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Contribution of nitrogen from urea applied at different rates and times on grapevine nutrition

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ABSTRACT

In Brazilian vineyards planted in sandy soils, nitrogen (N) should be applied at optimal rates and timing that correspond to greatest demand, thus minimizing N losses. The aim of this study was to evaluate the grapevine N distribution and recovery of urea-^{15}N applied at budding and bloom. In 2009, in a vineyard (*Vitis vinifera* cv. Cabernet Sauvignon) planted in Santana do Livramento, south Brazil, grapevines were treated with 10 kg N ha^{-1} at budding +10 kg N ha^{-1} at full bloom (10B + 10F); 20 kg N ha^{-1} at budding +20 kg N ha^{-1} at full bloom (20B + 20F); 20 kg N ha^{-1} at budding (20B); and 40 kg N ha^{-1} at full bloom (40F). Budding of grapevines in 2009 and 2010 was at the end of August and full bloom in November. In February 2010 and 2011, grapevine organs (leaves, berries, stem and roots) were collected, and in February 2011 soil samples were also collected in the profile. The wine-producing grapevines grown in the sandy soil took up more N derived from 20B treatment, compared with other N treatments, especially in the first crop season. The N derived from fertilizer applied at different rates and time was preferentially distributed in annual plant organs, but most N contained in the plant organs was derived from other sources than the fertilizer N. In the following season, ^{15}N applied in the previous year was recovered preferentially in leaves and fruits, again in low amounts. Nitrogen derived from fertilizer applied at different rates and time in a sandy soil apparently contributes little to grapevine nutrition.

1. Introduction

Sandy soils planted with vineyards normally have low to medium organic matter content which gives them a low capacity for supplying grapevines with mineral nitrogen (N) (Brunetto et al., 2007). Therefore, some wine producers maintain legumes in the inter-row spacing to promote the sumbiotic fixation of atmospheric N_2. During decomposition of shoots of cover crops in the soil surface and roots below the surface, N contained in the plant tissue is released within the root zone of grapevines and is taken up in small amounts by these crops (Brunetto et al., 2011, 2014). However, usually grapevines show low levels of N in the leaves (<16 g N kg^{-1}) (CQFS-RS/SC, 2004). This may cause a decline in crop yield and negatively affect the composition of grape. Thus, the addition of a mineral N source such as urea has been recommended.

Application of urea on the soil surface, it is rapidly hydrolyzed by urease extracellular enzymes produced by microorganisms such as bacteria, actinobacteria and soil fungi and produces ammonium carbonate $(NH_4^+)_2CO_3$ which is not stable in the soil. In the presence of water it decomposes into HCO_3^-, OH^- and ammonium (NH_4^+). The HCO_3^- may then decompose into CO_2 and OH^-. If the NH_4^+ reacts with OH^-, a loss of NH_3 can occur to the atmosphere. However, part of NH_4^+ is transformed through biological oxidation into nitrite (NO_2^-) followed by nitrate (NO_3^-) which may be taken up

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by plant roots or lost by leaching, especially in soils with a sandy texture (Barlow et al., 2009; Lorensini et al., 2012). However, the amount of N leached is especially dependent on the amount of N in the soil and the volume of rainfall (Nielsen et al., 1982; Hajrasuliha et al., 1998). A strategy for minimizing the NO_3^- leaching losses in the soil profile is to apply the fertilizer N at phenological stages with greater crop N demand (Conradie, 1990, 1991).

Literature shows contradicting results as to the most appropriate time for N application for grapevines production (Spayd et al., 1991). Some studies report that grapevines recover a satisfactory amount of N from fertilizer applied at the beginning of budding—this was shown by Conradie (1991) in South Africa, Löhnertz (1991) in Germany, Glad et al. (1994) in France, Araujo et al. (1995) in California, the United States and Brunetto et al. (2006b) in the south of Brazil. Most of these authors attribute this fact to mild temperatures and proper soil moisture at the end of winter which increases the activity of the microbial population in the soil and consequently the availability of mineral N in the soil. Also the emergence of active roots is greater at this time Brunetto et al. (2006b, 2014). However, Vos et al. (2004) in the United States and Schreiner and Scagel (2006) observed that grapevines recovered more N when fertilizer was applied from blooming to six weeks after blooming, in comparison with the application at the beginning of budding with a crop recovery of N greater than 20%.

