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Root morphology, yield and must composition of grapevine subjected to application of urea methods and rates

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Abstract

Nitrogen (N) is often applied to soil surface during the grapevine cycle, increases N losses. One way to reduce N losses lies on its application method, however how N dose and application mode impact on grapevines remain poorly investigated. The aim of this study is to evaluate the root growth, as well as grape must yield and composition, in a sandy soil and subjected to different N application doses and modes under subtropical climate conditions. Grapevines were implanted in 2011 and, since 2014, they were subjected to surface (N-Surf), and fertigation (N-Fert) N application, at rates of 0, 40 and 100 kg N ha^{-1} $year^{-1}$. Leaf N concentration, yield, must quality parameters and root system morphology were evaluated in the 2016/2017 and 2017/2018 seasons. The application of N in both rates (40 and 100 kg N ha^{-1} $year^{-1}$), in the N-Surf and N-Fert methods, increased the surface area of root, number of living roots and root production. The application of N rates, in both N application modes, provided adequate N supply to the soil and increased root system development, which contributed to the increase of soil N uptake by plants, higher N concentration in leaves and greater grape yield. However, N supply decreased the quality of the must, as observed in decreased TSS and total anthocyanins values and in increased total titratable acidity values, which are not desirable for red wine making processes.

Keywords: Nitrogen fertilization; Sandy soil; Grapes quality; Root system; Minirhizotron.

Introduction

Grapevines are grown worldwide in sandy soils that often have low organic matter (OM) content, a fact that leads to low nitrogen (N) availability in the soil (Gustavo Brunetto et al., 2016, 2014; Lorensini et al., 2017). Thus, applications of N fertilizer are necessary to increase soil mineral N availability, *i.e.* nitrate (NO_3^-) and ammonium (NH_4^+) forms, which are the ones uptake by plants. Soil surface (without incorporation) N applications are often recommended, however this approach may increase N losses, mainly due to volatilization (Lorensini et al., 2012; Viero, Bayer, Mara Vieira Fontoura, Paulo de Moraes, & Professor, 2014), and decrease grapevines N uptake (Gustavo Brunetto et al., 2016; Gustavo Brunetto, Kaminski, Melo, Brunning, & Mallmann, 2006). Therefore, it is necessary adopting alternative ways of supplying N to decrease losses and increase N use efficiency. Fertigation is one of these alternatives; it enables urea solubilization and hydrolysis reaction, decreases losses caused by volatilization, since it favors the migration of NO_3^- and NH_4^+ in soil profile (Comas, Bauerle, & Eissenstat, 2010).

Increased mineral N contents in soil subsurface layers can contribute plant root system growth and development (Centinari, Heuvel, Goebel, Smith, & Bauerle, 2016; Comas et al., 2010; Zhou, Yang, Ren, Huang, & An, 2014). Increased NO_3^- and NH_4^+ concentrations in soil solution activates NO_3^- (NRTs) and NH_4^+ (AMTs) transporters and, consequently, contributes for N uptake (Pii, Aldrighetti, Valentinuzzi, Mimmo, & Cesco, 2019; Tomasi, Monte, Varanini, Cesco, & Pinton, 2015; Xu, Fan, & Miller, 2012) and for the increase of grapevine root emission and root surface area (Krouk et al., 2010; Remans et al., 2006). In addition, high NO_3^- concentrations can inhibit auxin transport in the root system due to competition at the NRT1.1 transporter site; thus, auxin accumulates in plant roots and leads to an increase of lateral root growth in sites presenting high NO_3^- concentrations (Mounier, Pervent, Ljung, Gojon, & Nacry, 2014). On the other hand, high N doses can decrease soil pH due to NH_4^+ nitrification into NO_3^-. This phenomenon, which is often seen in vineyards (Lorensini et al., 2017), can solubilize minerals in the soil and release aluminum (Al^{+3}) into soil solution; consequently, Al^{+3} can be uptake by plant roots and lead to root length shortening and to root diameter increase (Inostroza-Blancheteau, Aquea, Reyes-Díaz, Alberdi, & Arce-Johnson, 2011; Riaz et al., 2018; Zhou et al., 2014). This outcome is undesirable because it reduces the volume of soil explored by the root system, as well as the absorption of water and nutrients (Riaz et al., 2018).

Mineral N provided by N fertilization can be uptake and transported to plant shoot, increasing N content in the leaves. This process occurs mainly in stages of intense root growth, such as at flowering, when the increased root volume contributes to great N uptake (Radville et al., 2016). Increased leaf N content can lead to an increased photosynthetic rate and CO_2 fixation, since RuBisCo corresponds to 50% of soluble proteins in the leaves of C3 plants (Stefano, Agyei, Njoku, & Udenigwe, 2018). This outcome favors cell division and elongation conditions in plant tissues and increases vegetative growth (Blank, Tittmann, Ghozlen, & Stoll, 2018; Moriwaki et al., 2019) in grape clusters, resulting in greater crop yield (Gustavo Brunetto et al., 2009a; Yu et al., 2012).

In addition to increased crop yield, there may be changes in oenological parameters associated with wine quality; these changes may be due to total anthocyanins and total soluble solids (TSS) dilution, as well as to increase in total titratable acidity values (Gustavo Brunetto et al., 2009a; Janjanin et al., 2016; Kelly et al., 2017; Spayd, Nagel, & Edwards, 1995). Thus, it is recommended adopting strategies, such as N doses and application modes, to stimulate root system growth in order to increase water and nutrient absorption by plants, as well as to increase crop yield without decreasing the quality of must. The aim of the current study was to investigate whether N rates and modes of application can change root system morphology, yield and the composition of grape must in grapevines (*Vitis vinifera* L.) grown in sandy soil under subtropical climate conditions.

