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Impact of application of urea modes and rates on yield and nitrous oxide emissions in grapevine (*Vitis vinifera L.*) on sandy soils in subtropical climate

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Summary

Grapevines subjected to applications of nitrogen (N) doses on the soil surface can use only a small amount of the nutrient, probably because of losses, such as nitrous oxide (N_2O) emissions to the atmosphere. An alternative to reduce these N losses may be the application of N via fertigation. The study aimed to evaluate the N_2O emissions and grape yield (*Vitis vinifera* L.), in grapevines submitted to the application of modes and doses of N cultivated in sandy soil in a subtropical climate. 'Alicante Bouschet' grapevines were subjected to a factorial scheme with three N rates: 0, 40 and 80 kg N ha^{-1} year^{-1}; and two application modes: surface (NS) and via fertigation (NF). Evaluations of N_2O emissions and ammonium (NH_4^+) and nitrate (NO_3^-) contents in the soil, N concentration in leaves, grape yield, and number of clusters per plant were carried out. Grapevines cultivated with applications of 40 and 80 kg N ha^{-1} yr^{-1}, in NF and NS modes, respectively, presented higher N_2O emissions. N_2O emission peaks occurred in the first 9 days after N application. Cumulative N_2O emissions ranged from 161.74 ± 34.67 to 496.18 ± 37.00 g ha^{-1} of N_2O-N, in soils that received 0 and 40 kg N ha^{-1} yr^{-1}, respectively, both in NF mode. Accumulated N_2O emissions had a linearly positive relation with the mineral N content in the soil (NH_4^+ and NO_3^-) and these had a negative relation with grape yield.

Keywords

N2O emissions, Nitrogen fertilization, Sandy soil, Denitrification, fertigation, viticulture

Introduction

Viticulture and enology play a strong role in the world economy (Fraga, 2019). Brazil is one of the most important grape-producing countries in South America, having more than 79,000 hectares cultivated with grapevine, producing 1,912,034 tons of grapes in 2017 (IBGE, 2020). Grapevines can be grown worldwide in sandy soils, with low levels of soil organic matter (SOM) and nitrogen (N) (Brunetto *et al.*, 2014, 2016; Lorensini *et al.*, 2017) but nitrogen fertilizers must be applied to supply the N demand by grapevines, enhancing the grape yield (Fig. 1) (Stefanello *et al.*, 2020). Urea is the most used nitrogen mineral fertilizer in the world, for it has the lowest cost per unit of N but with a high content in its composition and, also, ease of transport and storage (Glibert *et al.*, 2006; Guardia *et al.*, 2018). In grapevine cultivation in Brazil, the application of urea is normally on the soil surface (Brunetto *et al.*, 2016) which can lead to ammonia emissions (NH_3) due to the contribution to the increase in availability of N in the soil, as nitrate (NO_3^-) and ammonium (NH_4^+) (Lorensini *et al.*, 2017). The NH_4^+ made available by urea can follow three routes: (i) be absorbed by plant roots; (ii) react with OH^-, which may cause NH_3 volatilization to the atmosphere; and (iii) undergo a nitrification process by the biological oxidation of NH_4^+ to nitrite (NO_2^-) and then to NO_3^- (Brunetto *et al.*, 2016; Silva *et al.*, 2019). Nitrate in the soil can also follow three paths: (i) absorption by plants; (ii) leaching loss, especially in sandy textured soils (Barlow *et al.*, 2009; Lorensini *et al.*, 2012); or (iii) loss by denitrification, by the transformation of NO_3^- into gases such as nitrogen (N_2), nitric oxide (NO), or nitrous oxide (N_2O), through denitrifying bacteria when in anaerobic environments (Caranto and Lancaster, 2017; Guardia *et al.*, 2018; Hallin *et al.*, 2018). N_2O is a potent greenhouse gas (GHG) with a global warming potential about 298 times greater than carbon dioxide (CO_2) (IPCC, 2014). In the stratosphere, N_2O is the most aggressive gas for ozone layer depletion (Guardia *et al.*, 2018). Most anthropogenic N_2O emissions result from the intensification of agriculture with the increasing use of nitrogen fertilizers that stimulate microbial processes of nitrification and denitrification (Butterbach-Bahl

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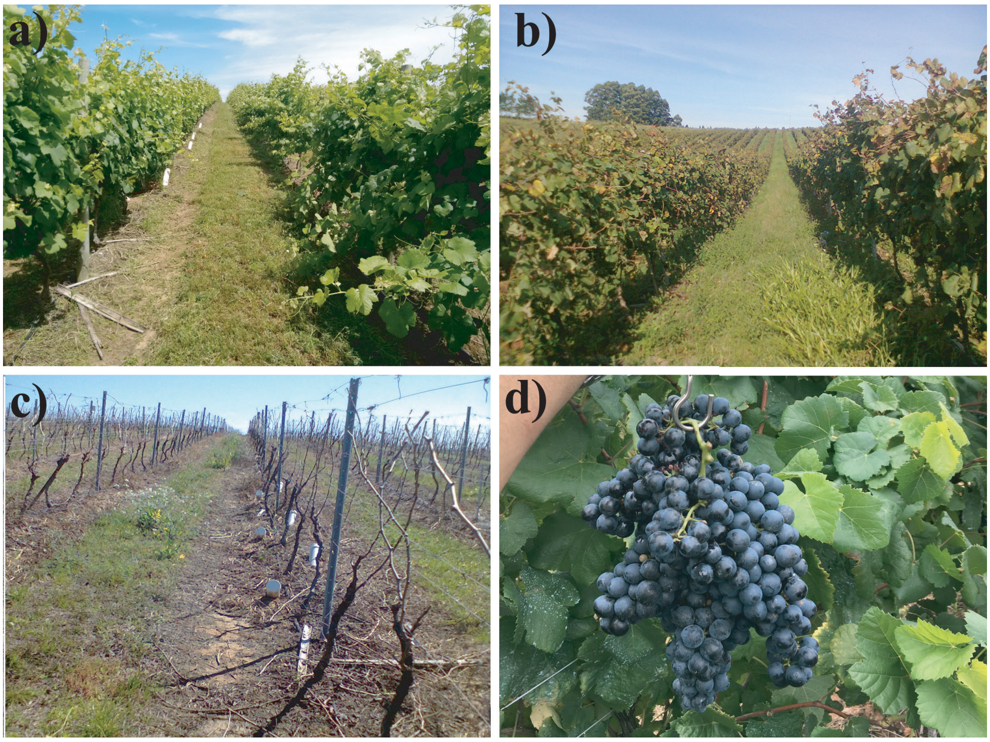