Part of N taken up by the crop is incorporated into the carbonate structures, such as allantoin (4N:4C), arginine (4N:4C), and citrulline (3N:3C), or, moreover, in mineral forms, such as NH_4^+ and NO_3^- transported through the stem and branches older than one year to the vegetative organs with active cell division and consequently with a higher dry matter increase, such as leaves, shoots, and bunches (berries + rachis) (Glad et al., 1994). Part of accumulated N in the annual organs throughout the period of leaf senescence may be redistributed to the perennial organs, especially to roots and stem/trunk (Bates et al., 2002; Zapata et al., 2004; Brunetto et al., 2005, 2006a, 2014). For studies on N recovery and accumulation in organs of fruit-bearing plants like grapevines, ^{15}N isotope has been used as a tracer since it allows a precise monitoring of fertilizer N taken up by the crop and its distribution in the plant (Brunetto et al., 2006a,b; Menino et al., 2007; Neto et al., 2008).

The aim of this study was to evaluate the grapevine N distribution and recovery of urea-^{15}N applied at budding and bloom. We hypothesized that fertilizer N applied at blooming in the previous year is remobilized for vegetative and flowering organs in the following season, whereas N applied at budding is especially used in the current season.

2. Materials and methods

2.1. Description of the experiment

The experiment was conducted from September 2009 to February 2012 in a vineyard at Santana do Livramento, Rio Grande do Sul—Brazil (longitude 655321.09 m E; latitude 6593897.74 m S). The vineyard (*Vitis vinifera*) was the Cabernet Sauvigon cultivar grafted on SO4 (*Vitis berlandieri* × *Vitis riparia*) rootstock. Plant density per hectare was 3703 (1.0 m × 2.7 m) on a spur pruned cordon system. Climate in the region is subtropical humid, Cfa2 type, according to the Köppen classification and is characterized by mild temperature and rainfall with little variation throughout the year. Mean annual rainfall for a long period is 1600 mm; the mean temperature of the hottest month (January) is 23.8 °C and the mean temperature of the coldest month (July) is 12.4 °C. Data on mean monthly temperature and accumulated rainfall throughout the experimental period are shown in Table 1. The soil is a Typic Hapludalf and the chemical properties in the 0–0.20 m layer are shown in Table 2. Plants in the inter-rows spacing were desiccated with non-residual herbicide. In this inter-rows, a mixture of plants was cultivated, especially the *Paspalum notatum*, *Trifolium repens* and *Lolium perenne*.

Table 1
Mean monthly values of rainfall (mm), air temperature (°C), and air relative humidity (%RH) during the experimental period.

Year/Month	Phenological Stage	Rainfall (mm)	Air temperature (°C)	Air RH (%)
2009				
August	Begin of budbreak	45.3	14.2	77.9
September	Budbreak	269.4	13.3	86.6
October	End of budbreak	135.9	16.9	77.8
November	Begin of bloom	540.8	20.8	86.7
December	End of bloom	219.0	21.7	79.2
2010				
January	Veraison	204.1	23.4	77.3
February	Veraison	240.7	23.1	82.8
March	Harvest	58.9	21.8	79.9
April	Start falling leaves	132.0	17.1	78.8
May	falling leaves	133.4	14.3	86.7
June	falling leaves	33.7	12.1	84.0
July	End of falling leaves	295.3	10.9	80.9
August	Budbreak	53.3	11.6	78.5
September	Budbreak	182.6	14.4	81.2
October	End of budbreak	19.4	16.2	68.1
November	Begin of bloom	29.0	19.7	59.8
December	End of bloom	56.0	23.7	57.3
2011				
January	Veraison	61.6	25.0	70.3

Table 2
Main physical and chemical characteristics of the soil in the experimental site at 0 – 0.20 m soil layer.

Soil characteristics	Unit	0–0.20 m
Clay	g kg^{-1}	63
Silt	g kg^{-1}	115
Sand	g kg^{-1}	822
Organic matter	g kg^{-1}	15.0
Total N	mg kg^{-1}	2.300
$pH_{(H_2O)}$	–	6.00
Exchangeable aluminum		0.00
Exchangeable magnesium	$cmol_c$ kg^{-1}	0.80
Exchangeable calcium	$cmol_c$ kg^{-1}	1.75
Exchangeable potassium	mg kg^{-1}	50.0
Available phosphorus	mg kg^{-1}	40.0

In 2009, grapevines received the following four treatments: 10 kg N ha^{-1} at budding +10 kg N ha^{-1} at full bloom (10B + 10F); 20 kg N ha^{-1} at budding +20 kg N ha^{-1} at full bloom (20B + 20F); 20 kg N ha^{-1} at budding (20B); and 40 kg N ha^{-1} at full bloom (40F). Budding of grapevines in 2009 and 2010 was at the end of August, and full bloom in November. The N source was urea enriched with 3% ^{15}N atom excess. The urea was incorporated into the soil surface in the row, below the of grapevine canopy. In addition, five plants without ^{15}N application were used as control plants. The experimental design was under randomized blocks with five replications, with three central plants in each plot being used for measurements. The leaves of grapevines collected in the veraison contained (%): 0.90 N, 0.2 P, 2.0 K, Ca 1.5 and 0.4 Mg.

