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Annual Urea Nitrogen Contribution to the Nutrition of Cabernet Sauvignon Grapevine Grown in Sandy and Clayey Soil

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Abstract: The timing of nitrogen fertilizer application in vineyards can determine the amount of nitrogen (N) absorbed, distributed, and accumulated in grapevine organs. The study aimed to evaluate the annual contribution of N from urea, applied at different times in Cabernet Sauvignon, grown in sandy and clayey soil in a subtropical climate. The sandy soil received 21.42 kg N ha^{-1} and the clayey soil 30 kg N ha^{-1}, both enriched with 3% excess ^{15}N atoms, applied at different times. The N derived from the fertilizer in grapevines, at all times of N application, and in sandy and clayey soil did not exceed 8%, with the highest values being observed in annual organs, especially in the leaves. The application of N marked at the phenological stages of IBB (50% at beginning of budbreak + 50% at full budbreak) enabled greater absorption of N derived from the fertilizer by the vines grown in both sandy and loamy soil. The N present in the annual organs (leaves, berries, stalks, and shoots) and in the perennial organs (stems and canes) of Cabernet Sauvignon grown in sandy and clayey soil was derived in greater percentages from the soil.

Keywords: N distribution; ^{15}N; N derived from fertilizer; N derived from soil

1. Introduction

Vineyard soil usually does not provide the amount of nitrogen (N) required to meet the grapevines' needs. For this reason, nitrogen fertilizers such as urea are applied to vineyards in production. However, the amount of N absorbed from the fertilizer depends on soil characteristics, such as organic matter and clay content [1]. In sandy soils with low levels of organic matter, part of the N in the fertilizer, especially nitrate (NO_3^-), can be lost, for example, through leaching [2–5], reducing the amount of N absorbed by the grapevines. On the other hand, in clayey soils with a medium organic matter content, part of the N in the fertilizer is expected to remain complex in the soil's organic matter or be adsorbed into the functional groups of reactive inorganic particles [6,7]. Thus, N losses are expected to be lower, which may increase the likelihood of N absorption by the vines [7].

The amount of N that the vine absorbs from the fertilizer also depends on the time of application throughout the annual biological cycle. The literature presents contradictory results on the best times to apply N in vineyards. Some studies report that the dose of N should preferably be applied at the beginning of budbreak in [3,8–11] because soil temperatures and humidity can stimulate soil microbial activity, which increases the mineralization of organic matter and, consequently, the availability of mineral forms of N, in addition to fertilizer. In addition, at the beginning of budbreak, the grapevines emit young roots, which can actively absorb water and nutrients, such as forms of N [12,13]. However, other studies report that N should preferably be supplied at flowering and within six weeks of flowering [14,15]. More recent studies suggest that N should be applied at two moments, at budbreak and at full bloom [3]. However, it may be appropriate to spread the dose of N over a greater number of periods than those reported, such as berry growth, where the vine shows intense growth of aerial organs, such as leaves, shoots, and bunches, which can increase the demand for N [16,17]. Defining suitable times to supply N is one of the most efficient strategies for increasing the absorption of N from the fertilizer, reducing the potential for losses of N forms, especially nitrate (NO_3^-) [3,18]. This is not sufficiently known in vineyards cultivated in subtropical regions, where rainfall is frequent and in high volumes, which increases the likelihood of N losses through leaching, especially in sandy soils, which can contaminate subsurface waters, or through surface runoff, especially in soils located on undulating terrain, potentially contaminating surface waters [19,20].

In addition, the time of application of the nitrogen fertilizer can determine the amount of N accumulated in annual organs, such as leaves and fruit, and in perennial organs, such as canes, stems, and roots [3,17,21]. At harvest, the N present in bunches is removed from the vineyard [22]. Senescent leaves are deposited on the ground, and during decomposition, the N is released into the soil [23,24]. On the other hand, part of the N applied can be accumulated in stems and canes, which is desirable because part of the N can be redistributed in the next productive vegetative cycle to annual organs with intense growth [17,22]. When this occurs, the amount of N from the fertilizer absorbed tends to be lower, especially when the fertilizer is applied during periods when the plant is starting to emit roots and grow the aerial part, such as the beginning of budbreak. However, in vineyards grown in subtropical climates, the distribution of N fertilizer in annual and perennial organs is still not sufficiently known, especially in vines grown in soils with different characteristics, such as lower and higher organic matter and clay content.

The isotopes of ^{15}N can be used to define with high reliability the best time to apply N and to which organs the N provided by the fertilizer is preferentially directed in peach [18], coffee [25], citrus [26], and even in grapevines [3,16,17]. In this way, this method allows the labeled N that has accumulated in the vine's organs to be traced and quantified, making it possible to know the fate of the N applied to the plants [22]. The present study aimed to evaluate the annual contribution of N from urea when applied at different times to Cabernet Sauvignon grown in sandy and clayey soil in a subtropical climate.

2. Materials and Methods

2.1. Location of Experiments 1 and 2

Two experiments were installed. Experiment 1 was installed in a commercial vineyard on a farm in Santana do Livramento, Rio Grande do Sul (RS), Campanha Gaúcha region, Southern Brazil (Latitude 30°48′31″ S and Longitude 55°22′33″ W). Experiment 2 was set up in a commercial vineyard in Bento Gonçalves, Rio Grande do Sul (RS), Serra Gaúcha region, southern Brazil (Latitude 29°09′44″ S and Longitude 51°31′50″ W) (Figure 1).