Fig. 1: Commercial vineyard in southern Brazil on sandy soil under subtropical climate during phenological stages of flowering (a); color of the grape berries/veraison (b); leaf senescence/vegetative rest (c); and high-yield grape bunch (d) of grapevines submitted to application of urea modes and doses. Photograph by Matheus S. Kulmann and Lincon O. Stefanello.

et al., 2013; Cowan *et al.*, 2020). In addition, edaphoclimatic conditions, such as temperature, precipitation, air velocity, relative humidity of soil and air, SOM content, soil pH value, and concentration of NO_3^- and NH_4^+ in the soil, are favorable environmental conditions for the action of denitrifying microorganisms (Jensen *et al.*, 2011). It was estimated that agriculture contributes to 37% of the emission of anthropogenic N_2O in Brazil (Brasil, 2014) and 60-70% globally (Tian *et al.*, 2019). This suggests that practices of application of nitrogen fertilizers by winegrowers, such as modes and doses of N applied, can influence the availability of NO_3^- and NH_4^+ in soil and, consequently, the N_2O soil emissions to the atmosphere. Thus, it was necessary to adopt alternative ways of supplying N doses that reduce N_2O-N losses and contribute to N uptake by grapevine plants, reflecting an increase in grape productivity (Fig. 1d). Fertigation promotes the solubilization and hydrolysis reaction of urea, contributing to the migration of NO_3^- and NH_4^+ in the soil profile (Comas *et al.*, 2010; Stefanello *et al.*, 2020). Losses of N in the form of N_2O should be assessed regionally to improve inventories on GHG emissions. However, data on N_2O emissions in vineyards around the world are scarce (Carlisle *et al.*, 2010; Fentabil *et al.*, 2016; Steenwerth and Belina, 2010) and, as to our knowledge, there is none on grapevines under subtropical climate. The study aimed to evaluate the productivity of *Vitis vinifera* L. vines subject to different application modes and doses of mineral N in a sandy soil under a subtropical climate and the respective N_2O emissions.

Material and Methods

Description of study and experimental area

The study was carried out in a vineyard in Santana do Livramento (30° 48′ 31″ S; 55° 22′ 33″ W), State of Rio Grande do Sul, Southern Brazil. The sandy soil was classified as a Typical Hapludalf (Soil Survey Staff, 2014). Main chemical properties of the soil from the 0.0-0.10 m layer, prior to the installation of the experiment are presented in Table 1. The climate in the region was humid subtropical, type Cfa, according to Köppen's classification, characterized by mild temperatures and rain, with little variation throughout the year. The average annual rainfall was 1,600 mm. The average temperature in the hottest month (January) was 23.8 °C and in the coldest month (July) was 12.4 °C (Alvares *et al.*, 2013). The annual sunshine duration was approximately 2,500 hours and the relief of the area was gently undulating. The average values of air temperature and monthly precipitation accumulated during the study period are shown in Fig. 2. Meteorological data were obtained by the meteorological station of the National Institute of Meteorology (INMET). The vineyard was established in 2011 with the cultivar 'Alicante Bouschet' (*Vitis vinifera* L.), grafted onto Paulsen 1103 rootstock. Planting density was 2.976 plants hectare^{-1} (2.8 m between rows × 1.2 m between plants), conducted in espalier system. The inter-row spacing is covered by native grasses, such as *Paspalum notatum*, *Paspalum plicatulum*, and an exotic grass, such as *Lolium multiflorum*. Periodic application of a non-selective herbicide with systemic action (*i.e.* glyphosate) was carried out to maintain the planting line without cover crops. The cover crops in the inter-row spacing were mowed five times per season and their residues were deposited on the soil surface. In 2011, before the installation of the experiment, the vines were submitted to annual applications of 45 kg P_2O_5 ha^{-1} year^{-1} and 45 kg K_2O ha^{-1} year^{-1}, for four years (CQFS-RS/SC, 2016). The source of P was triple superphosphate (42% P) and the source of K was potassium chloride (60% K). Nitrogen fertilizers were applied annually according to the treatments.

Table 1: Chemical characteristics of soil in experimental site at 0-0.10 m soil layer of the treatments: (NS – Nitrogen surface; and NF – Nitrogen fertigation; 0, 40 and 80 kg N ha^{-1} $year^{-1}$).

Property	0 NS	0 NF	40 NS	40 NF	80 NS	80 NF
Total N (g kg^{-1})	1.6 ± 0.0	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.0	2.0 ± 0.1
TOC (g kg^{-1})	6.5 ± 0.1	7.2 ± 0.1	7.8 ± 0.2	8.5 ± 0.3	11.4 ± 0.1	8.4 ± 0.0
pH in water (1:1 ratio)	5.7 ± 0.0	5.5 ± 0.0	5.7 ± 0.1	6.0 ± 0.2	5.7 ± 0.0	5.3 ± 0.1
Available P [(1)] (mg kg^{-1})	15.9 ± 3.1	28.4 ± 4.8	21. 2 ± 2.1	30.6 ± 3.0	27.2 ± 2.8	17.2 ± 0.7
Exchangeable K [(1)] (mg kg^{-1})	67.0 ± 8.1	78.0 ± 13.2	112.2 ± 13.5	187.2 ± 20.0	94.0 ± 16.8	96.5 ± 15.9
Exchangeable Ca [(2)] (cmolc kg^{-1})	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.01	0.9 ± 0.1	0.8 ± 0.1	0.6 ± 0.1
Exchangeable Mg [(2)] (cmolc kg^{-1})	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.3 ± 0.1
Density	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1

[(1)] Extracted by Mehlich-1; [(2)] Extracted by KCl 1 mol L^{-1}. The values are presented as mean ± standard deviation (n = 3).