In February 2010 and 2011, eight bunches of grapes were collected randomly from each grapevine. Then, berries from the upper, middle and lower part of each bunch were separated and frozen in liquid N, dried in a freeze-dryer until constant weight, and reserved for testing. The eight rachis of bunches were also reserved for analysis. Afterwards, mature leaves were collected from the middle third of three shoots chosen at random in each plant. Leaves and rachis

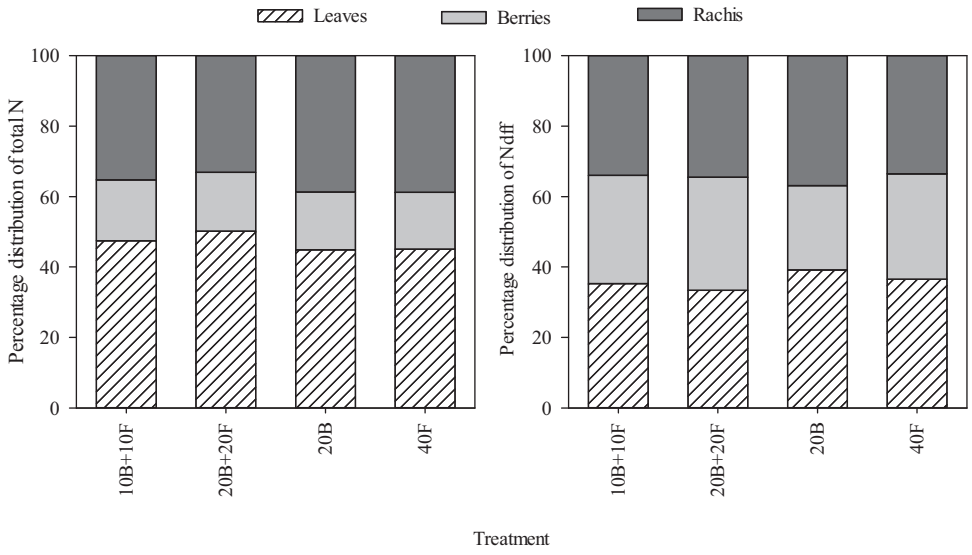

Fig. 1. Percentage distribution of total N (a) and N derived from fertilizer (Ndff) (b), in Cabernet Sauvignon grapevine organs as response to urea-^{15}N applied this season (2009/2010). 10B + 10F = 10 kg N ha^{-1} applied at budding +10 kg N ha^{-1} applied at full bloom; 20B + 20F = 20 kg N ha^{-1} applied at budding +20 kg N ha^{-1} applied at full bloom; 20B = 20 kg N ha^{-1} applied at budding; 40F = 20 kg N ha^{-1} applied at budding.

Table 3
Total N (%), atom% ^{15}N excess, N derived from fertilizer (%Ndff), and N derived from other sources (%Ndfs) in Cabernet Sauvignon grapevine organs as response to urea-^{15}N applied this season (2009/2010).

Treatment	Leaves	Berries	Rachis	CV (%)
	Total N (%)			
10B + 10F	2.33 bA[a]	0.85 aC	1.73 aB	22.74
20B + 20F	3.03 aA	1.01 aC	2.00 aB	10.58
20B	2.10 bA	0.77 aB	1.81 aA	7.89
40F	2.37 bA	0.85 aB	2.04 aA	6.73
CV (%)	8.78	16.11	16.78	
	Atom% ^{15}N excess			
10B + 10F	0.120 bA	0.104 aA	0.116 bA	13.53
20B + 20F	0.133 bA	0.128 aA	0.137 bA	5.90
20B	0.218 aA	0.133 aB	0.205 aA	6.46
40F	0.127 bA	0.104 aA	0.116 bA	3.72
CV (%)	4.90	8.42	6.78	
	%Ndff			
10B + 10F	4.00 bA	3.48 aA	3.85 bA	13.48
20B + 20F	4.42 bA	4.26 aA	4.57 bA	5.71
20B	7.27 aA	4.45 aB	6.84 aA	6.36
40F	4.23 bA	3.47 aA	3.88 bA	3.82
CV (%)	4.95	8.39	6.72	
	%Ndfs			
10B + 10F	96.00 aA	96.52 aA	96.15 aA	0.53
20B + 20F	95.58 aA	95.74 aA	95.43 aA	0.26
20B	92.73 bB	95.56 aA	93.16 bB	0.42
40F	95.77 aA	96.53 aA	96.12 aA	1.12
CV (%)	0.92	0.34	0.34	

[a] Mean values followed by the same lowercase in the column and uppercase in the line do not differ among them according to the Scott-Knott test at 5% probability. 10B + 10F = 10 kg N ha^{-1} applied at budding +10 kg N ha^{-1} applied at full bloom; 20B + 20F = 20 kg N ha^{-1} applied at budding +20 kg N ha^{-1} applied at full bloom; 20B = 20 kg N ha^{-1} applied at budding; 40F = 20 kg N ha^{-1} applied at budding; CV = coefficient of variation.

were dried in an air circulation oven at 65 °C until constant weight, and were sieved (<0.05 mm) and reserved for testing.