Materials and Methods

Experimental site description

The experiment was conducted in a vineyard established in 2011, in Santana do Livramento County, Rio Grande do Sul State (RS), Southern Brazil (30º 48’ 31” S; 55º 22’ 33” W). Alicante Bouschet (*Vitis vinifera* L. grafted onto Paulsen 1103 rootstock) was the adopted cultivar. Plant density was 2,976 hectare^{-1} (2.8 m between rows × 1.2 m between plants), based on the espalier system. Soil in the experimental site was classified as Typic Hapludalf (Soil Survey Staff, 2014); the main chemical properties of the 0.0-0.20 m layer before the experiment are shown in Table 1.

Based on Köppen classification, the climate in the region is subtropical humid (Cfa); it is featured by mild temperatures and rainfall rates, with little variation throughout the year. Mean annual rainfall is 1,600 mm, whereas mean temperature is 23.8° C in the warmest month (January) and 12.4° C in the coldest month (July) (Alvares, Stape, Sentelhas, De Moraes Gonçalves, & Sparovek, 2013). Annual insolation reaches approximately 2,500 hours; the site presents gently undulating terrain. Two crop production cycles, 2016/17 and 2017/18, were evaluated. According to data from the National Institute of Meteorology (INMET), the experimental area recorded 513.2 mm and 189.6 mm of rainfall from November 2016 to February 2017 and from November 2017 to February 2018 (period between treatment application and harvest), respectively (Fig. 1).

Weeds in the grapevine row were desiccated with two to four sequential applications of 2 L ha^{-1} y^{-1} of non-selective systemic herbicide (*i.e.*, glyphosate), depending on cover plant development. Inter-row cover plants were cut near the soil surface, five times throughout each crop season and the residues were deposited on soil surface between rows. Grapevines were subjected to four applications of 45 kg P_2O_5 ha^{-1} year^{-1} and 45 kg K_2O ha^{-1} year^{-1} prior to experiment installation (CQFS-RS/SC, 2016). Triple superphosphate (42% P) and KCl (60% K) were used as P and K sources, respectively. Nitrogen fertilizers were applied to the crop based on each treatment.

Experimental design and treatments

Starting from September 2014, two N application methods: surface (N-Surf) and fertigation (N-Fert) and three rates 0, 40 and 100 kg N ha^{-1} year^{-1} were evaluated. The experiment followed a complete randomized block experimental design with three repetitions (plot) with 2 x 3 factorial arrangement (*i.e.*, two N application modes and three N rates). Each plot consisted of five plants were only the three central plants were used for data collection. In both application methods, urea was employed as N source (45% N); in N-Surf treatment, N was applied on the surface of the soil, within plant canopy projection, without incorporation. Every year, N application was performed during flowering (Gustavo Brunetto et al., 2009a). In N-Fert treatment, N was applied via drip irrigation (Netafim™ 132 Dripnet PC AS 16250), at a flow rate of 1.6 L h^{-1}; drippers were spaced 0.6 m a part along the tree row, within canopy projection. The applications were performed at four different times, at 7-day intervals, according to the following procedure: 10 minutes of water; 10 minutes of water + N; and 10 minutes of water. Applications N-Fert provided 6.4 L of water per plant per crop season.

Minirhizotron installation

Minirhizotron tubes (Supplementary material 1) were installed in the ground of four plants per treatment in May 2016 to enable scanning the grapevine root system *in situ*. For each assessed grapevine plant, a hole was made with a spiral drill (70 mm diameter and 1,500 mm length), pulled by motorized ground driller (Branco, Bps 52), 0.5 m away from the trunk, inside the canopy projection. Each individual hole was made at an angle of 45° to soil surface and a clear acrylic tube (70 mm x 1050 mm) was inserted. Three images were taken along each tube, referring to the soil layers: 0-20; 20-40; and 40-60 cm deep. Each image had fixed dimensions of 216 mm × 196 mm. A flexible, lightweight, waterproof and non-toxic plastic polyethylene rod (CID Bio-Science, Inc. Camas, WA, USA) internally protected the tubes. PVC pipes (100 mm diameter) were used to protect the external part of the tubes from damage caused by weather, handling or pest attacks. Soil disturbance during the hole drilling process made it necessary waiting eight months for soil-plant-tube system stabilization, before the beginning of the scanning activities.

Image collection and measurements of root morphological parameters

Root images were taken at grapevine flowering stage (in October) in the 2016/17 and 2017/18 crop seasons. This phenological stage was selected because it coincides with a period of intense root growth and nutrient absorption, as reported in several studies (Comas, Anderson, Dunst, Lakso, & Eissenstat, 2005; Radville et al., 2016). Images of the root system were generated *in situ* with a CI-600™ cylindrical scanner (CID Bio-Science, Inc. Camas, WA, USA).