Experiment 1 was set up in a vineyard established in 1978. The cultivar was Cabernet Sauvignon, grafted onto SO_4 rootstock. The plants were grown at a density of 1525 plants ha^{-1} (3.5 m × 2.0 m), and the conduction system was espalier. The soil is a Argissolo Vermelho [27], Hapludalf soil [28]. Experiment 2 was set up in a vineyard planted in 1986. The cultivar was Cabernet Sauvignon, grafted onto SO_4 rootstock. The plants were grown at a density of 2666 plants ha^{-1} (1.5 m × 2.5 m). The overhead trellis

system was used. The soil is classified as Neossolo Litólico [27] and Udorthent soil [28]. The physical and chemical characteristics of the soils are shown in Table 1.

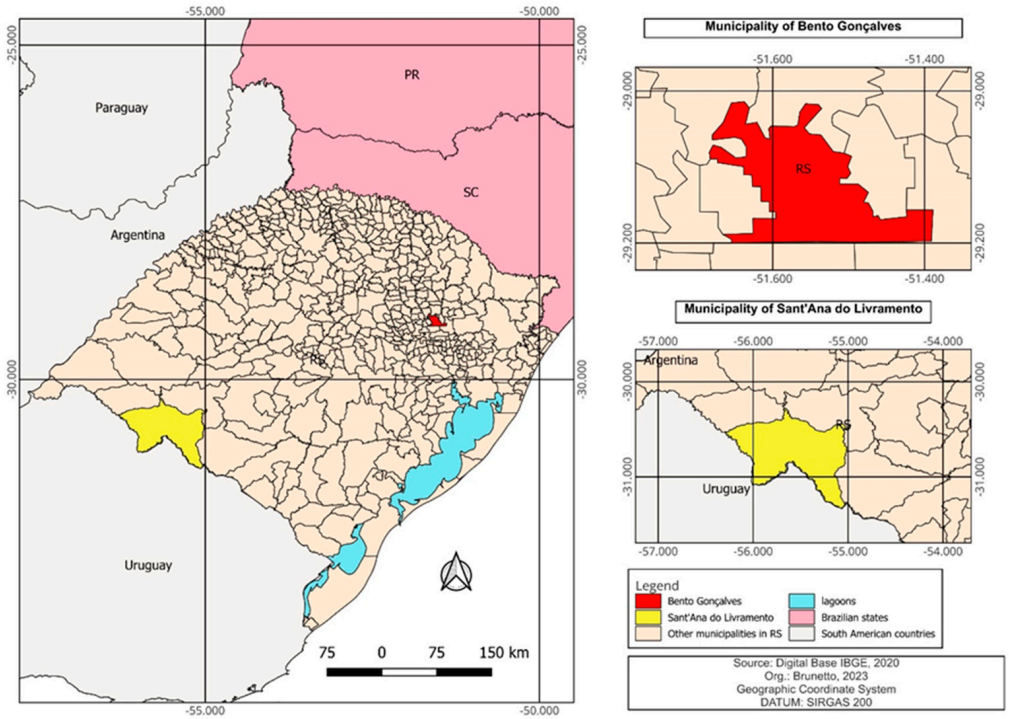

Figure 1. Geographical indication of the cities of Santana do Livramento (Campanha Region) and Bento Gonçalves (Serra Region) in Rio Grande do Sul (RS) state, Brazil.

Table 1. Main physical and chemical characteristics of the soils in the 0–0.20 m deep layer.

Soil Characteristics	Value	
	Experiment 1	Experiment 2
Clay (pipette method) (g kg^{-1})	63	162
Silt (pipette method) (g kg^{-1})	115	558
Sand (granulometric analysis) (g kg^{-1})	822	280
Organic matter (Walkley–Black method) (g kg^{-1})	15.0	29.8
pH in H_20 (1:1 ratio) [29]	5.78	6,93
Exchangeable Al (extractor KCl 1 mL^{-1}) [29] ($cmol_c$ dm^{-3})	0.00	0.00
Exchangeable Mg (extractor KCl 1 mL^{-1}) [29] ($cmol_c$ dm^{-3})	0.82	4.46
Exchangeable Ca (extractor KCl 1 mL^{-1}) [29] ($cmol_c$ dm^{-3})	1.74	7.11
Available P (extractor $Mehlich^{-1}$) (mg dm^{-3})	54	63
Available K (extractor $Mehlich^{-1}$) (mg dm^{-3})	42	110
Total N (Kjeldahl method) [29] (%)	0.04	0.20

The region's climate is humid subtropical, type Cfa, according to the Köppen–Gaiger classification. The climate is characterized by mild temperatures and rainfall with little variation throughout the year. The average annual rainfall is 1100 mm; the average temperature of the warmest month (January) is 22.6 °C, and the average temperature of the coldest month (July) is 11.5 °C [30]. The monthly temperature, rainfall, and radiation data are shown in Table 2.

Table 2. Mean monthly values of rainfall (mm), air temperature (°C), and radiation during the experimental period.

Year/Month	Phenological Stage	Rainfall (mm)	Air Temperature (°C)	Radiation
		Experiment 1—Campanha Gaúcha		
2004				
September	Budbreak	132.8	14.9	149.3
October	Beginning of bloom	155.8	17.9	234.1
November	End of bloom	122.9	20.1	302.6
December	Veraison	147.0	22.6	390.3
2005				
January	Veraison	127.9	24.6	453.5
February	Harvest	167.9	23.2	379.3
		Experiment 2—Serra Gaúcha		
2004				
September	Budbreak	185	14.9	162
October	Beginning of bloom	156	17.0	192
November	End of bloom	140	18.9	219
December	Veraison	144	20.7	239
2005				
January	Veraison	140	21.8	231
February	Harvest	139	21.7	199

2.2. Treatments

In Experiment 1, 30 kg N ha^{-1} was applied, enriched with 3% atoms of ^{15}N in excess, in four ways: 25% at beginning budbreak + 25% at full budbreak + 25% end of bloom + 25% at berries still hard and green (IBBFC); 50% at beginning budbreak + 50% at full budbreak (IBB); 33.33% at full budbreak + 33.33% end of bloom + 33.33% at berries still hard and green (BFC); and 50% end of bloom + 50% at berries still hard and green (FC).