In February 2011, shoots and leaves were collected at random from each plant. The 1-year-old shoots were also collected each plant and prepared for analysis. For each stem, a strip of about 0.05 m length, representing around 1/4 of the diameter of the stem was collected at 0.4 m from the soil surface. Roots were also taken from a soil volume collected in two pits of 0.20 m width × 0.20 m length × 0.20 m depth. Pits were opened beside the grapevine row, corresponding to the area beneath the plant canopy, where ^{15}N fertilizer has been applied. Soil was removed from the roots by washing the roots with distilled water and roots were reserved for analysis. Leaves, shoots, 1-year-old shoots, stems, and root samples were oven-dried with air circulation at 65 °C until constant weight, and were sieved (0.05 mm sieve) and kept for analysis.

In the same pits where roots were taken, soil samples were collected in February 2011 at 0–0.05, 0.05–0.10 and 0.10–0.20 m depth. Soil samples were air dried, sieved and analyzed.

2.2. Plant and soil analysis

Total N and atom% ^{15}N enrichment in the plant organs and soil samples were analyzed by dry combustion and mass spectrophotometer (Hydra 20/20 model, PDZ Europa, Crewe, UK), respectively. The ^{15}N dilution technique was used to estimate the atom% ^{15}N excess in the plant organs and soil, and the%^{15}N recovery in each organ and soil layer, as follows (Brunetto et al., 2014):

The atom% ^{15}N excess was calculated according to Eq. (1):

$$Atom^{15}\ N\ excess\ in\ sample\ (\%) = \%atom^{15}\ N\ in\ sample - 0.3663\% \quad (1)$$

where ^{15}N from natural abundance in the atmosphere is 0.3663% enrichment and is assumed as reference.

The nitrogen derived from fertilizer (%Ndff) was calculated using Eq. (2):

$$Nitrogen\ derived\ from\ fertilizer\ (\%) = \frac{\%atom^{15}N\ excess\ in\ sample}{\%atom^{15}N\ excess\ in\ fertilizer} \times 100 \quad (2)$$

The nitrogen derived from other sources, especially from the soil (%Ndfs) was calculated using Eq. (3):

$$Nitrogen\ derived\ from\ othersources(\%) = 100 - nitrogen\ derived\ from\ fertilizer \quad (3)$$

2.3. Statistical analysis

Data were subjected to Analysis of Variance and when this analysis indicated statistically significant differences, data were subjected to the Scott–Knott mean comparison test for $p < 0.05$.

Table 4
Total N (%), atom% ^{15}N excess, N derived from fertilizer (%Ndff), and N derived from other sources (%Ndfs) in Cabernet Sauvignon grapevine organs as response to urea-^{15}N applied in the previous season.

Treatment	Leaves	Berries + Rachis	Shoots	1-year-old shoots	Stem	Roots	CV (%)
	Total N (%)						
10B + 10F	1.41 aA[a]	0.47 aD	0.70 aC	0.67 aC	0.41 aD	1.17 bB	8.10
20B + 20F	1.41 aA	0.44 aC	0.69 aB	0.60 aB	0.38 aC	1.44 aA	10.35
20B	1.25 bA	0.59 aB	0.64 aB	0.62 aB	0.39 aC	1.17 bA	14.23
40F	1.21 bA	0.50 aC	0.68 aB	0.59 aB	0.36 aC	1.32 aA	14.39
CV (%)	13.10	17.89	5.22	11.52	4.94	4.00	
	Atom% ^{15}N excess						
10B + 10F	0.033 aC	0.030 aB	0.072 cA	0.045 aB	0.057 aA	0.060 bA	9.40
20B + 20F	0.038 aC	0.034 aC	0.096 bA	0.070 aB	0.066 aB	0.080 aB	15.11
20B	0.020 bD	0.016 bD	0.121 aA	0.059 aC	0.072 aC	0.086 aB	19.40
40F	0.042 aC	0.041 aC	0.094 bA	0.055 aC	0.068 aB	0.053 bC	19.95
CV (%)	14.15	22.15	16.31	15.51	13.82	13.90	
	%Ndff						
10B + 10F	1.10 aC	1.00 aB	2.40 cA	1.50 aB	1.91 aA	2.01 bA	9.33
20B + 20F	1.27 aC	1.13 aC	3.19 bA	2.33 aB	2.19 aB	2.68 aB	15.19
20B	0.68 bD	0.54 bD	4.04 aA	1.96 aC	2.39 aC	2.88 aB	19.31
40F	1.40 aC	1.37 aC	3.12 bA	1.83 aC	2.27 aB	1.75 bC	19.65
CV (%)	13.74	21.81	16.20	15.32	13.68	13.84	
	%Ndfs						
10B + 10F	98.90 bA	99.00 bA	97.60 aB	98.50 aA	98.09 aB	97.99 aB	0.16
20B + 20F	98.73 bA	98.87 bA	96.81 bC	97.67 aB	97.81 aB	97.32 bB	0.33
20B	99.32 aA	99.46 aA	95.96 cC	98.04 aB	97.61 aB	97.12 bD	0.41
40F	98.60 bA	98.63 bA	96.88 bC	98.17 aA	97.73 aB	98.25 aA	0.39
CV (%)	0.15	0.22	0.53	0.30	0.31	0.33	