Subsequently, images were captured at different soil layers (0-20, 20-40 and 40-60 cm deep) and individually analyzed. The CI-600 scanner was connected to a computer by USB cable. Images captured in TIFF format (600 dpi resolution) were analyzed in the RootSnap!® CI-690 software version 1.3.2.25 (CID Bio-Science, Inc. Camas, WA, USA), which enables seeing the fine roots (≤ 2 mm). Parameters evaluated in each tube based on generated images were (i) root surface area (mm^2 tube^{-1}); (ii) mean root diameter (mm tube^{-1}); (iii) number of living roots; (iv) number of dead roots; and (v) root production as the difference between the total length of living and dead roots (mm tube^{-1}).

Leaf collection for nitrogen analysis

Six fully expanded leaves located in the opposite direction of the grape clusters, in the middle third of the annual branches, were collected at flowering (October 2016 and 2017) and at berry veraison (December 2016 and 2017). According to CQFS-RS/SC (2016), these leaves are the diagnostic organ used to assess the nutritional status of grapevines. Leaves were washed in distilled water and dried in forced-air-circulation oven at 65 ºC, until they reached constant weight. Then, they were milled in Wiley mill and sieved through 2-mm mesh. Subsequently, the leaf tissue was prepared and subjected to sulfuric digestion (Tedesco, Gianello, Bissani, Bohnen, & Volkweiss, 1995). Total N concentration was determined in Kjeldahl semi-micro steam distiller (Tecnal, TE-0363, Brazil).

Grape yield

The number of grape clusters per plant was counted in the 2016/17 and 2017/18 grape harvests. All grape clusters were harvested and weighed in a portable electronic scale (WalMur, 50 K, Brazil), to measure grape yield (Mg ha^{-1}). Berries were collected at the upper, middle and lower third of the grape clusters, thus totaling 100 berries per repetition and 9 repetitions per treatment. They were weighed in scale (Bel Engineering, Precision Scale L, Brazil) to determine the weight of 100 berries, before storing for chemical analysis (in triplicate) of the must.

Chemical analysis of the must

The stored berries were peeled and only the pulp and seeds were analyzed. A benchtop refractometer (Cosmo, Type K - 32) was used to determine the total soluble solids (TSS) based on standards set by AOAC (2005). Total titratable acidity was determined by transferring 10 mL of extract to a 100 mL volumetric flask, whose volume was completed with distilled water. For the titration of the resulting solution 0.1 mol L^{-1} of sodium hydroxide (NaOH) was used (IAL, 2008).

Berry peel was used to determine total anthocyanin contents through a contact with a 70:30 acidified (1% HCl) ethanolic solution (Ju and Howard 2003) at peel/solution ratio of 1:3 (m/v) and crushed in a blender (Arno, Clic Lav Top, Brazil) at low (1500 RPM) and high speed (3500 RPM) for 40 seconds each. The solution resulting from the aforementioned extraction was placed in a 250 mL beaker and left to rest for 30 minutes at room temperature (20 ± 1 ºC) before it was centrifuged at 3500 RPM for 5 minutes. Extract supernatant was removed and stored in amber bottle, which was placed in refrigerator (4-10ºC). Subsequently, triplicate readings of these extracts were performed.

Total anthocyanin content was determined based on the pH difference method (Giusti and Wrolstad 2001), according to which samples were dissolved in two buffer systems: 0.025 mol L^{-1} potassium chloride at pH 1.0 and 0.4 mol L^{-1} sodium acetate at pH 4.5. Maximum absorption readings were taken at 515 nm and 700 nm, with the aid of a UV-visible spectrophotometer (FEMTO, 600 plus, Brazil). Absorbance was calculated through equation (1):

$$A = (A515vis - A700nm)_{pH1.0} - (A515vis - A700nm)_{pH4.5} \quad (1)$$

Pigment concentration in the extract was calculated and represented in cyanidine-3-glycoside through equation (2).

$$Anthocyanins\ (mg\ 100\ mL^{-1}) = (A\ x\ MM\ x\ DF\ x\ 10000)/(\varepsilon\ x\ 1) \quad (2)$$

wherein: A = absorbance; MM = molecular mass; DF = dilution factor; and ε = molar absorptivity.

Statistical analysis

Data were subjected to analysis of variance by the R Studio software (R Core Team, 2019); whenever the analysis of variance showed a significant effect, means were compared through Scott-Knott test, at 5% significance level ($P < 0.05$). In addition, data were subjected to multivariate principal component analysis (PCA) in the R software (R Core Team, 2019) in order to investigate correlation effects between response variables and treatments' distribution along the evaluated crop seasons.

Results

Root morphological parameters

In the 2016/17 crop season, the highest root surface areas, recorded in the 0-20 cm layer, were observed in grapevines subjected to the application of 40 kg N ha^{-1} year^{-1} as N-Surf and of 100 kg N ha^{-1} year^{-1} as N-Fert (Fig. 2a). As for the 2017/18 crop season, the highest root surface areas were observed in grapevines subjected to the application of 40 kg N ha^{-1} year^{-1} as N-Surf and of 100 kg N ha^{-1} year^{-1} as N-Surf and N-Fert (Fig. 2b). In the 2016/17 crop season, the highest root surface area, recorded in the 20-40 cm and 40-60 cm layers was observed in grapevines subjected to the application of 40 kg N ha^{-1} year^{-1} as N-Surf (Fig. 2a); whereas in the 2017/18 crop season, the highest values recorded were observed in grapevines subjected to application of 100 kg N ha^{-1} year^{-1} and 40 kg N ha^{-1} year^{-1} as N-Fert and N-Surf, respectively (Fig. 2b).