In Experiment 2, 21.42 kg N ha^{-1}, enriched with 3% atoms of ^{15}N in excess, were applied in the same four treatments of Experiment 1, namely, IBBFC, IBB, BFC, and FC. For the N applications, 22 days were considered between the beginning budbreak and full budbreak, 28 days between full budbreak and end of bloom, and 21 days between end of bloom and berries still hard and green.

The doses of N applied are the quantities normally used in vineyards in each of the regions. Before applying the N, the weeds were removed manually in an area measuring 0.50 × 0.50 m (0.25 m^2), with the vine stem in the center of the area. Urea (45% total N) was then applied to the surface of the soil and incorporated manually. Irrigation was carried out shortly afterward to speed up the solubilization of the urea and enhance the migration of the urea solubilization products in the soil profile to reduce the potential for NH_3 volatilization. During the course of the experiments, the 0.25 m^2 area was sprayed with a non-residual herbicide to prevent the presence of spontaneous weeds and their ^{15}N absorption.

The experimental design was randomized blocks with three replications, and each replicate was made up of three plants with an equal number of productive shoots. In addition, three replicates, made up of three vines, did not receive the fertilizer enriched with ^{15}N, and another three plants were left as a border between the treatments. These plants were evaluated to determine the natural abundance of ^{15}N. During the course of the experiments, the vines were subjected to fertilizer application (except N) in accordance with the regional recommendation proposed by the Soil Chemistry and Fertility Commission [31].

2.3. Sampling of Vine Organs and Soil

When the grapes were fully ripe, all the bunches were collected. Ripening was considered complete when a sample of bunches analyzed at random reached 14 to 18 °Brix in the Serra Gaúcha region and 21 to 25 °Brix in Campanha Gaúcha for the Cabernet Sauvignon cultivar. Four bunches were chosen at random from each grapevine. A total of 30 berries were dried in a vacuum oven at a temperature of 80 °C and a pressure of 20 kPa

until constant mass [32]. The remaining berries were removed, and the stalks set aside. The vines were then cut off close to the surface of the ground and separated into leaves, shoots, canes, and stems. The vines were all cut on the same day, in sequence across treatments, for each of the experiments.

The leaves, shoots, canes, stems, and stalks were dried in a forced-air oven at 65 °C until constant mass and weighed to determine dry matter. The leaves, shoots, canes, and stems were then ground in a grinder. Afterward, the samples of leaves, shoots, canes, stems, berries, and stalks were ground in a Willey-type macro and micro mill and prepared for analysis of total N and ^{15}N.

After the vines had been harvested, soil samples were taken from the 0.50 × 0.50 m patch previously individuated per each vine at 0–0.10, 0.10–0.20, and 0.20–0.40 m. The soil was air-dried and macerated in an agate stone grater. The soil was set aside for analysis of total N and ^{15}N [29,33].

2.4. Total N and 15N Analysis

Samples of grapevine organs and soil were analyzed for total N (Nt) and ^{15}N by mass spectrometry (Finnigan MAT® mass spectrometer, Delta Plus model, Bremen, Germany) [29,33].

2.5. Calculations and Statistical Analysis

With the results of the tissue analyses obtained, the atoms of excess ^{15}N, fertilizer-derived N (Ndff), and soil-derived N (Ndfs) were calculated in the parts of the vines and in the soil, according to the procedure described by [34].

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

$$\text{Atom } ^{15}\text{N excess in sample (\%)} = \text{\% atom } ^{15}\text{N in sample} - 0.3663\% \text{ (natural abundance of atmospheric } ^{15}\text{N isotope)} \quad (1)$$

The N derived from fertilizer (%Ndff) was calculated using Equation (2):

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

The N derived from soil (%Ndfs) was calculated using Equation (3):

$$\text{N derived from soil (\%)} = 100 - \text{N derived from fertilizer} \quad (3)$$

The results were subjected to analysis of variance, and when the effects were significant, they were subjected to the mean comparison test using the minimum significant difference test ($p < 0.05$).

3. Results

3.1. Experiment 1—Annual Urea Nitrogen Contribution to the Nutrition of Cabernet Sauvignon Grapevine Grown in Sandy Soil

Among the vine annual organs, the leaves had the highest Nt (Total N) values, followed by the stalk, at all times of N application (IBBFC, IBB, BFC, and FC), with a significant difference between the seasons, with a higher percentage of recovery in the BFC season, where the leaves showed approximately 9% more Nt when compared to the application in the IBBFC seasons, which showed the lowest percentage of Nt (2.23%). Between the two types of perennial organs, the previous canes had more Nt values at all N application times (Table 3).

The highest values of excess ^{15}N atoms were found in leaves at all times of N application when compared to the other organs. However, the IBB application times showed the highest recovery percentages in leaves, followed by the shoots and berries, where they exceeded the recovery percentage obtained when N was applied in the BFC times for leaves and shoots and FC for berries by 120.3%, 124.2% and 143.4%, respectively (Table 3). The lower Nt values in the stem, compared to the values observed in the canes, indicate that the organ is the flow of N to the growing organs. However, the values of excess ^{15}N atoms

in leaves did not differ statistically from the values observed in shoots, canes, and stems when N was applied in IBBFC (Table 3).