Experimental design and treatments

In September 2014, 18 plots were demarcated consisting of five plants each. The experimental design was randomized blocks, in a 2 × 3 factorial scheme, with three replications. Each repetition was composed of five plants, the three central plants of each repetition being evaluated. The first factor was the way in which N was applied: N applied to the soil surface (NS) and N applied via fertigation (NF). The second factor was dose of N: 0, 40 and 80 kg N ha^{-1} $year^{-1}$. The N source used was urea (45% N). Due to the residual effect of N in the previous harvest, in this study, we presented parameters referring to 2016/17, 2017/18, and 2018/19 harvests. The applications of N in the NS mode were carried out on the soil surface without incorporation in the projection of the plant canopy,. At the stage of full bloom (Brunetto *et al.*, 2009) the doses of N were applied annually in a single dose. In NF mode, N doses were applied from the solubilization of urea in water in a cistern and followed the procedure below: (a) 10 minutes of water slide only; (b) 10 minutes of water + N-urea; and (c) 10 minutes of water slide only. The solution containing N was applied via drippers (Netafim™ 132 Dripnet PC AS 16250) to the projection of the canopy of the plants, spaced at 0.6 m, with a flow of 1.6 L h^{-1}.

Measurements of N_2O fluxes

In the period between the application of N (November 2018) and the grape harvest (February 2019), soil N_2O fluxes were measured by the static chamber method (Pinheiro *et al.*, 2019),. Following the methodology proposed by Reeves *et al.* (2016), measurements were taken between 9:00 and 12:00

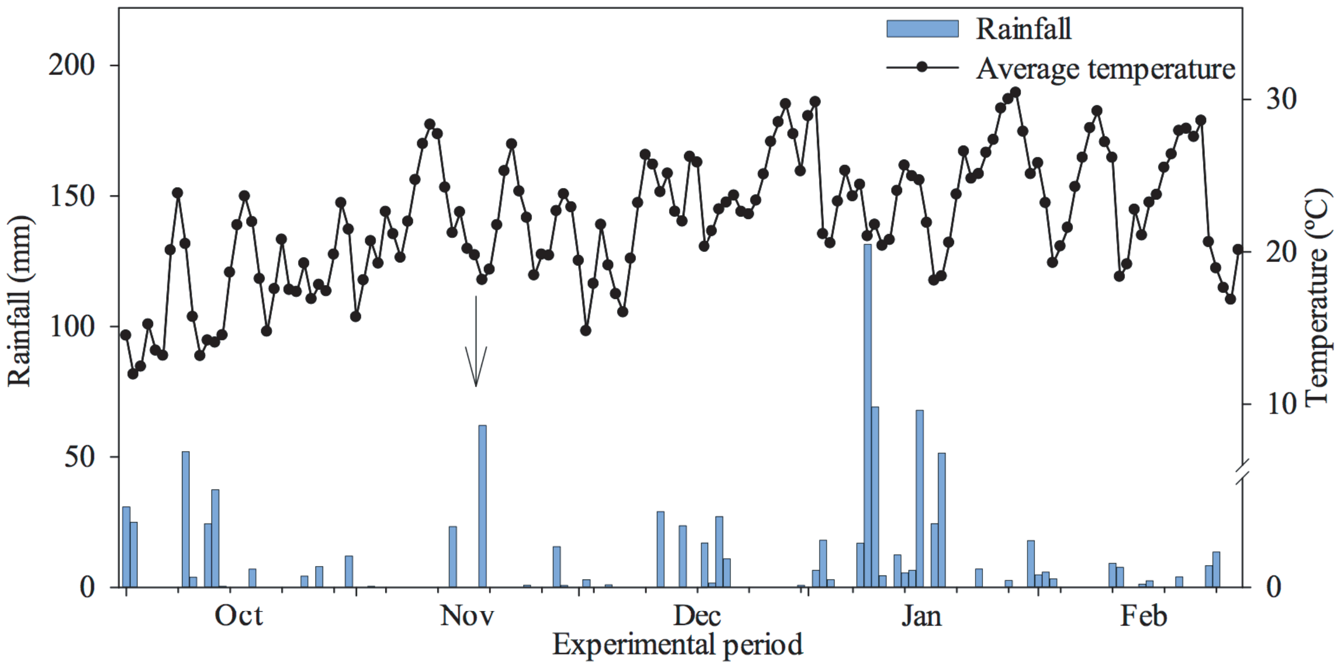

Fig. 2: Average daily air temperature and rainfall values throughout the grape season. Dashed arrows indicate N fertilization.

a.m.. The gas collection device consisted of a chamber (70 cm length × 40 cm width × 20 cm height) and a base (70 cm length × 40 cm width × 10 cm height), both in galvanized steel. The bases were inserted into the soil at a depth of 5 cm, in the grapevine planting line, where they remained throughout the study. To minimize the disturbances caused by the allocation of the metallic base on the N_2O fluxes, the bases were installed about a month before N application. On the base, there was a channel which was filled with water moments before the beginning of the collections, to avoid gas exchange with the environment outside the chamber. Gas samples were collected manually, using a 20 mL polypropylene syringe, exactly at 0, 20 and 40 min after coupling the chambers to the gutters in all treatments. Before gas sampling, the air present inside the chambers was homogenized for 30 seconds and the internal temperature was measured. After collections, the samples were transferred to evacuated bottles (Labco Exetainer®, Lampeter, United Kingdom). N_2O concentrations were determined in a gas chromatograph (GC-2014, Shimadzu Corp., Kyoto, Japan), within a maximum period of 24 hours after sample collection. N_2O emissions were calculated according to the variation in the concentration of N_2O in the chamber as a function of the time it remained closed, adjusted by the chamber volume, floor area occupied by the chamber, and molecular weight of N_2O (Jantalia *et al.*, 2008). Cumulative N_2O emissions were obtained by linear interpolation between N_2O collections. Thus, the average emissions between two consecutive sampling events were calculated by the value resulting from the interval of days between the two collections.