In the 2016/17 crop season, the highest mean root diameters, recorded in the 0-20 cm layer were observed at rate of 0 kg N ha^{-1} year^{-1} and 100 kg N ha^{-1} year^{-1}, as N-Surf and N-Fert, respectively (Fig. 2c). On the other hand, the highest root diameters, recorded in the 20-40 cm and 40-60 cm layers in the 2016/17 crop season, were observed in grapevines subjected to all N rates (0, 40 and 100 kg N ha^{-1} year^{-1}) applied as N-Surf. In the 2017/18 crop season, the highest root diameters in the three soil layers were observed in plants subjected to 40 kg N ha^{-1} year^{-1} in the N-Surf mode and to 100 kg N ha^{-1} year^{-1} as N-Fert mode (Fig. 2d).

Root production and mortality

In 2016/17 and 2017/18 crop seasons, the highest number of living roots, recorded in the 0-20 cm layer, were observed in plants subjected to 40 kg N ha^{-1} year^{-1} as N-Surf and to 100 kg N ha^{-1} year^{-1} as N-Fert mode (Fig. 3a, b). In the 2016/17 crop season, the highest number of living roots, recorded in the 20-40 cm and 40-60 cm layers, were observed in grapevines subjected to 40 kg N ha^{-1} year^{-1} as N-Surf and N-Fert modes (Fig. 3a). In the 2017/18 crop season, the highest number of living roots, recorded in the 20-40 cm layer were observed in grapevines subjected to 100 kg N ha^{-1} year^{-1} as N-Surf and N-Fert (Fig. 3b). In 40-60 cm layer, the highest number of living roots was in the 100 kg N ha^{-1} year^{-1} as N-Fert (Fig. 3b).

In the 2016/17 crop season, the highest number of dead roots, recorded in the 0-20 cm layer were observed in grapevines subjected to 40 kg N ha^{-1} year^{-1} as N-Surf mode and to 100 kg N ha^{-1} year^{-1} as N-Fert mode (Fig. 3c). In the 2017/18 crop season, the highest number of dead roots, recorded in the 0-20 cm layer, were observed in grapevines subjected to 100 kg N ha^{-1} year^{-1} as N-Fert and to 0 kg N ha^{-1} year^{-1} and 40 kg N ha^{-1} year^{-1} as N-Surf (Fig. 3d). In the 2016/17 crop season, the highest number of dead roots, recorded in the 20-40 cm layer were observed in plants subjected to 40 kg N ha^{-1} year^{-1} as N-Surf and N-Fert and to 0 kg N ha^{-1} year^{-1} and 100 kg N ha^{-1} year^{-1} as N-Surf mode (Fig. 3c). In the 2017/18 crop season, the highest number of dead roots, recorded in the 20-40 cm layer, were observed in grapevines grown in control soil (0 kg

N ha^{-1} $year^{-1}$) N-Fert and in soil subjected to the application of 40 kg N ha^{-1} $year^{-1}$ as N-Surf mode (Fig. 3d). In the 2016/17 crop season, the largest total number of dead roots recorded in the 40-60 cm layer, was observed in grapevines grown in the control soil N-Fert and N-Surf (Fig. 3c). In the 2017/18 crop season, the highest number of dead roots recorded were observed in grapevines subjected to 100 kg N ha^{-1} $year^{-1}$ as N-Fert and to 40 kg N ha^{-1} $year^{-1}$ as N-Surf (Fig. 3d).

In the 2016/17 crop season, the highest root production, recorded in the 0-20 cm layer, was observed in grapevines grown in soil subjected to the application of 40 kg N ha^{-1} $year^{-1}$ as N-Surf and to 100 kg N ha^{-1} $year^{-1}$ as N-Fert (Fig. 4a). In the 2017/18 crop season, the highest root production, recorded in the 0-20 cm, 20-40 cm and 40-60 cm layers, were observed in grapevines subjected to 100 kg N ha^{-1} $year^{-1}$ as N-Surf and N-Fert (Fig. 4b).

Leaf N concentration

In the 2016/17 crop season, the highest N concentrations, in leaves collected at flowering and at berry veraison were observed in grapevines subjected to application of 40 kg N ha^{-1} $year^{-1}$ as N-Surf and N-Fert (Fig. 5a, c). In the 2016/17 crop season, N concentration in leaves, collected at flowering were significant difference between N supply methods, except for rate of 100 kg N ha^{-1} $year^{-1}$, and at veraison there was a significant difference between the N supply methods for 0 kg N ha^{-1} $year^{-1}$. In the 2017/18 crop season, the highest N concentration in leaves collected at flowering and at veraison was observed in grapevines grown in soil subjected to 100 kg N ha^{-1} $year^{-1}$ as N-Fert (Fig. 5b, d). In 2017/18 crop season, N concentrations in leaves collected at flowering and at berries veraison in grapevines subjected to N-Surf did not show significant difference among N rates (Fig. 5b, d).

Yield and its components

In the 2016/17 crop season, the lowest weight of 100 berries was observed in grapevines grown in soil subjected to the application of 0 and 100 kg N ha^{-1} $year^{-1}$, than 40 kg N ha^{-1} $year^{-1}$, as N-Fert (Fig. 6a). The weight of 100 berries recorded for grapevines subjected to N-Surf in the 2016/17 crop season did not statistically differ with N doses (Supplementary material 2). In the 2017/18 crop season, the weight of 100 berries did not show statistically significant difference among N doses and modes (Fig. 6b).