Table 3. Total N, atom% ^{15}N excess, N derived from fertilizer (%Ndff), and N derived from soil (%Ndfs), in parts of Cabernet Sauvignon grapevine after application of 21.42 kg N ha^{-1}.

Treatment	Leaves	Shoot	Berries	Stalk	Cane	Stem	CV (%)
			Total N (%)				
IBBFC	2.23 Ab *	0.47 Dc	0.81 Cb	1.62 Bb	0.38 Dns	0.33 Da	24.38
IBB	2.30 Ab	0.43 Dc	0.91 Cab	1.76 Bb	0.36 D	0.26 Db	18.51
BFC	2.43 ABa	0.52 Db	0.94 Ca	2.13 Ba	0.38 E	0.31 Fab	4.91
FC	2.25 Ab	0.59 Da	0.96 Ca	1.79 Bb	0.42 D	0.33 Ea	15.71
CV(%)	3.60	6.64	5.52	11.54	17.85	14.56	
			Atom% ^{15}N excess				
IBBFC	0.1458 Ab	0.1297 Ab	0.1450 Ab	0.1542 Aa	0.0514 Bb	0.0419 Bb	20.44
IBB	0.2383 Aa	0.2001 Ca	0.2134 Ba	0.1911 Ca	0.0912 Da	0.0970 Da	6.35
BFC	0.1082 Ab	0.0894 Bb	0.1048 ABbc	0.0780 Cb	0.0249 Dc	0.0278 Db	19.97
FC	0.1453 Ab	0.1170 Bb	0.0877 Cc	0.0994 BCb	0.0583 Db	0.0495 Db	19.97
CV(%)	20.32	25.51	24.56	20.86	26.66	26.74	
			%Ndff				
IBBFC	4.86 Ab	4.32 Ab	4.83 Ab	5.14 Ab	1.71 Bb	1.39 Bb	20.45
IBB	7.94 Aa	6.67 Ca	7.11 Ba	6.37 Ca	3.04 Da	3.23 Da	6.36
BFC	3.61 Ab	2.98 Bb	3.49 ABbc	2.60 Cb	0.83 Dc	0.93 Db	19.97
FC	4.84 Ab	3.93 Bb	2.92 Cc	3.32 BCb	1.94 Db	1.65 Db	19.05
CV(%)	20.30	25.49	24.57	20.85	26.63	26.77	
			%Ndfs				
IBBFC	95.14 Ba	95.67 Ba	95.17 Bb	94.86 Bb	98.29 Ab	98.60 Aa	0.78
IBB	92.05 Db	93.33 Bb	92.89 Cc	93.63 Bb	96.96 Ac	96.77 Aa	0.39
BFC	96.39 Da	97.02 BCa	96.51 Cab	97.40 Ba	99.17 Aa	99.07 Aa	0.49
FC	95.16 Da	96.09 Ca	97.07 Ba	96.69 BCa	98.06 ABb	98.35 Aa	0.60
CV(%)	1.70	1.19	1.18	1.41	0.51	0.67	

(*) Mean values followed by the same letter, lower case between N application times and upper case between plant parts, do not differ among minimum significant difference (MSD) test at 5% probability. IBBFC: 25% beginning of budbreak + 25% full budbreak + 25% end of bloom + 25% berries still hard and green; IBB: 50% beginning of budbreak + 50% full budbreak; BFC: 33.33% full budbreak + 33.33% end of bloom + 33.33% berries still hard and green; FC: 50% end of bloom + 50% berries still hard and green. Ndff: N derived from fertilizer; Ndfs: N derived from soil. CV = coefficient of variation. ns: effect not significant.

The highest Ndff values were observed in the annual organs (leaves, berries, stems, shoots), especially in the leaves, when N was applied to IBBFC, IBB, and FC, with a significant percentage difference of 7.9% in relation to the berries for the IBB seasons and 23.2% in relation to the branches of the year when applied in FC, with no significant difference between the annual organs for the IBBFC harvest. However, when N was applied in BFC, the highest Ndff values were found in the stalk. The berries showed the second highest Ndff value when N was applied in IBBFC, IBB, and BFC. The stem and shoots showed the highest Ndff values when N was applied in IBB, with 3.23% and 3.04%, respectively, corresponding on average to 151% less than the percentage found in the leaves, the organ that showed the highest recovery of N derived from the fertilizer (Table 3). We point out that most of the Ndff was observed in annual organs, but the values did not exceed 8% (Table 3).

The highest Ndfs values were observed in the stem when N was applied in IBBFC (98.60%) and FC (98.35%) (Table 3). However, when N was applied in IBB (96.96%) and BFC (99.17%), the highest Ndfs values were found in the cane, with no statistical difference between these two perennial organs in all the treatments (Table 3).

The Ndff was accumulated preferentially in annual organs (leaves, shoots, berries, and stalks) at all times of N application (Figure 2) rather than in perennial parts, such as the previous canes and stems.