Measurements of mineral N in the soil

Soil samples for quantification of mineral N forms in the soil were carried out between the application of treatments (November 2018) and the grape harvest (February 2019). Three soil samples randomly distributed in each plot from the 0-0.10 m layer were withdrawn using a stainless-steel auger (3 cm in diameter), on the same dates as the gas sampling. Immediately after collection, the samples were stored in coolers and transported to the laboratory where they were kept in a freezer (-4 °C) for further analysis. In the laboratory, the mineral N forms of the soil, ammonium (NH_4^+), and nitrate (NO_3^-) were extracted from the addition of 20 g of moist soil and stirred with 80 mL of KCl 1 mol L^{-1} for 30 min. Thereafter, it was decanted for 30 min, the supernatant extract was collected to determine the concentrations of NH_4^+ and NO_3^- in a steam drag distiller (Tecnal, TE-037, Brazil), following the methodology proposed by Tedesco *et al.* (1995). The gravimetric soil moisture was determined by drying the samples at 105 °C in an oven with forced air circulation for 24 h (Tedesco *et al.*, 1995). The water-saturated pore space (WFPS) in the 0-0.10 m layer was calculated by the ratio of gravimetric moisture to total soil porosity, determined from the bulk density (Siqueira Neto *et al.*, 2009).

Sampling of leaves for N analysis

During the flowering of the vines (October/November), complete and fully expanded leaves were collected in opposition to the bunches, in the middle third of the branches of the year. The leaves were collected in the 2016/17, 2017/18, and 2018/19 seasons. The leaf opposite the bunch is considered the diagnostic organ to assess the nutritional status of grapevines (CQFS-RS/SC, 2016). Later in the laboratory, the leaves were carefully washed with distilled water and dried in an oven with forced air circulation at 65 °C until reaching a constant mass. The leaves were ground in a Willey mill and passed through a 2 mm mesh sieve. Subsequently, the plant tissue was prepared and subjected to sulfuric digestion. The concentration of total N was determined in a micro-Kjeldahl steam drag distiller (Tecnal, TE-0363, Brazil) (Tedesco *et al.*, 1995).

Grape yield

At the grape harvest (February) all bunches of five plants per treatment were counted, collected and weighed using a portable digital scale (WalMur, 50 K, Brazil) to determine the number of clusters per plant and the grape production per plant and conversion to yield (Mg ha^{-1}). Similarly, to the leaves collection, grape yield was measured in 2016/17, 2017/18, and 2018/19 crop seasons.

Statistical analysis

Data on leaf N concentration, number of clusters per plant and grape yield were analysed for normality of residues and homogeneity of variances. Subsequently, the data were subjected to analysis of variance using the R Studio software (R Core Team, 2019). When the effects of the application of doses and modes of N application were significant, a comparison of means was performed using the Scott-Knott test ($P<0.05$). Based on the standard error of the mean, the effects of N doses and modes of application on N_2O emissions were evaluated through descriptive data analysis. Accumulated N_2O emission data were analysed for homogeneity and normality and subsequently compared by Tukey's test ($P<0.05$) using the R Studio statistical software (R Core Team, 2019). In addition, to verify the relation between variables and treatments, a multivariate Principal Component Analysis (PCA) was performed using the FactoExtra package (Kassambara and Mundt, 2017) and according to the main Principal Components (PC1 and PC2), which comprised the standardised and orthogonal linear combination to explain the original data variance.

Results

Climatic conditions and WFPS

The average air temperature varied between 11.9 and 30.4 °C during the 2018/19 crop season (October 2018 to February 2019). Rainfall ranged from 60.2 (February 2019) to 445.1 mm (January 2019) and the accumulated rainfall was 927.7 mm (Fig. 2). In the soil, the variation of water-filled pore space (WFPS) occurred according to the intensity of rainfall. The maximum value observed occurred in the soil that received 80 kg N ha^{-1} $year^{-1}$, in the NF mode, (91%) at 4 days after application of N, while the minimum value was observed in the soil submitted to 40 kg N ha^{-1} year^{-1}, in NS mode, (13%) at 64 days after application of N (Fig. 4a). WFPS values in NS modes ranged from 13 to 77%, while in NF mode doses ranged from 22 to 91%.

Nitrous oxide emissions

N application increased N_2O fluxes, especially in the first nine days (Fig. 3a). A significant increase in N_2O fluxes was already observed in the 1st day after application of N. The highest N_2O flux was measured 2 days after 80 kg N ha^{-1} yr^{-1} application and reached 61.47 ± 12.52 and 57.64 ± 22.03 µg N m^{-2} h^{-1} in NS and NF application modes, respectively (Fig. 3a). The highest N_2O emissions from the soils that received 40 kg N ha^{-1} yr^{-1} were measured one day after N application and reached 30.37 ± 18.95 and 37.41 ± 3.29 µg N m^{-2} h^{-1} in NS and NF application mode, respectively. After 31 days past N application, N_2O fluxes decreased and did not differ among treatments. The accumulated emissions of N_2O throughout the grapevine productive cycle was higher ($P<0.05$) in the soil that received 40 kg N ha^{-1} yr^{-1}, under the NF application mode (496.18 ± 37.00 g N ha^{-1}) and 80 kg N ha^{-1} yr^{-1}, in the NS application mode (477.15 ± 69.41 g N ha^{-1}). The lowest accumulated N_2O emissions throughout the cycle ($P<0.05$) were observed in treatments without N fertilization (0 kg N ha^{-1} yr^{-1}) as expected, with values of 227.27 ± 35.53 and 161.74 ± 34.67 g N ha^{-1} for NS and NF application modes, respectively. At 31 and 64 days after N application, periods that coincide with the change of color of the grape berries and with the grape harvest, respectively, the accumulated N_2O emissions represented 40 and 72% of measurements taken during the entire season (Fig. 3b).

Soil inorganic N

The application of N fertilizer provided an increase in the soil concentrations of NH_4^+ and NO_3^- up to 9 days after application (Fig. 4b, c). The highest concentrations of NH_4^+ in the soil were measured two days after application of 80 kg N ha^{-1} yr^{-1}, for both modes of N fertilization (NF and NS) (Fig. 4b). Nine days after N application, the concentrations of NH_4^+ in the soil that received 80 kg N ha^{-1} yr^{-1} were reduced by 96% in the NS mode and 92% in the NF mode, compared to the initial values. The highest concentrations of NO_3^- in the soil were observed at 5 and 6 days after application of 40 kg N ha^{-1} yr^{-1}, in the NF and NS application modes, respectively (Fig. 4c). Statistical differences among treatments in soil N concentration were observed at 1, 3, 6, and 9 days after N fertilization at 1-9 days after fertilizer N application to soil NH_4^+ concentration. Nine days after N fertilization, the concentrations of NH_4^+ and NO_3^- in the soil decreased for all treatments and remained stable. Thereafter, with no significant differences among treatments.