In 2016/17 crop season, the largest number of grape clusters per plant fertilized as N-Fert was observed at the rate of 100 kg N ha^{-1} $year^{-1}$ (Fig. 6c). The number of grape clusters per plant fertilized as N-Surf were not affected by N application rates (Supplementary material 2). In 2017/18 crop season, the smallest numbers of grape clusters per plant were observed in control grapevines (Fig. 6d). In the two crop seasons - 2016/17 and 2017/18, the number of grape clusters per plant did not statistically differ between N supply modes (Supplementary material 2).

In the 2016/17 crop season, the lowest yield of N-Fert plant was observed in control (12.49 Mg ha^{-1}) (Fig. 6e), whereas yield in N-Surf plants was not affected by application rates. In the 2017/18 crop season, the highest yields recorded were observed in grapevines grown in soil subjected to the application of 100 kg N ha^{-1} $year^{-1}$ as N-Surf and N-Fert (29.77 and 27.86 Mg ha^{-1}, respectively) (Fig. 6f). In the 2016/17 and 2017/18 seasons, crop yield was not statistically affected by N application modes (Supplementary material 2).

Chemical parameters of the must

In the 2016/17 crop season, the highest total titratable acidity was observed in grape musts subjected to the application of 100 kg N ha^{-1} $year^{-1}$ as N-Fert (Fig. 7a), whereas in the 2017/18 crop season, the highest total titratable acidity was recorded in grape musts subjected to the application of the same N dose, in both N-Fert and N-Surf modes (Fig. 7b). In the 2016/17 and 2017/18 crop seasons, the highest TSS values in must were observed in grapevines that were not fertilized (0 kg N ha^{-1} $year^{-1}$) (Fig. 7c, d). In the 2016/17 crop season, the highest total anthocyanin were observed in grape that were not fertilized (Fig. 7e), whereas in the 2017/18 crop season, the highest total anthocyanin values recorded were observed in grapevines subjected to the application of 0 kg N ha^{-1} $year^{-1}$and 40 kg N ha^{-1} $year^{-1}$, for both N application modes (N-Surf and N-Fert modes) (Fig. 7f).

Principal Component Analysis (PCA)

The sum of components PC1 and PC2 explained 54.18% of original data variability (Fig. 8). The PC1 explained 32.22% of data variability and enabled observing treatment trends to show repeated behavior throughout crop seasons, a fact that was evidenced by the constant position of the 0 kg N ha^{-1} $year^{-1}$ dose (at the right side) in the spatial distribution. These results differed from those recorded for N doses of 40 kg N ha^{-1} $year^{-1}$ and 100 kg N ha^{-1} $year^{-1}$, which alternated in the most productive position between crop seasons and always headed left in the spatial distribution.

The PC2 explained 21.96% data variability and it was efficient in separating grapevines' productive behavior in each crop season, which was delimited by different ellipses. Thus, plants subjected to the application of 40 kg N ha^{-1} $year^{-1}$ and 100 kg N ha^{-1} $year^{-1}$ were mostly influenced by production variables such as yield, number of grape clusters and weight of 100 berries; as well as by several root parameters such as surface area, diameter, number of living roots and root production, which were positively correlated to each other. On the other hand, grapevines that were not subjected to N application mainly showed the influence of variables on quality parameters such as total anthocyanins and TSS. This variable of quality parameters also showed negative linear correlation to grape yield.

Discussion

The increased root surface area observed in plants subjected to N application may have happened due to increased content of mineral N forms (*i.e.* NH_4^+ and NO_3^-) in the soil which derived from the applied urea (Radville et al., 2016; Yan et al., 2017). Nitrogen application as fertigation probably promoted the movement of NO_3^-, a form of N common in subtropical soils (Barlow, Bond, Holzapfel, Smith, & Hutton, 2009; G. Brunetto et al., 2017), through to deeper soil layers and, consequently, reduced N losses due to volatilization. This outcome most likely resulted from the downward flow of water deriving from fertigation (Castellanosa et al., 2013), considering that the investigated soil was sandy with low OM content, a fact that facilitates the ion approximation through mass flow until the outer surface of the root system (Comas et al., 2010).

Increased NO_3^- concentration in soil solution stimulates the development of plant root system components, such as root diameter and surface area, two components sensitive to NO_3^- concentrations in the soil (Othman and Leskovar 2019). It also enables activating transporters, such as NRT1.1, in the root system, a fact that increases the amount of NO_3^- uptake by plants (Krouk et al., 2010; Pii et al., 2013; Remans et al., 2006). This NO_3^- root transporter is also capable of transporting auxin, under the control of NO_3^- in root-growth medium. Roots grown at high NO_3^- concentrations shows the preference for transporting NO_3^- over auxin, which accumulates in the roots and favors root growth (Mounier et al., 2014). Increased NO_3^- concentrations in root tissues also increases cytokinins metabolism rates (Silva and Delatorre 2009); cytokinines are important cell division regulators that lead to increased root system surface area (Centinari et al., 2016; Comas et al., 2010).