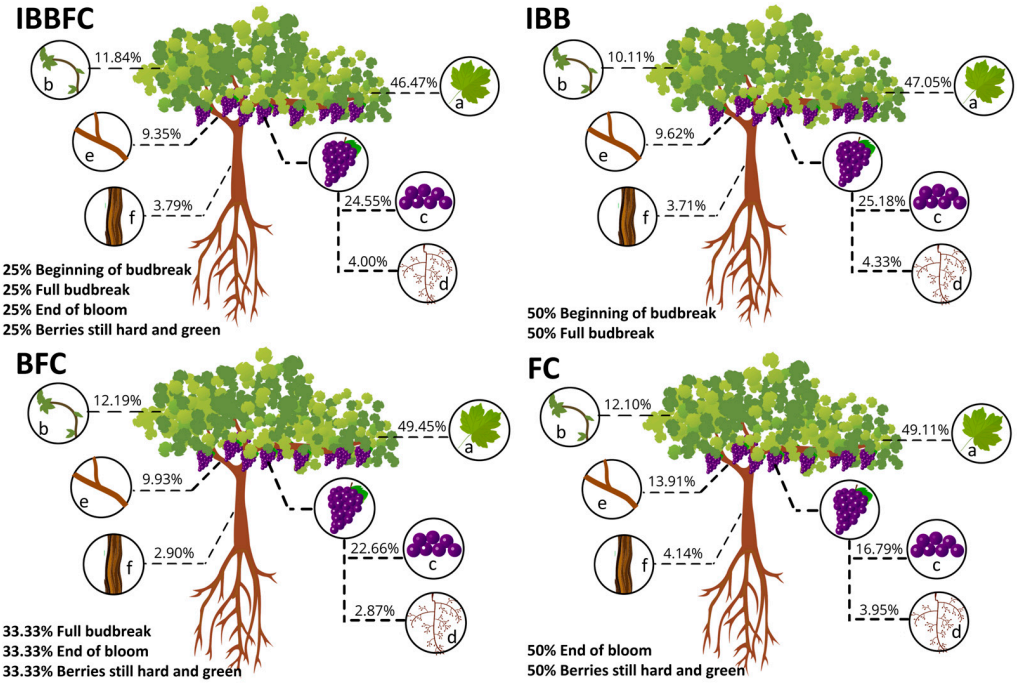

Figure 2. Percentage distribution of fertilizer-derived nitrogen (Ndff) in leaves (a), shoot (b), berries (c), stalk (d), cane (e), and stem (f) in Cabernet Sauvignon vines subjected to the application of 21.42 kg N ha^{-1}. IBBFC: 25% beginning of budbreak + 25% full budbreak + 25% end of bloom + 25% berries still hard and green; IBB: 50% beginning of budbreak + 50% full budbreak; BFC: 33.33% full budbreak + 33.33% end of bloom + 33.33% berries still hard and green; FC: 50% end of bloom + 50% berries still hard and green.

The highest soil Nt values were observed in the 0–0.10 m layer of soil when N was applied in IBBFC, BBF, and BFC. When N was applied in FC, the highest total N values were observed in the 0.10–0.20 and 0.20–0.40 m layers (0.50%). The highest values of ^{15}N atoms and Ndff (%) were observed in the 0–0.10 m layer when N was applied in IBBFC, IBB, BFC, and FC (Table 4).

Table 4. Total N (%), atom% ^{15}N excess, and N derived from fertilizer (%Ndff) measured in soil with Cabernet Sauvignon grapevine after application of 21.42 kg N ha^{-1}.

Treatment	Layer (m)			CV (%)
	0–0.10	0.10–0.20	0.20–0.40	
		Total N (%)		
IBBFC	0.07 a [a]	0.05 a	0.04 b	19.64
IBB	0.07 a	0.05 b	0.05 b	20.16
BFC	0.11 a	0.04 b	0.05 b	17.58
FC	0.11 a	0.50 b	0.50 b	23.30
		Atom% ^{15}N excess		
IBBFC	0.2396 a	0.0808 b	0.0565 c	8.58
IBB	0.1555 a	0.0519 b	0.0763 c	15.51
BFC	0.2203 a	0.0560 c	0.1491 b	23.89
FC	0.1598 a	0.0862 b	0.0732 b	21.26
		%Ndff		
IBBFC	7.98 a	2.69 b	1.88 c	8.59
IBB	5.18 a	1.73 b	2.54 b	15.47
BFC	7.34 a	1.86 c	4.97 b	23.86
FC	5.33 a	2.88 b	2.44 b	21.29

[a] Within the line mean values followed by the same lowercase in the line do not differ among them according to the minimum significant difference (MSD) test at 5% probability. IBBFC: 25% beginning of budbreak + 25% full budbreak + 25% end of bloom + 25% berries still hard and green; IBB: 50% beginning of budbreak + 50% full budbreak; BFC: 33.33% full budbreak + 33.33% end of bloom + 33.33% berries still hard and green; FC: 50% end of bloom + 50% berries still hard and green. Ndff: N derived from fertilizer. CV = coefficient of variation.

3.2. Experiment 2—Annual Urea Nitrogen Contribution to the Nutrition of Cabernet Sauvignon Grapevine Grown in Clayey Soil

The leaves, among the annual organs, showed the highest Nt values when N was applied to IBBFC, IBB, BFC, and FC (Table 5). When we look at the effect of N application at different times, for the leaves, recovery is greatest in the IBB season (1.79%), with no statistically significant difference from the IBBFC (1.71%) and FC (1.74%) seasons. The berries were the second annual organ to show the highest Nt values when N was applied in IBBFC (0.76%) and FC (0.76%). However, it recovered on average 127% less than the leaves. The stalk was the second annual organ to show the highest Nt values when N was applied in IBB and BFC (Table 5).

Table 5. Total N, atom% ^{15}N excess, N derived from fertilizer (%Ndff), and N derived from soil (%Ndfs) in parts of Cabernet Sauvignon grapevine after application of 30 kg N ha^{-1}.