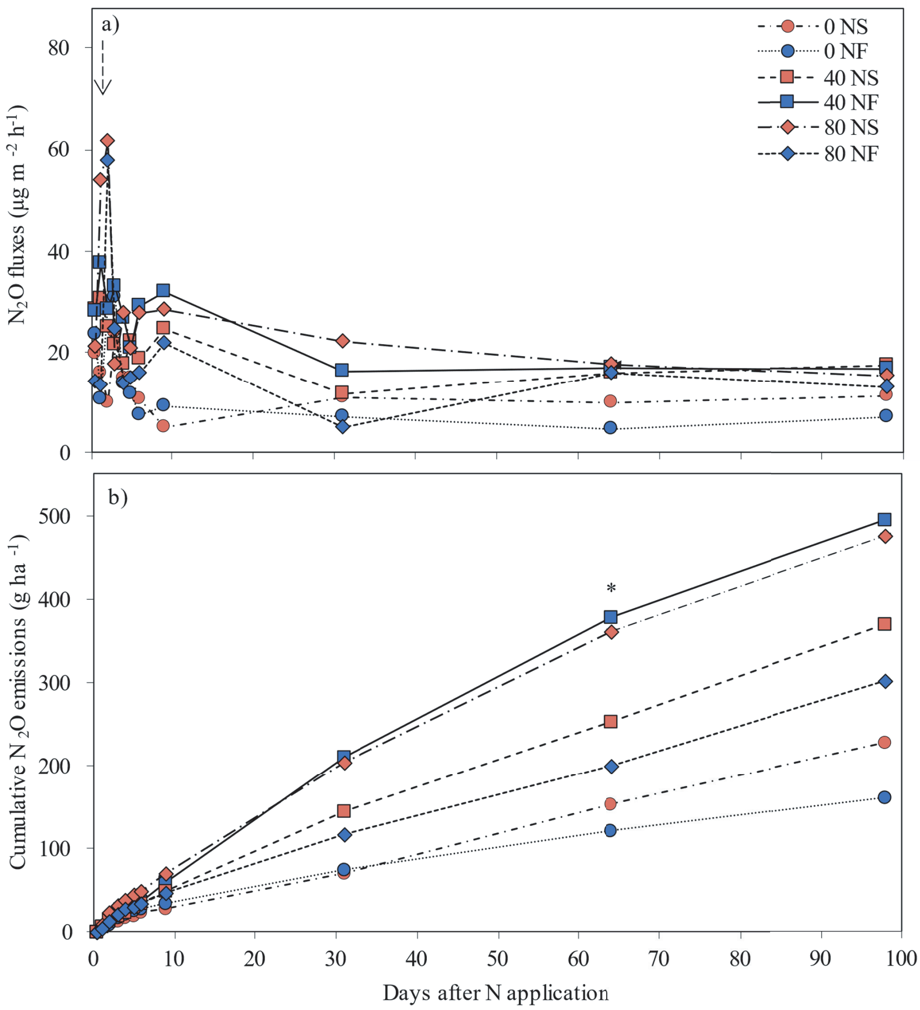

Fig. 3: Fluxes (a) and cumulative emission (b) of N2O-N in soil of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevine subjected to different N doses (0, 40 and 80 kg N ha^{-1} year^{-1}) and application modes (NS – Nitrogen surface; and NF – Nitrogen fertigation. Asterisk at the top indicates difference between treatments by least significant difference (LSD) test (p < 0.05).