[a] Mean values followed by the same lowercase in the column and uppercase in the line do not differ among them by according to the Scott-Knott test at 5% probability. 10B + 10F = 10 kg N ha^{-1} applied at budding + 10 kg N ha^{-1} applied at full bloom; 20B + 20F = 20 kg N ha^{-1} applied at budding +20 kg N ha^{-1} applied at full bloom; 20B = 20 kg N ha^{-1} applied at budding; 40F = 20 kg N ha^{-1} applied at budding; CV = coefficient of variation.

3. Results and discussion

3.1. N distribution in the grapevine

In the 2009/2010 crop season, the greatest concentration of total N was observed in the leaves of grapevines that received 20 kg N ha^{-1} at budding +20 kg N ha^{-1} at full bloom (20B + 20F) in comparison with other treatments (Table 3). Yet, the total N concentration in berries and rachis was not statistically different among treatments. By the addition of 10B + 10F and 20B + 20F at full bloom, a higher ($p < 0.05$) concentration of total N was observed in leaves as compared with berries and rachis (Table 3). Similarly, in grapevines with application of 20 kg N ha^{-1} at budding (20B) and 40 kg N ha^{-1} at full bloom (40F), a higher concentration of total N was observed in leaves and rachis as compared with berries (Table 3, Fig. 1).

Among grapevine organs, the highest atom% ^{15}N excess and %Ndff applied in the year was measured in the leaves and rachis with the addition of 20B, and no statistical difference was observed for other treatments (Table 3, Fig. 1). As expected, the lowest concentration of N derived from other sources (%Ndfs) was observed in the leaves and rachis of grapevines fertilized with 20B (Table 3). Thus, leaves and rachis represent a physiological N sink during the vegetative and productive growth of grapevines (Glad et al., 1994; Schreiner and Scagel, 2006; Brunetto et al., 2014). However, the %Ndff in leaves, rachis and berries was low, smaller than 7.3% for all treatments indicating that more than 92.7% of N in these organs was derived from other sources than the urea-^{15}N, including internal N, the soil N, plant N reserves, irrigation water, etc. The low %Ndff recovered in grapevine organs in the year of urea-^{15}N application is attributed to the dilution effect of fertilizer N in the plant which is mobilized to these new organs (Nikolaidou et al., 2010; Agnelli et al., 2014), assuming that roots are active at these growth phases. In previous studies, Conradie (1990, 1991), Löhnertz (1991), Glad et al. (1994), Araujo et al. (1995) and Eissenstat (2007) observed a large number of young roots which were a little active but were responsible for the crop water and fertilizer N uptake.

In the following season (2010/2011), the highest total N concentration was observed, especially in leaves of grapevines with the addition of 10B + 10F and 20B + 20F (Table 4, Fig. 2), confirming the results observed in the previous season. Unlike, roots presented the highest total N concentration by the addition of 20B + 20F and 40F. The total N concentration in berries + rachis, 1-year-old shoots, shoots and stem was not affected by treatments. For most treatments in this season, total N concentration followed the sequence: leaves > roots > 1-year-old shoots = shoots > berries + rachis = stem.

The highest atom% ^{15}N excess and %Ndff applied in the previous season was observed in the leaves and berries + rachis of grapevines by the addition of 10B + 10F, 20B + 20F and 40F (Table 4, Fig. 2). In the shoots, the highest atom% ^{15}N excess and %Ndff was measured in grapevines under the 20B treatment and in 20B + 20F, and in roots for 20B treatment. In the 1-year-old shoots, shoots and stem no significant response to treatments was observed. As to treatment 10B + 10F, the atom% ^{15}N excess and %Ndff varied in the plant organs according to the sequence: shoots = stem = roots > 1-year-old shoots = berries + rachis > leaves, similar to the application of double amount of N (20B + 20F): shoots > 1-year-old shoots = stem = roots > leaves = berries + rachis. With the addition of 20B, the order was similar: shoots > roots > 1-year-old shoots = stem > leaves = berries + rachis. Finally, by the addition of 40F in the previous year, the atom% ^{15}N excess and %Ndff in plant organs again followed a similar sequence: shoots > stem > roots = 1-year-old shoot = leaves = berries + rachis.