Treatment	Leaves	Shoots	Berries	Stalks	Canes	Stems	CV (%)
			Total N (%)				
IBBFC	1.71 Aab *	0.45 Da	0.76 Ba	0.67 Cb	0.38 Eb	0.33 Ea	8.50
IBB	1.79 Aa	0.46 Da	0.53 Cb	0.62 Bb	0.39 Eab	0.29 Eb	7.56
BFC	1.68 ABb	0.40 Db	0.49 Cb	0.54 Bc	0.41 Dab	0.28 Eb	4.60
FC	1.74 Aab	0.47 Ca	0.76 Ba	0.84 Ba	0.42 Ca	0.28 Db	10.41
CV (%)	3.82	5.33	10.67	7.58	5.61	8.33	
			Atom% ^{15}N excess				
IBBFC	0.1678 Ab	0.1562 Ab	0.1549 Ans	0.1398 Ab	0.0551 Bb	0.0502 Ba	24.09
IBB	0.2179 Aa	0.1953 Aa	0.1735 A	0.2064 Aa	0.0732 Ba	0.0510 Ba	25.65
BFC	0.0577 ABc	0.0526 ABc	0.0989 A	0.0457 Bc	0.0272 Bc	0.0114 Bb	22.67
FC	0.1503 Ab	0.1380 Ab	0.1237 AB	0.1021 Bb	0.0738 Ca	0.0538 Ca	21.03
CV (%)	22.69	19.19	25.56	25.41	16.49	29.42	
			%Ndff				
IBBFC	5.59 Ab	5.21 Ab	5.16 Ans	4.66 Ab	1.84 Bb	1.67 Ba	24.07
IBB	7.26 Aa	6.51 Aa	5.78 A	6.88 Aa	2.44 Ba	1.70 Ba	25.64
BFC	1.92 ABc	1.76 ABc	3.29 A	1.53 Bc	0.91 Bc	0.38 Bb	22.67
FC	5.01 Ab	4.60 Ab	4.12 AB	3.40 Bb	2.46 Ca	1.79 Ca	21.02
CV (%)	22.71	19.18	25.55	25.42	16.46	29.45	
			%Ndfs				
IBBFC	94.41 Bb	94.79 Bb	94.84 Bns	95.34 Bb	98.16 Ab	98.33 Ab	1.00
IBB	92.74 Bc	93.49 Bc	94.22 B	93.11 Bc	97.56 Ac	98.29 Ab	1.91
BFC	98.08 ABa	98.24 ABa	96.70 B	98.47 Aa	99.09 Aa	99.62 Aa	1.37
FC	94.99 Cb	95.39 Cb	95.88 BC	96.59 Bb	97.54 Ac	98.21 Ab	0.77
CV (%)	1.18	0.90	2.67	1.52	0.32	0.41	

(*) Mean values followed by the same letter, lower case between N application times and upper case between plant parts, do not differ among minimum significant difference (MSD) test at 5% probability. IBBFC: 25% beginning of budbreak + 25% full budbreak + 25% end of bloom + 25% berries still hard and green; IBB: 50% beginning of budbreak + 50% full budbreak; BFC: 33.33% full budbreak + 33.33% end of bloom + 33.33% berries still hard and green; FC: 50% end of bloom + 50% berries still hard and green. Ndff: N derived from fertilizer; Ndfs: N derived from soil. CV = coefficient of variation. ns: effect not significant.

The highest values of excess ^{15}N atoms were found in leaves when N was applied to IBBFC, IBB, and FC (Table 5), with a statistical difference between the seasons, where application in IBB was 190.6% higher than application in IBBFC, which had the second highest value of excess ^{15}N atoms. However, when N was applied in BFC, the highest values of excess ^{15}N atoms were observed in berries (0.989%), but there was no statistical difference between the application times. The shoots, among the perennial organs, showed the highest values of ^{15}N atoms in excess, but without differing statistically from the values found in the stem, at all times of N supply (Table 5), with the IBB and FC seasons showing the highest percentages of excess ^{15}N atoms for both perennial organs.

The highest Ndff values were observed in leaves when N was applied to IBBFC, IBB, and FC, with a significant difference between the application times, with the highest percentage achieved with the application of N in IBB (7.26%). However, when the N dose

was applied in BFC, the highest Ndff values were observed in berries (3.29%), 71.5% higher than the percentage obtained in the leaves. The shoots showed the second highest Ndff value when N was applied in IBBFC, IBB, and FC (Table 5).

The highest Ndfs values were observed in the perennial organs (canes and stem) when N was applied in the IBBFC, IBB, BFC, and FC phenological stages. However, if we look at the BFC treatment, the stalk and annual organ did not differ statistically from the highest values found for the perennial organs (Table 5). This showed that the annual plant organs tend to recover the N derived from the fertilizer (Table 5).

Inside the vine, the Ndff was distributed especially to annual organs (leaves, shoots, berries, and stems) (Figure 3). The percentages of Ndff in perennial organs (canes and stems) were small.