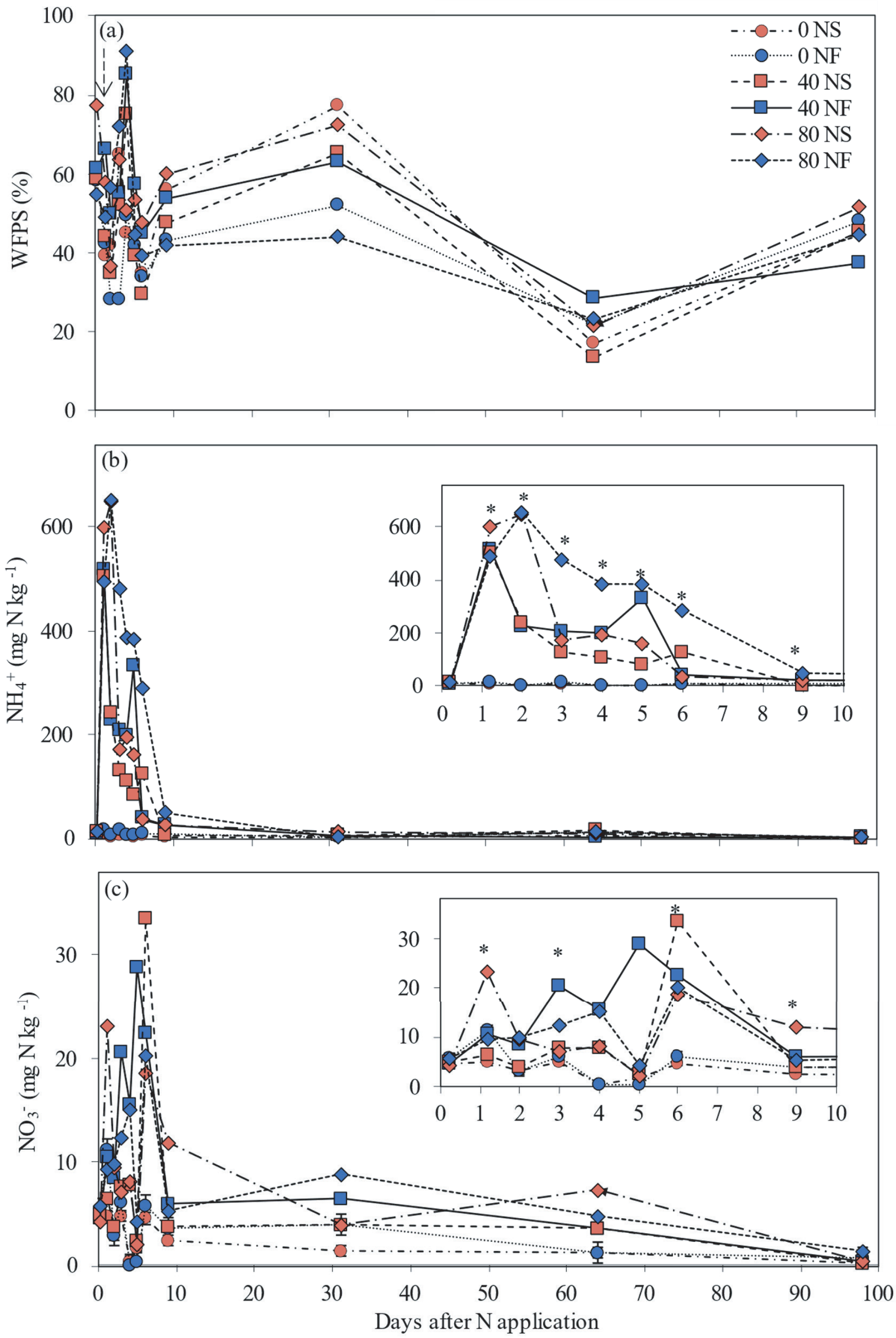

Fig. 4: Water-filled pore space (a); soil ammonium (b) and nitrate concentrations (c) of the 0-0.10 m soil layer in 'Alicante Bouschet' (Vitis vinifera L.) grapevine subjected to different N doses (0, 40 and 80 kg N ha^{-1} year^{-1}) and application modes (NS – Nitrogen surface; and NF – Nitrogen fertigation; Asterisk at the top indicates difference between treatments by least significant difference (LSD) test ($p < 0.05$).

Leaf N concentration

The highest leaf N concentrations among N doses were observed in grapevines subjected to the application of 40 kg N ha^{-1} yr^{-1} under the NS mode in the 2016/17 and 2017/18 growth seasons, and under the NF mode in 2016/17 growth season (Fig. 5a). In the 2017/18 crop season, leaf N concentration under the NF mode, did not differ significantly between N doses. In the 2016/17 and 2017/18 growth seasons, grapevines cultivated in soils submitted to application of 40 kg N ha^{-1} yr^{-1} the highest leaf N concentrations, especially in the NS mode. In these crop seasons, the grapevines fertilized with 0 and 80 kg N ha^{-1} yr^{-1} did not vary between the NS and NF treatments. In 2018/19, the leaf N concentration did not differ statistically among doses and modes of N supply.

Grape yield and components

In the 2017/18 and 2018/19 seasons, the number of clusters per plant under the NS treatment was higher in the grapevines submitted to 80 kg N ha^{-1} yr^{-1}. Under the NF mode, there was no significant difference among the N rates (Fig. 5b). For the same crop seasons, the grapevines submitted to 80 kg N ha^{-1} yr^{-1} had the highest grape yield under the NS mode; as to the unfertilized grapevines, no significant crop seasons and yield differences between NF and NS modes were observed. The highest grape yields of the plants under the NS mode were observed in 2017/18 growth season for 40 and 80 kg N ha^{-1} yr^{-1}, (Fig. 5 h), but did not vary for the NF treatment. The number of bunches per plant did not vary significantly among modes and rates of N in the 2016/17 growth season.

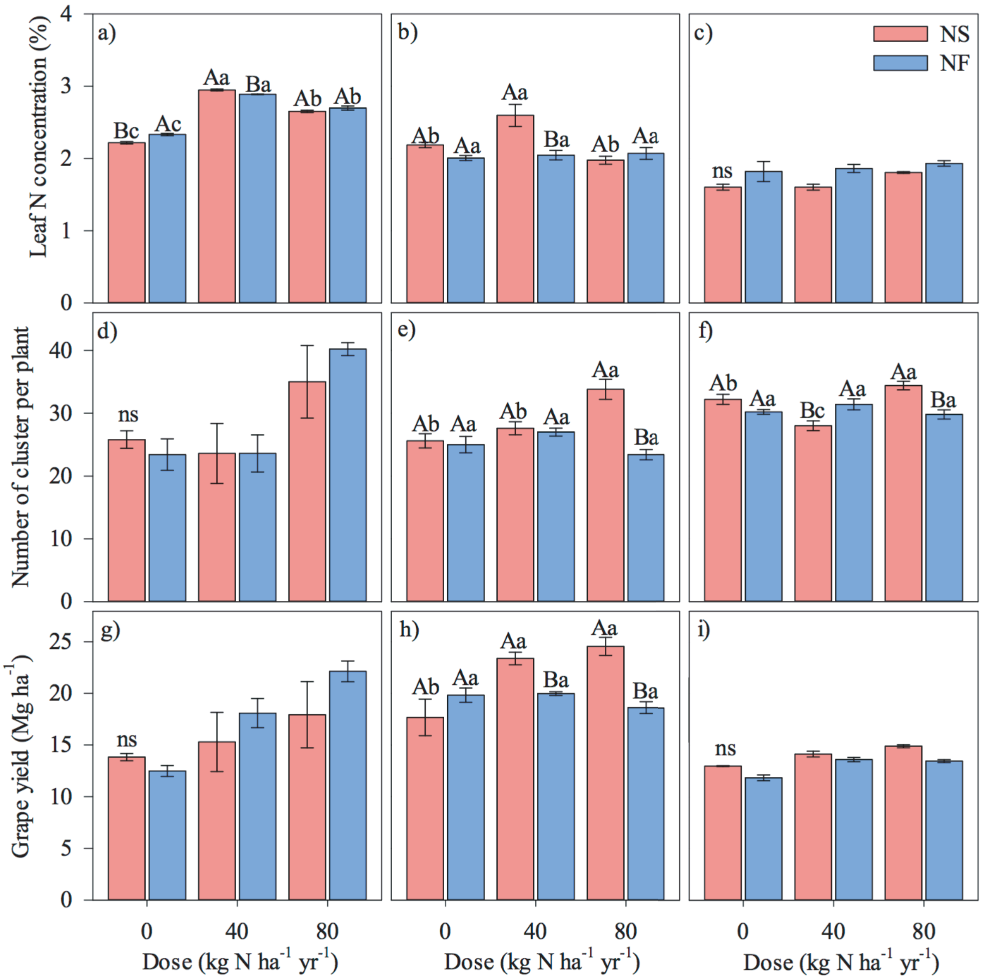

Fig. 5: Leaf N concentration, number of clusters per plant and grape yield of 2016/17 crop season (a, d, g), 2017/18 (b, e, h) and 2018/19 (c, f, i) of 'Alicante Bouschet' (Vitis vinifera L.) grapevine subjected to different N doses (0, 40 and 80 kg N ha^{-1} $year^{-1}$) and application modes (NS – Nitrogen surface; and NF – Nitrogen fertigation; The vertical bars indicate the standard error (n = 5). Different lower-case letters indicate a significant difference among N doses in the same application mode, and different upper-case letters indicate a significant difference among the N application modes in the same N dose by the Scott-Knott test (P<0.05). ns = not significant.