The greatest %Ndfs was observed in leaves and berries + rachis in the grapevines provided with 20B in the previous year (Table 4), in the shoots by the addition of 10B + 10F, and in the roots by addition of 10B + 10F and 40F. Treatments did not affect the %Ndfs in 1-year-old shoots and stems. The treatment 10B + 10F affected significantly %Ndfs in the following order: leaves = berries + rachis = 1-year-old shoots = shoots = stem = roots. The %Ndfs in plant organs followed the order: leaves = berries + rachis > 1-year-

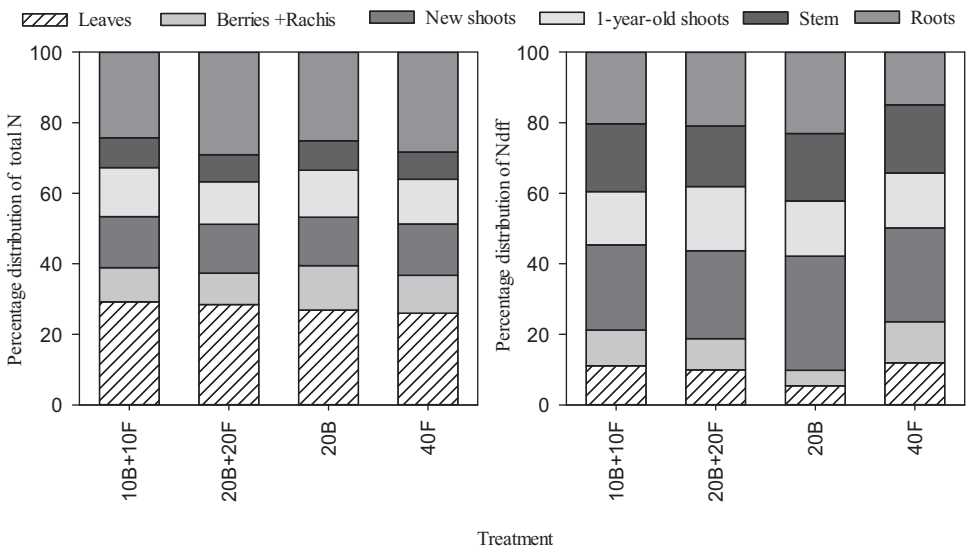

Fig. 2. Percentage distribution of total N (a) and N derived from fertilizer (Ndff) (b), in Cabernet Sauvignon grapevine organs as response to urea-^{15}N applied this season (2009/2010). 10B + 10F = 10 kg N ha^{-1} applied at budding +10 kg N ha^{-1} applied at full bloom; 20B + 20F = 20 kg N ha^{-1} applied at budding + 20 kg N ha^{-1} applied at full bloom; 20B = 20 kg N ha^{-1} applied at budding; 40F = 20 kg N ha^{-1} applied at budding.

old shoots = stem = roots, and was the opposite of %Ndff in plant organs by double N fertilizer (20B + 20F) and the 20B. This shows that most N in these plant organs came from other sources, as the internal N reserves and soil N (Brunetto et al., 2006b; Menino et al., 2007; Neto et al., 2008; Nikolaidou et al., 2010; Agnelli et al., 2014). Fertilizer N applied in the previous season was accumulated mostly in the reserve organs: stem, roots and shoots. This ^{15}N accumulated in the reserve organs was remobilized for the new organs in the following season. As demonstrated by Menino et al. (2007) for perennial orange trees and Neto et al. (2008) for pear trees, fertilizer N applied in the year is mobilized to the young organs during the vegetative and flowering phases, and when is applied at harvest, N is accumulated in the reserve organs (stem, roots, shoots) to be translocated for the new flush in the following year. Leaves are the most enriched plant N organ from fertilizer N applied in the year, especially at bloom.

3.2. N distribution in the soil

In the soil, the highest ($p < 0.05$) concentration of total N for all treatments was observed in the surface layer (0.0–0.05 m) and the lowest level was measured for the 40B treatment added in the previous season (Table 1). In the 0.05–0.10 and 0.10–0.20 m soil layers, the total N concentration did not vary ($p \geq 0.05$) among treatments (Table 5).