In the present study, the increased mineral N availability in the soil contributed to increase the mean root diameter in grapevines grown in soil subjected to N application rate of 40 and 100 kg N ha^{-1} year^{-1}. It may have happened due to decreased soil pH values, which reduced cell division and elongation (Barlow et al., 2009; Yan et al., 2017). Such process often takes place because H^+ is released during nitrification and stimulates the solubilization of Al^{+3} from soil native minerals (Miotto et al., 2019). Released Al^{+3} can bind to carboxylic groups and to pectic materials in the cell wall. This process increases the stiffness and decreases the extensibility of root cell walls; consequently, it can lead to disorganized cell division, which impairs root elongation and enables larger root diameter (Riaz et al., 2018; Zhou et al., 2014).

Nitrogen applications increased mineral N contents in the soil, a fact that stimulated root emission, as observed in the total number of living roots in grapevines subjected to N addition in both application modes. Roots preferentially grow in soil regions presenting the highest nutrient contents (Centinari et al., 2016), including mineral N (Lima, Kojima, Takahashi, & von Wirén, 2010). This because NO_3^- available in the soil acts as a signaling molecule that can regulate the expression of genes involved in plant hormonal balance (Lee et al., 2016; Xuan, Beeckman, & Xu, 2017). This process contributes to the production and development of new roots, mainly in soil regions where NO_3^- is available (Krouk et al., 2010; Remans et al., 2006).

The increased production and number of living roots in grapevines subjected to the highest N dose application (100 kg N ha^{-1} year^{-1}) can also be associated with strategies adopted by plants to uptake and accumulate N, such as amino acids and proteins, in perennial organs (Ortiz-Lopez, Chang, & Bush, 2000). The increased N availability in the soil increases the likelihood of nutrient uptake; however, in addition to N supply, root emission and renewal processes also depend on the internal carbon (C) balance of grapevines (Jagodzinski and Kalucka 2011; Yan et al. 2017). In periods of higher metabolic activity, such as intense annual plant growth, part of N can be redistributed to shoots and leaves, in order to enable biochemical and physiological processes in grapevines (Gustavo Brunetto et al., 2016; Zambrosi et al., 2012). However, senescence is expected to happen in part of the roots when most of the C found in plants is allocated to the shoot, as it happens in vegetative growth periods (Kou, Chen, et al., 2015; Kou, Guo, Yang, Gao, & Li, 2015). This was observed in grapevines subjected to 40 kg N ha^{-1} year^{-1} as N-Surf mode and to 100 kg N ha^{-1} year^{-1} as N-Fert mode, in the two evaluated crop seasons.

Increased N concentrations in leaves collected at flowering and at berry veraison can be attributed to increased mineral N content in the soil, in grapevines subjected to N applications. Part of N uptake by plants can be incorporated into C skeletons, such as amino acids, amines and proteins, which can be transported to leaves, which undergo intense cell division and elongation processes (Radville et al., 2016; Yu et al., 2012). This phenomenon, in association with the application of higher N rates such as 100 kg N ha^{-1} year^{-1}, led to increase of N concentrations in leaves of plants subjected to this treatment. In addition, large rainfall volumes recorded in concomitance of N applications, as observed in the 2016/17 crop season (Fig. 1), have stimulated NO_3^- movement in the soil solution, since NO_3^- links itself to reactive particles in the soil by means of external sphere complex (Comas et al., 2010; Li, Wang, & Stewart, 2013; Lorensini et al., 2012). This process may have stimulated the emission of new in-depth roots and increased grapevine N uptake (Gustavo Brunetto et al., 2016).

The increased number of grape clusters per plant and yield of grapevines subjected to the highest N application (100 kg N ha^{-1} $year^{-1}$) might be associated to a higher mineral N availability in the soil (Brunetto et al., 2016; Steenwerth and Belina, 2010), a fact that stimulated root growth to 60 cm. On the one hand, increased N concentrations in plant leaves stimulated photosynthetic processes, since higher concentrations of chlorophylls and chloroplast proteins, such as RuBisCO, enabled increased CO_2 fixation in leaf tissues, as well as increased plant growth (Blank et al., 2018; Moriwaki et al., 2019). On the other hand, plants needed to be photosynthetically active in order to enable NO_3^- uptake and reduction. Therefore, higher rates of photosynthesis can also favor the uptake of N to be increased, since enzymes responsible for reducing N were regulated by light, whereas the energy used in this process derived from chloroplasts (Krouk et al., 2010).

The highest total titratable acidity in the must of grapevines grown in soil subjected to the highest N applications may be explained by the inverse relationship between the total titratable acidity in the must and the vegetative growth of grapevine shoots. Increased leaf yield also increases the shading of clusters inside plants, which, in its turn, delays the ripening of grapes and decreases organic acid degradation in berries (Centinari et al., 2016; Vilanova, Fandiño, Frutos-Puerto, & Cancela, 2019). Decreased TSS values observed in the must of grapevines grown in soil subjected to the application of higher N doses resulted from the increased number of grape clusters per plant and, mainly, from the increased grape yield, which contributed to dilute the sugars in the berries (Guilpart, Metay, & Gary, 2014). In addition, decreased TSS levels in the must may have resulted from increased leaf area in grapevines subjected to higher N applications, since excessive leaf growth reduces direct sunlight incidence on berries, a fact that leads to lower sugars concentration (Sadras and Moran 2012). Nitrogen applications at the highest dose (100 kg N ha^{-1} $year^{-1}$) and N supply modes (N-Surf and N-Fert) led to TSS values lower than 14 ºBrix, which are not appropriate for winemaking processes (Chiarotti, Guerios, Cuquel, & Biasi, 2011). The application of 40 and 100 kg N ha^{-1} $year^{-1}$, in both N supply methods, also reduced total anthocyanin values in grape must. This outcome may be associated with increased number of grape clusters per plant and with grape yield, which is often associated with increased berry size and pulp/peel ratio. Thus, the concentration of important compounds, such as total anthocyanins in the must, which account for the reddish color of the must and, consequently, of the wine, gets diluted (Brunetto et al. 2009b; Sadras and Moran, 2012; Sofo et al. 2012). In addition, as plants' vegetative growth increases, part of the anthocyanins found in the clusters may have been redistributed to other growth sites and had their contents decreased in berries (Terrier, Poncet-Legrand, & Cheynier, 2009).