In 2016/17 and 2018/19, the grape yield did not vary significantly among modes and N doses.

Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed extracting only the first two components (PC1 and PC2), which explained 87.3% of the original data variability (Fig. 6). PC1 explained 61.5% of the variability and allowed the observation of the effects of N doses on the response variables, evidenced by the positioning of the treatments 80 kg N ha^{-1} yr^{-1}, under NS mode and 40 kg N ha^{-1} yr^{-1}, under NF mode, on the right in the spatial distribution, indicating a positive relation with the cumulative emission of N_2O (Cumulative N_2O), the concentration of NO_3^- (NO_3^-) and NH_4^+ (NH_4^+) in the soil, and the grape yield (Yield). These results are opposite to NS and NF treatments without fertilization (0 kg N ha^{-1} yr^{-1}), being observed to the left of the spatial distribution, and indicating a negative relation with the referred parameters. PC2 explained 25.8% of the data variability and was efficient in separating the response variables such as leaf N concentration (Leaf N) and number of clusters per plant (Cluster), which were influenced by N fertilization (40 and 80 kg N ha^{-1} yr^{-1}) and the NF mode. This behavior showed an inverse relation to the 80 kg N ha^{-1} yr^{-1} treatment, in the NF mode, and demonstrated a linearly inverse relation.

Discussion

The highest N_2O emissions in the initial evaluation period, at 9 days after application of N in the soil, were observed mainly because of the greater availability of N in the soil, derived from nitrogen fertilizer, combined with the C content in the soil, which favored the population's microbial activity. This contributed to the microbial processes of nitrification and denitrification, which are carried out by certain bacteria such as *Nitrobacter* spp. and *Pseudomonas denitrificans*, respectively, increasing the N_2O emissions (Toma and Hatano, 2007; Hayakawa *et al.*, 2009; Frimpong *et al.*, 2011). These results corroborate a study carried out by Fentabil *et al.* (2016), who observed that the application of 40 kg N ha^{-1} in grapevines favored N_2O emissions, especially in the first 15 days after application (DAA) of N. Initially, the highest N_2O emissions may be related to the nitrification process, due to the high concentration of NH_4^+, made available by the nitrogen fertilizer. The NH_4^+ in the soil is transformed by the nitrifying microorganisms to NO_2^- and then to NO_3^-, using the reducing (electron) equivalents of NH_4^+ to assimilate CO_2. During this oxidation reaction, there may have been losses of N molecules via N_2O gas transformation (Carlisle *et al.*, 2010; Fentabil *et al.*, 2016). After this initial period, denitrification was probably the predominant process in N_2O emission, as the NO_3^- produced by nitrification can be made available to denitrifying microorganisms. Microorganisms use NO_3^- as an electron acceptor, under anaerobic conditions, during phosphorylation

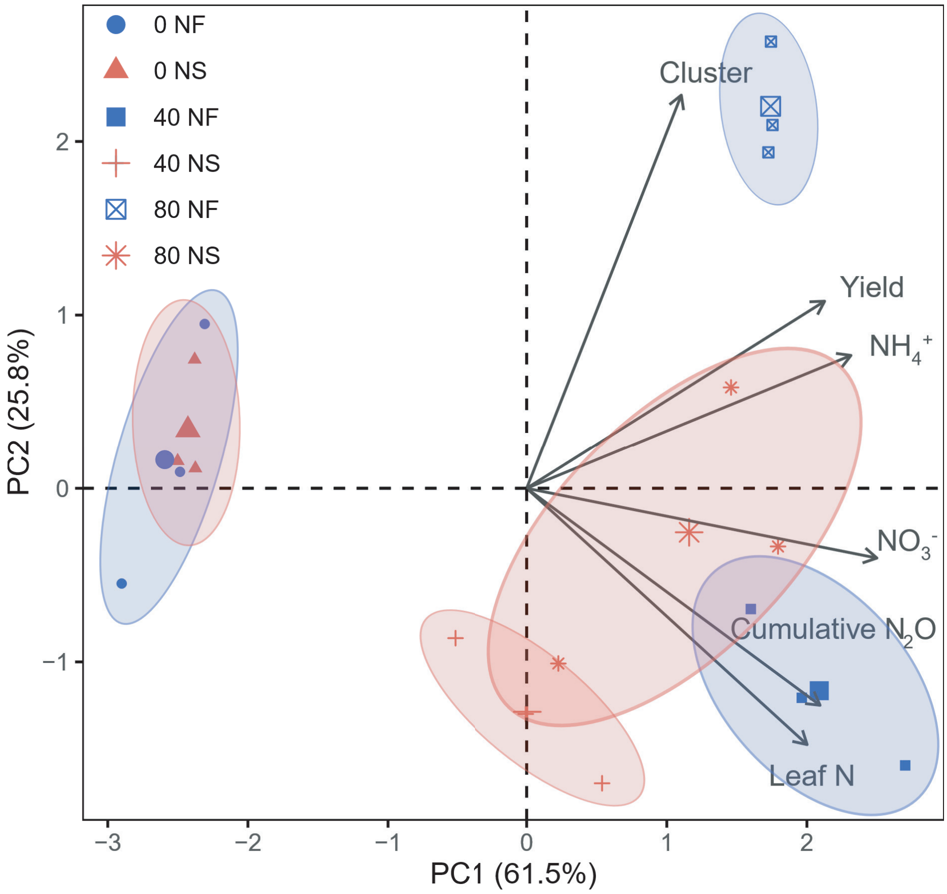

Fig. 6: Relation between principal component 1 (PC1) and principal component 2 (PC2) of the parameters of nutritional status (leaf N concentration (Leaf N)), yield parameters (grape yield (Yield), number of cluster per plant (Cluster)), soil parameters (ammonium (NH4+) and nitrate (NO3-) concentrations) and cumulative N2O emission (Cumulative N2O)) of 'Alicante Bouschet' (Vitis vinifera L.) grapevine subjected to different N doses (0, 40 and 80 kg N ha^{-1} year^{-1}) and application modes (NS – Nitrogen surface; and NF – Nitrogen fertigation.