The higher %Ndff was observed in the surface layer regardless of treatment. In the surface layer (0–0.05), the highest atom% ^{15}N excess and %Ndff was measured under the highest N rates applied in the previous season (20B + 20F and 40F) (Table 5). In contrast, the lowest atom% ^{15}N excess and %Ndff was observed under the 20B treatment applied in the previous year. In the 0.05–0.10 m soil layer, atom% ^{15}N excess and %Ndff were higher ($p < 0.05$) under the 20B and 40F treatment. In the 0.10–0.20 m soil layer, no significant differences of atom% ^{15}N excess and %Ndff were found for all treatments applied.

The lowest atom% ^{15}N excess and %Ndff in the surface layer with 20B may be attributed to the greater fertilizer N uptake by grapevines in the first crop season (2009/2010), diagnosed by the increase in the concentration of atom% ^{15}N excess and %Ndff in the leaves and berries + rachis (Table 3), but also to some movement of ^{15}N in the soil profile to the 2nd layer.

Table 5
Residual total N (%), atom% ^{15}N excess, and N derived from fertilizer (%Ndff) measured on February 2011 in soil planted with Cabernet Sauvignon grapevine after application of urea-^{15}N in 2009.

Treatment	Layer (m)			CV (%)
	0–0.05	0.05–0.10	0.10–0.20	
	Total N (%)			
10B + 10F	0.07aA[a]	0.04aB	0.03 aB	20.68
20B + 20F	0.07 aA	0.03 aB	0.04 aB	9.33
20B	0.06 aA	0.04 aB	0.03aC	6.88
40F	0.04bA	0.03aB	0.03aB	8.69
CV (%)	14.08	10.19	11,56	
	Atom% ^{15}N excess			
10B + 10F	0.129 bA	0.038 bB	0.026 aB	22.79
20B + 20F	0.177 aA	0.068 bB	0.050 aB	15.76
20B	0.085 cA	0.112 aA	0.024 aB	14.46
40F	0.194 aA	0.137 aB	0.055 aC	14.47
CV (%)	23.15	7.81	16.04	
	%Ndff			
10B + 10F	4.29 bA	1.26 bB	0.87 aB	22.38
20B + 20F	5.91 aA	2.27 bB	1.66 aB	15.86
20B	2.83 cA	3.72 aA	0.78 aB	14.14
40F	6.47 aA	4.56 aB	1.84 aC	18.60
CV (%)	23.11	7.74	16.59	

[a] Mean values followed by the same lowercase in the column and uppercase in the line do not differ among them according to the Scott-Knott test at 5% probability. 10B + 10F = 10 kg N ha^{-1} applied at budding + 10 kg N ha^{-1} applied at full bloom; 20B + 20F = 20 kg N ha^{-1} applied at budding + 20 kg N ha^{-1} applied at full bloom; 20B = 20 kg N ha^{-1} applied at budding; 40F = 20 kg N ha^{-1} applied at budding; CV = coefficient of variation.

4. Conclusions

Grapevines for wine production grown in a sandy soil took up more N derived from fertilizer applied at a rate of 40 kg N ha^{-1} split at budding and full bloom in the first season. This fertilizer N was preferentially recovered in annual plant organs (leaves and berries + rachis). Unlike, in the following season (2010/2011), fertilizer N applied in the previous year was recovered especially in reserve organs, such as the stem, roots and shoots, whereas leaves and berries + rachis showed the lowest ^{15}N recovery. Nitrogen in both seasons was mainly derived from other sources than the fertilizer N.

Conflicts of interest

The authors declare no financial or other competing conflicts of interest.

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References

Agnelli, A., Bol, R., Trumborec, S.E., Dixond, L., Coccoe, S., Corti, G., 2014. Carbon and nitrogen in soil and vine roots in harrowed and grass-covered vineyards. Agric. Ecosyst. Environ. 193, 70–82.

Araujo, F., Williams, L.E., Matthews, M.A.A., 1995. Comparative study of young Thompson Seedless grapevines (*Vitis vinifera* L.) under drip and furrow irrigation. II. Growth, water use efficiency and nitrogen partitioning. Sci. Hortic. 60, 251–265.

Barlow, K., Bond, W., Holzapfel, B., Smith, J., Hutton, R., 2009. Nitrogen concentrations in soil solutionand surface run-off on irrigated vineyards in Austrália. Aust. J. Grape Wine Res. 15, 31–143.

Bates, T.R., Dunst, R.M., Joy, P., 2002. Seasonal dry matter starch, and nutrient distribution in Concord grapevine roots. HortScience 37, 313–316.

Brunetto, G., Kaminski, J., Melo, G.W.B., Gatiboni, L.C., Urquiaga, S., 2005. Absorção e redistribuição do nitrogênio aplicado via foliar em videiras jovens. Rev. Bras. Frutic. 27, 110–114.