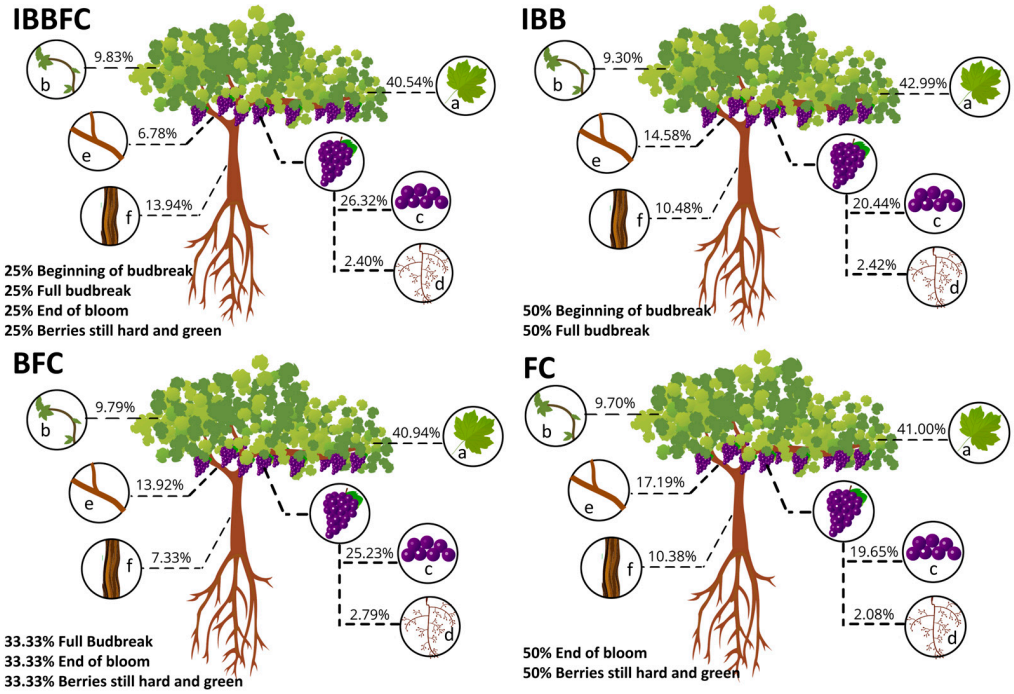

Figure 3. Percentage distribution of fertilizer-derived nitrogen (Ndff) in leaves (a), shoot (b), berries (c), stalk (d), cane (e), and stem (f) in Cabernet Sauvignon vines subjected to the application of 30 kg N ha^{-1} at different times. IBBFC: 25% beginning of budbreak + 25% full budbreak + 25% end of bloom + 25% berries still hard and green; IBB: 50% beginning of budbreak + 50% full budbreak; BFC: 33.33% full budbreak + 33.33% end of bloom + 33.33% berries still hard and green; FC: 50% end of bloom + 50% berries still hard and green.

The highest values of total N, excess atoms of ^{15}N, and Ndff were observed in the soil layer 0–0.10 m deep at all times of N application (Table 6).

Table 6. Total N (Nt), atom% ^{15}N excess, and N derived from fertilizer (%Ndff) measured in soil with Cabernet Sauvignon grapevine after application of 30 kg N ha^{-1}.

Treatment	Layer (m)			CV (%)
	0–0.10	0.10–0.20	0.20–0.40	
	Total N (%)			
IBBFC	0.14 a [a]	0.13 a	0.08 b	19.56
IBB	0.18 a	0.11 b	0.10 b	22.74
BFC	0.22 a	0.12 b	0.10 b	23.97
FC	0.23 a	0.11 b	0.12 b	10.42

Table 6. *Cont.*

Treatment	Layer (m)			CV (%)
	0–0.10	0.10–0.20	0.20–0.40	
		Atom% ^{15}N excess		
IBBFC	0.0957 a	0.0305 b	0.0091 b	23.23
IBB	0.0447 a	0.0127 b	0.0047 c	15.18
BFC	0.1302 a	0.0259 b	0.0052 c	15.54
FC	0.1702 a	0.0125 b	0.0026 b	20.80
		%Ndff		
IBBFC	3.19 a	1.02 b	0.30 b	23.20
IBB	1.49 a	0.42 b	0.16 c	15.47
BFC	4.34 a	0.87 b	0.18 c	15.54
FC	5.68 a	0.42 b	0.09 b	20.77

[(a)] Within the line, mean values followed by the same lowercase in the line do not differ among them according to the minimum significant difference (MSD) test at 5% probability. IBBFC: 25% beginning of budbreak + 25% full budbreak + 25% end of bloom + 25% berries still hard and green; IBB: 50% beginning of budbreak + 50% full budbreak; BFC: 33.33% full budbreak + 33.33% end of bloom + 33.33% berries still hard and green; FC: 50% end of bloom + 50% berries still hard and green. Ndff: N derived from fertilizer. CV = coefficient of variation.

4. Discussion

The highest percentages of total N, atoms of excess ^{15}N and Ndff, were observed in annual organs, especially in leaves, berries, and shoots, at all N application times (IBBFC, IBB, BFC, and FC) and sites (Experiments 1 and 2). This may have been because annual organs such as leaves, bunches, and shoots undergo intense cell division and elongation throughout the vine's vegetative and productive cycle, which can increase dry matter production and the demand for nutrients such as N [3,22]. N is a primary nutrient that is highly required and absorbed by grapevines [22]. In addition, throughout the vegetative and productive cycle, vines can emit new roots, which can absorb water and forms of mineral N from the soil. Part of the N can be incorporated into carbon chains, which can be distributed to growing organs such as leaves and bunches [3,18].