Results of the principal component analysis showed intrinsic climate variation in each crop season, which resulted in plants' productive response to the application of different treatments. It also showed annual variations in fruit yield and nutritional status. It happened because fruit plant species have a cyclical dynamics of C allocation to different organs that can favor yield in a given year and species maintenance in the following year, due to reserve accumulation in organs that require more energy for protein synthesis, mainly in plants subjected to N applications (Ceccon et al. 2016; Artacho and Bonomelli 2017). The PCA enabled a better understanding of the positive relationship among root system, plants yield, N application rates and modes. These results showed that as photoassimilates accumulated in grapevines roots, water and nutrient intake increased, a fact that improved crop yield (Antolín et al., 2003; Barlow et al., 2009; Comas et al., 2010). In addition to this process, there was a decrease in the oenological quality of the must, which was diagnosed based on decreased total anthocyanins and total soluble solids values, as well as on increased total acidity values.

Conclusion

The application of 40 kg N ha^{-1} year^{-1}, as N-Surf mode, provided adequate N supply to the soil and increased root system development, which enabled larger root surface area, larger number of living roots and greater root production. This process enabled an increase of soil N uptake by plants, higher N concentration in leaves and greater grape yield. However, N supply decreased the quality of the must, as observed in decreased TSS and total anthocyanins values and in increased total titratable acidity values, which are not desirable for red wine making processes.

Author contribution statement

Conceptualization, MSSK; data duration, MSSK, LOS and RS; formal analysis, MSSK, LOS and RS; funding acquisition, MT, EB and TLT; investigation, WSA, GAS, CFP, GS, JPJS and ROSS; project administration, GB; supervision, GB; writing—original draft, MSSK; writing—review and editing, LOS, RS, MT, EB, TLT and GB.

Conflicts of interest

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

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Table 1 Main physical and chemical characteristics of the soil in the experimental site at 0.0-0.20 m soil layer

Soil characteristics	0.0-0.20 m
Clay (Pipette method) (g kg^{-1})	63
Silt (Pipette method) (g kg^{-1})	107
Sand (Pipette method) (g kg^{-1})	830
Organic matter (Walkley Black method) (g kg^{-1})	10.0
pH in water (1:1 ratio)	5.5
Available P (Extracted by Mehlich-1) (mg kg^{-1})	20.0
Exchangeable K (Extracted by Mehlich-1) (mg kg^{-1})	65.0
Exchangeable Ca (Extracted by KCl 1 mol L^{-1}) ($cmol_c$ kg^{-1})	1.7
Exchangeable Mg (Extracted by KCl 1 mol L^{-1}) ($cmol_c$ kg^{-1})	0.8
Exchangeable Al (Extracted by KCl 1 mol L^{-1}) ($cmol_c$ kg^{-1})	0.0