of electron transport, and finally converts NO_3^- into N_2O and N_2, that are emitted into the atmosphere (Carlisle *et al.*, 2010; Müller *et al.*, 2019). On the other hand, at the time of application of N doses, the fertilizer was disposed on the soil surface and, therefore, the formation of microenvironments or microsites saturated with N may have occurred (Müller *et al.*, 2019), exceeding the absorption capacity of plants and adsorption of soil particles. This process represents one of the main sources of agricultural N_2O production (Carlisle *et al.*, 2010). In addition, the management employed for the deposition of plant residues on the soil surface between the lines may have increased the levels of C in the soil, which contributes to the occurrence of another phenomenon that enhances the production of agricultural N_2O, which the increase in the number of microaggregates (Loss *et al.*, 2015). This favors the formation of anaerobic sites, through the consumption of oxygen (O_2) by heterotrophic bacteria (Siqueira Neto *et al.*, 2009; Li *et al.*, 2015). Another factor that may have contributed to the high N_2O emissions in the initial period after N application in the soil was the occurrence of high daily average temperatures (> 25 °C), which leverages such emissions (Carlisle *et al.*, 2010). In addition, significant rainfall was observed a few days before N application, which contributed to increasing soil moisture and, with this, increasing the pore space saturated with water (WFPS). In general, nitrification predominates when WFPS is between 30 and 60%, favoring aerobic conditions for microorganisms. The increase in WFPS, on the other hand, contributes to the occurrence of the denitrification process, which is slightly higher when the WFPS exceeds 60-65% and, thus, anaerobic conditions prevail (Davidson *et al.*, 2000; Carlisle *et al.*, 2010; Weiler *et al.*, 2018), especially in the first few weeks when a large amount of substrate is available. Although the WFPS values during most of the experimental period were below 60%, in which nitrification predominates, the C content available from the deposition of plant residues on the soil surface between the vine rows must be considered, which contributes to reduce the availability of O_2 from the soil and favors denitrification. Thus, the combination of WFPS values close to 60% or higher after rainfall events and the intense microbial activity during the decomposition of plant residues may have contributed to the emission of N_2O (Chen *et al.*, 2013; Weiler *et al.*, 2018).

The highest values of cumulative N_2O emissions at 31 and 64 days after application of N represented 40 and 72% of the average emissions of the treatments, respectively. This fact can be attributed to a prolongation of NO_3^- availability throughout the experimental period and also to voluminous precipitation events combined with the high temperatures that occurred during this period, favoring microbial activity. This contributes to making NO_3^- available in the soil, which enhances the emission of N_2O (Carlisle *et al.*, 2010). In general, cumulative N_2O emissions were lower than those observed in other studies, such as that reported by Marques *et al.* (2018), who observed cumulative values of 860 ± 0.22 and 620 ± 0.14 g ha^{-1} of N_2O-N in grapevines submitted to surface application of 50 kg N ha^{-1} and application of 50 kg N ha^{-1} with the preparation of the soil between the rows (10 cm deep), respectively. Also, the study conducted by (Fentabil *et al.* 2016), observed cumulative values of 1590 g ha^{-1} of N_2O in grapevines submitted to application of 50 kg N ha^{-1}. One of the possible explanations for the low cumulative N_2O emissions verified in our study may be related to the physical characteristic of the soil, which has a sandy texture (83 g kg^{-1}) and low cation exchange capacity (CEC) (2.67 cmolc dm^{-3}). This enhances the migration of NO_3^- deep into the soil, reducing the possibility of denitrification by microorganisms (Steenwerth and Belina, 2010). In general, the grape yield values were higher in the grapevines subjected to the application of N doses. It may have happened because of the increase in the NH_4^+ and NO_3^- contents in the soil, provided by the addition of N, which stimulates the absorption of the element through the roots of the grapevines (Steenwerth and Belina, 2010; Brunetto *et al.*, 2016). With increased N uptake, grapevines tend to accumulate N in photosynthetically active tissues, which can be seen in the concentration of N in plant leaves (Fang *et al.*, 2013; Arrobas *et al.*, 2014). Thus, the higher concentration of N in leaves may have contributed to important physiological processes, such as photosynthesis. This is because, the greater the leaf N concentration, the greater the concentrations of chlorophylls and chloroplast proteins, which conditions the greater interception of the sun's rays. Thus, the fixation of CO_2 in the leaf tissue is increased, as well as the processes of cell division and elongation of plant tissues and organs (Blank *et al.*, 2018; Moriwaki *et al.*, 2019) and the productivity of grapevines fruits. Studies that consider only the area-scale N_2O emission factor cannot be used to recommend adequate management practices to mitigate N_2O emission without compromising the productivity of the crop studied (Fentabil *et al.*, 2016). In our study, we found that even with the increase in N_2O emissions, there were no reductions in the grape yield parameters. Thus, a negative linear relation between these parameters (N_2O emissions *vs*. grape yield) cannot be considered.

Conclusion

Grapevines cultivated in soil with applications of 40 and 80 kg N ha^{-1} year^{-1}, in NF and NS modes, respectively, resulted in higher N_2O emissions, which had emission peaks up to 9 DAA of N. That's because, these modes of application and N doses provide higher levels of NH_4^+ and NO_3^- in the soil, which conditioned a favorable environment for microbial activity and, consequently, higher N_2O emission. The application of N at doses 40 and 80 kg N ha^{-1} year^{-1}, in the NS and NF modes, respectively, are the most efficient doses and modes for N application in grapevines. These management practices are the most suitable because they combine adequate levels of NH_4^+ and NO_3^- in the soil, which conditions low N_2O emissions and provides higher grape yields.

Conflicts of interest

The authors declare that they have no competing interests.

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