The lowest percentages of Ndff and ^{15}N atoms in excess were found in perennial organs (stems and canes) at all times of N application for both sites (Experiments 1 and 2). This may be because these organs have fewer active cells compared to annual organs. They can act as a "channel" for directing and distributing N to annual organs [3,4]. On the other hand, sometimes perennial organs, such as stems and canes, can have little active tissue, sometimes necrotic, which hinders the transport, accumulation, and redistribution of N derived from the fertilizer, which can be absorbed in the same year as the nitrogen fertilizer is applied to the soil [15,17]. It is desirable for perennial organs to behave as accumulators of N, including that derived from fertilizer, because part of the N can be redistributed to other organs in the next vegetative and productive cycle [35], reducing dependence on the absorption of N applied to the soil in the same year. This can reduce the dose or frequency of nitrogen fertilizer application in vineyards [3,17]. This is desirable because it reduces the cost of purchasing fertilizers and the potential for soil and water contamination in vineyards, especially as nitrate (NO_3^-), which has low binding energy with functional groups of reactive soil particles and, consequently, has high mobility in the soil profile [3,18].

The vines subjected to N application in IBB in Experiment 1 had higher values of ^{15}N atoms in excess and Ndff when considering all plant organs (annuals and perennials). This greater absorption of N from the fertilizer may have occurred because the fertilizer dose was applied at budbreak and split in two moments, 50% of the dose at the start of budbreak and 50% during budbreak. As a result, a greater amount of N was applied in a shorter time, which can increase the concentration of N forms in the solution, which enhances their approach to the outer surface of the roots, increasing the likelihood of N absorption from the fertilizer [35]. In addition, during budbreak and in periods close to it, grapevines can absorb a greater amount of N, including that from the fertilizer [3,36], because mild

temperatures and suitable soil moisture conditions favor the availability and absorption of forms of N by the plants [22,37]. Thus, it can be inferred that the application of N shortly after budbreak and during this phenological stage is an appropriate strategy for Cabernet Sauvignon grapevines grown in sandy soil.

The lower total values of Ndff and atoms of ^{15}N in excess in grapevines subjected to N application in BFC in both experiments, when considering all the organs evaluated, may have been because N was applied in a greater number of periods compared to the other treatments. Thus, the amount of N applied in each season was lower, which can increase the concentration of N in the solution slightly, reducing the amount of N approaching the root system and, consequently, N absorption [17]. The amount absorbed can also be reduced if the rootstock or the cultivar values of the uptake kinetic parameters (e.g., K_m, C_{min}, V_{max}, and Influx) confer low N absorption efficiency [12]. Also, part of the N applied in BFC may have been lost through leaching, surface runoff, and volatilization [3,19,38]. We also point out that N applied at a greater number of moments throughout the vine cycle can stimulate the activity of the soil's microbial biomass, increasing the mineralization of organic N in the soil [22]. This will increase the amount of mineral N forms native to the soil present in the solution, which can reach the root system in greater concentration than the N derived from the fertilizer [13,38].

In the grapevines grown in the sandy soil (Experiment 1), especially when N was applied in IBB, the Ndff values were higher than those observed in the plants grown in the clayey soil (Experiment 2). This may have been because the dose of N applied in Experiment 1 was higher (30 kg N ha^{-1}). But also, in sandy soils, the organic matter and clay contents are lower, which decreases the complexation of N from the fertilizer in the organic matter or adsorption on functional groups of reactive inorganic particles [6,22]. Thus, greater availability of mineral forms of N to plants is expected [5,36], which can be absorbed, especially during periods of greater demand for the nutrient (Budbreak). We would also point out that the vines in Experiment 1 are older, which may indicate a greater demand for N, as can be seen from the higher values of Nt, Ndff, and excess ^{15}N atoms. On the other hand, in Experiment 2, the soil has a higher organic matter content, which hypothetically leads to greater mineralization of organic N, with an increase in the availability of inorganic forms of N native to the soil [38], partly explaining the lower recovered values of Nt, Ndff, and excess ^{15}N atoms in the grapevines.

We highlight that the Ndff in grapevines, at all times of N application and in both experiments, did not exceed 8%, with the highest values being observed in annual organs. This clearly indicates that most of the N present in grapevines is derived from other sources, such as the mineralization of organic N present in the soil's organic matter or the decomposition of plant residues present in the soil (for example, cover crop residues—aerial part and senescent roots; grapevine residues—leaves, pruned shoots, and senescent roots), or even derived from reserve organs such as roots [17,22,35]. However, it is worth noting that even though the value of Ndff in grapevines was small, it was allocated preferentially in the leaves when N was applied at IBB and BFC. Thus, the N applied during the year is preferentially absorbed and directed toward leaf growth.

In the soil, at all times of N application, the highest values of excess ^{15}N atoms and Ndff were observed in the 0–0.10 m layer. This may be because part of the N applied (enriched with ^{15}N) replaces the unmarked inorganic N in the soil, adsorbed to the functional groups of reactive inorganic soil particles, or incorporated into the microbial biomass or organic compounds in the soil [38,39]. As a result, part of the inorganic N derived from the mineralization of organic matter is made available to the soil solution and can then be absorbed by the vines [3,40]. This may partly explain the high percentages of Ndfs observed in all the organs of the vines grown in Experiments 1 and 2.

5. Conclusions

The N applied at different times in sandy and clayey soil after absorption by roots was preferentially directed to annual organs of Cabernet Sauvignon, especially to leaves.

Cabernet Sauvignon vines grown in clayey soil absorbed N derived from fertilizer preferentially when 50% was applied at beginning of budbreak + 50% at full budbreak (IBB).

Cabernet Sauvignon vines grown in sandy soil absorbed N derived from fertilizer preferentially when 50% was applied at beginning of budbreak + 50% at full budbreak (IBB).

The N present in annual organs (leaves, berries, stalks, and shoots) and perennial organs (canes and stems) of Cabernet Sauvignon grown in sandy and clayey soil was derived especially from the soil.

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