roots	Thick roots
50B+50F	9.52	62.8	94.4	5.60	7.84	26.2	3.21	51.9
100B	5.11	39.0	64.4	8.53	6.14	15.9	3.21	40.5
Significance	*	*	*	*	*n.s.*	*n.s.*	*n.s.*	*

n.s., *: effect not significant and significant at P≤0.05, respectively.

488
489

490

Table 6. Effect of urea application on organ N deriving from fertilizer (g pt^{-1}) at the end of the experiment.

Treatment	Stone	Pulp	Leaves	Shoot	Shoot (1 year old)	Stem	Fine roots	Thick roots
50B+50F	0.140	1.12	0.964	0.056	0.129	0.397	0.114	1.24
100B	0.053	0.528	0.877	0.100	0.115	0.240	0.074	0.638
Significance	*	*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	**

n.s., *, **: effect not significant, significant at P≤0.05 and P≤0.01, respectively.

491
492

493

Table 7. Effect of urea application on organ N deriving from soil (g pt^{-1}) at the end of the experiment.

Treatment	**Stone**	**Pulp**	**Leaves**	**Shoot**	**Shoot (1 year old)**	**Stem**	**Fine roots**	**Thick roots**
50B+50F	9.38	61.6	93.5	5.55	7.72	25.8	3.10	50.6
100B	5.05	38.4	63.5	7.84	6.01	15.6	3.14	39.9
Significance	*	*	*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*

n.s., *: effect not significant and significant at P≤0.05, respectively.

494
495

496

Table 8. Effect of urea application and depth (m) on soil total N content (g kg^{-1}) in 2016 and 2017.

Treatment	**0-0.025**	**0.026-0.05**	**0.051-0.01**	**0.011-0.02**
		2016		
50B+50F	20.5	19.5	14.6	12.7
100B	19.0	15.6	13.4	13.4
Significance		*2SEM=0.412*		
		2017		
50B+50F	20.7	18.3	16.8	15.5
100B	18.7	17.0	15.7	15.3
Significance		*2SEM=0.146*		

Values differing by 2 standard error of means (SEM) are statistically different. Interaction treatment*depth significant at P <0.05 in 2016 and P <0.01 in 2017.

497
498

499

Table 9. Effect of urea application and depth (m) on soil ^{15}N atom excess (%) in 2016 and 2017.

Treatment	**0-0.025**	**0.026-0.05**	**0.051-0.01**	**0.011-0.02**
		2016		
50B+50F	0.032	0.018	0.006	0.003
100B	0.043	0.032	0.024	0.010
Significance		*2SEM=0.0011*		
		2017		
50B+50F	0.019	0.009	0.007	0.005
100B	0.029	0.021	0.011	0.009
Significance		*2SEM=0.0010*		

Values differing by 2 standard error of means (SEM) are statistically different. Interaction treatment*depth significant at $P < 0.05$ for both 2016 and 2017.

500
501

Table 10. Effect of urea application and depth (m) on soil N deriving from fertilizer (%) in 2016 and 2017.

Treatment	**0-0.025**	**0.026-0.05**	**0.051-0.01**	**0.011-0.02**
		2016		
50B+50F	1.44	1.05	0.806	0.333
100B	1.08	0.602	0.211	0.103
Significance		*2SEM=0.038*		
		2017		
50B+50F	0.965	0.715	0.353	0.291
100B	0.634	0.295	0.231	0.181
Significance		*2SEM=0.035*		

Values differing by 2 standard error of means (SEM) are statistically different. Interaction treatment*depth significant at $P < 0.05$ for both 2016 and 2017.

502

503

504

505
506
507

508

Suplementary

Table S1. Mean monthly rainfall, air temperature and relative humidity during the experimental months in 2016 and 2017.

Month	Phenological stage	Average air temperature (ºC)		Average rainfall (mm)		Relative air humidity (%)	
		2016	2017	2016	2017	2016	2017
February	Start of senescence	22.6	22.2	146	147	79.2	79.7
June	Budding	10.0	13.8	7	160	78.2	81.5
July	Flowering	12.8	14.5	192	29	77.5	69.5
September	Fruit Growing	14.2	19.1	84	107	74.1	73.9
November	Start of fruiting	18.6	18.2	104	1620	70.9	70.4

509