Brunetto, G., Kaminski, J., Melo, G.W.B., Rheinheimer, D.S., 2006a. Recuperação e distribuição do nitrogênio fornecido a videiras jovens. Pesq. Agropec. Bras. 41, 1299–1304.

Brunetto, G., Kaminski, J., Melo, G.W.B., Bruning, F.S., Mallmann, F.J.K., 2006b. Destino do nitrogênio em videiras Chardonnay e Riesling Renano quando aplicado no inchamento das gemas. Rev. Bras. Frutic. 28, 497–500.

Brunetto, G., Ceretta, C.A., Kaminski, J., Melo, G.W.B., Lourenzi, C.R., Furlanetto, V., Moraes, A., 2007. Aplicação de nitrogênio em videiras na Campanha Gaúcha: Produtividade e características químicas do mosto da uva. Ciência Rural 37, 389–393.

Brunetto, G., Ventura, M., Scandellari, F., Ceretta, C.A., Kaminski, J., Melo, G.W.B., Tagliavini, M., 2011. Nutrients release during the decomposition of mowed perennial ryegrass and white clover and its contribution to nitrogen nutrition of grapevine. Nutr. Cycl. Agroecosyst. 90, 299–308.

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

CQFS-RS/SC (Comissão de Química e Fertilidade do Solo—RS/SC), 2004. Manual de adubação e calagem para os Estados do Rio Grande do Sul e de Santa Catarina. SBCS—Núcleo Regional Sul/UFRGS, Porto Alegre.

Conradie, W.J., 1990. Distribution and translocation of nitrogen absorbed during late spring by two-year-old grapevines grown in sand culture. Am. J. Enol. Vitic. 41, 241–250.

Conradie, W.J., 1991. Distribution and translocation of nitrogen absorbed during early summer by two-year-old grapevines grown in sand culture. Am. J. Enol. Vitic. 42, 180–190.

Eissenstat, D.M., 2007. Dinamica di crescita delle radici nelle colture da frutto. Italus Hortus 14, 1–8.

Glad, C., Farineau, J., Regnard, J.L., Morot-Gaudry, J.L., 1994. The relative contribution of nitrogen originating from two seasonal ^{15}N supplies to the total nitrogen pool present in the bleeding sap and in whole *Vitis vinifera* cv. Pinot noir grapevines at bloom time. Am. J. Enol. Vitic. 45, 327–332.

Hajrasuliha, S., Rolston, D.E., Louie, D.T., 1998. Fate of 15N fertilizer applied to trickle-irrigated grapevines. Am. J. Enol. Vitic. 49, 191–198.

Löhnertz, O., 1991. Soil nitrogen and the uptake of nitrogen in grapevines. In: International Symposium on Nitrogen in Grapes and Wine, 1991, Washington. The American Society for Enology and Viticulture, Washington, pp. 1–11.

Lorensini, F., Ceretta, C.A., Girotto, E., Cerini, J.B., Lourenzi, C.R., De Conti, L., Trindade, M.M., Melo, G.W.B., Brunetto, G., 2012. Lixiviação e volatilização de nitrogênio em um Argissolo cultivado com videira submetida à adubação nitrogenada. Ciência Rural 42, 1173–1179.

Menino, M.R., Carranca, C., Varennes, A., 2007. Distribution and remobilization of nitrogen in young non-bearing orange trees grown under mediterranean conditions. J. Plant Nutr. 30, 1083–1096.

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.

Nielsen, D.R., Biggar, J.W., Wierenga, P.J., 1982. Nitrogen transport process in soil. In: Stevenson, P.J. (Ed.), Nitrogen in Agriculture Soils. American Society of Agronomy, Madison, pp. 423–448.

Nikolaidou, A.E., Pavlatou-Ve, A.K., Kostopoulou, S.K., Mamolos, A., Kalburtji, K.L., 2010. Litter quality and decomposition of *Vitis vinifera* L. residues under organic and conventional farming systems. Eur. J. Soil Biol. 46, 208–217.

Schreiner, R.P., Scagel, C.F., 2006. Nutrient uptake and distribution in a mature 'Pinot Noir' vineyard. HortScience 41, 336–345.

Spayd, S.E., Wample, R.L., Nagel, C.W., Stevens, R.G., Evans, R.G., 1991. Vineyard nitrogen fertilization effects on must and wine composition and quality. In: International Symposium on Nitrogen in Grapes and Wine. The American Society for Enology and Viticulture, pp. 196–199.

Vos, R.J., Zabadal, T.J., Hanson, E.J., 2004. Effect of nitrogen application timing on N uptake by *Vitis labrusca* in a short-season region. Am. J. Enol. Vitic. (Davis) 55, 246–252.

Zapata, C., Deléens, E., Chaillou, S., Magné, C., 2004. Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). J. Plant Physiol. 161, 1031–1040.