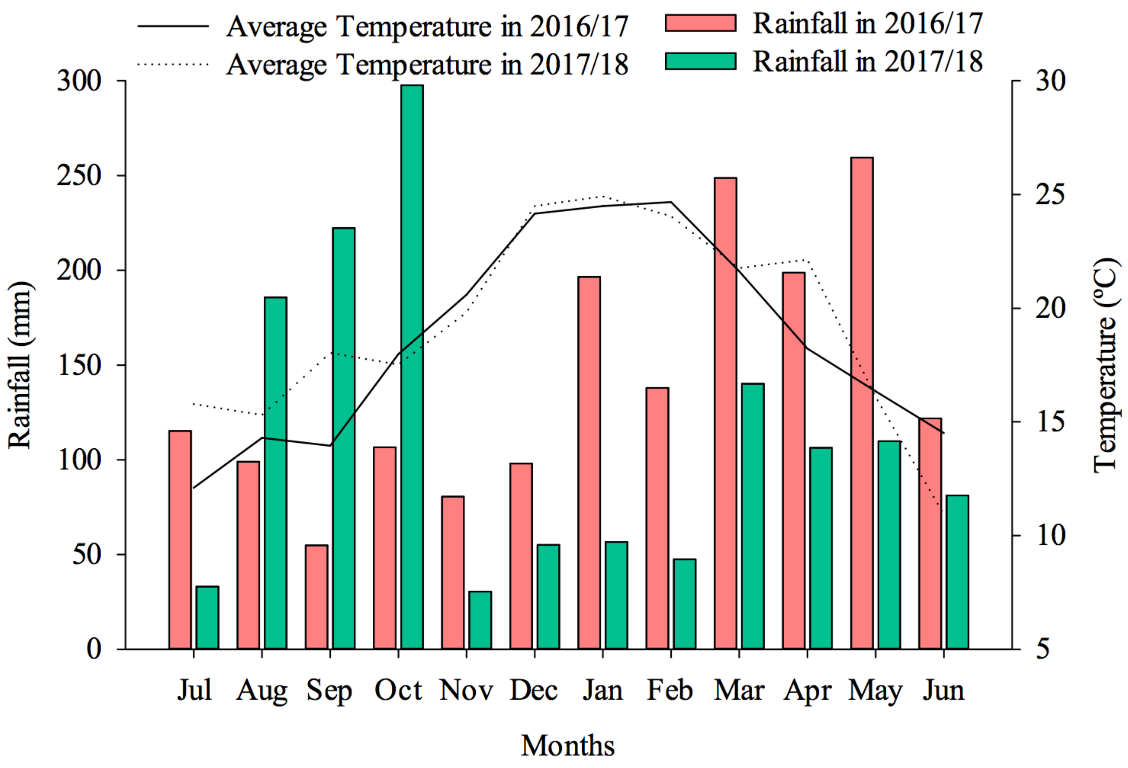


Fig. 1 Monthly accumulated rainfall (mm) and average monthly temperature (ºC) of the 2016/17 and 2017/18 crops season obtained at the experimental station in Santana do Livramento, state of Rio Grande do Sul, Brazil

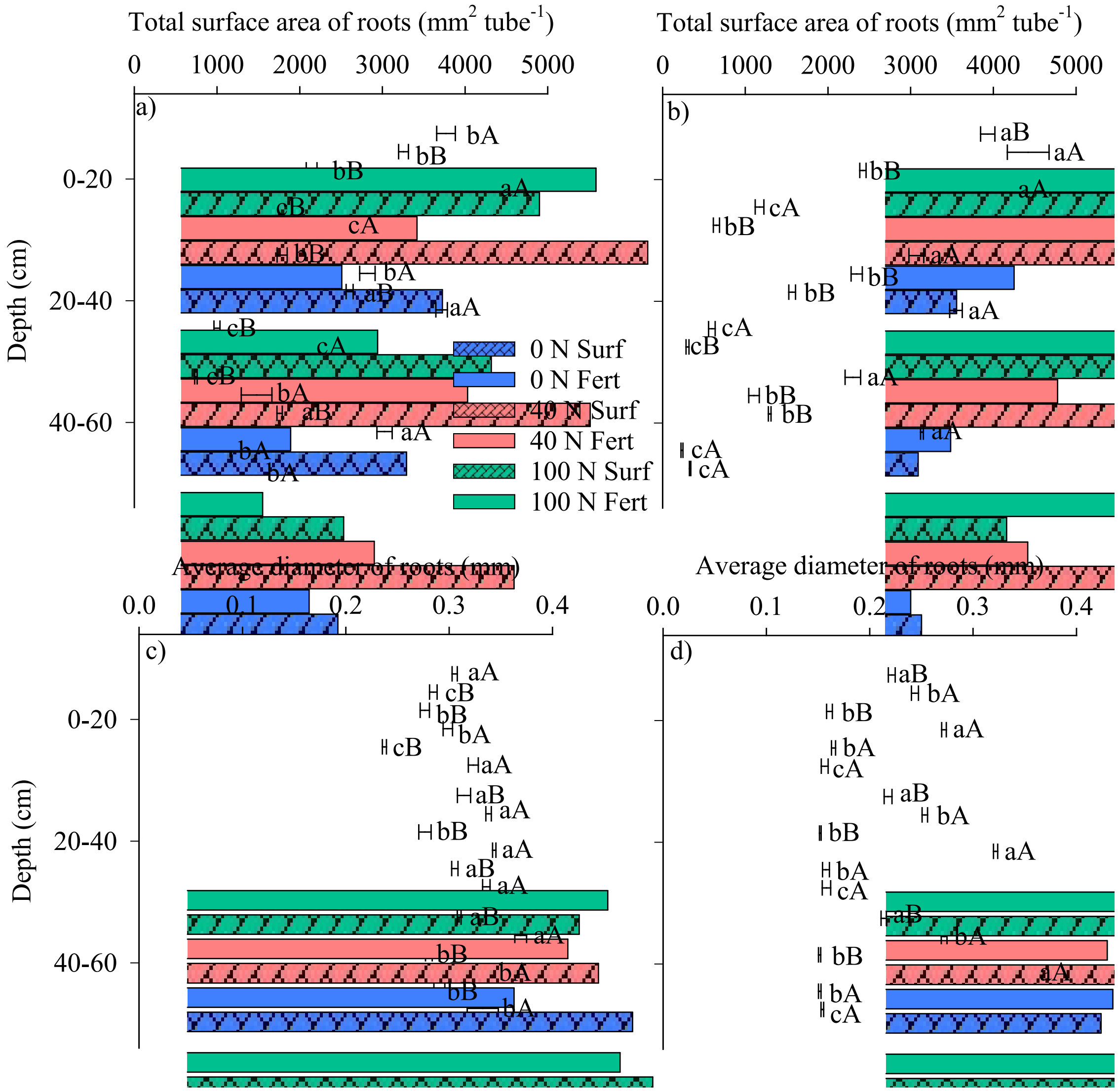


Fig. 2 Effect of N application rate and method on root total surface area in the 2016/17 (a) and 2017/18 crop season (b), average diameter of roots in the 2016/17 (c) and 2017/18 crop season (d) in 0-20, 20-40 and 40-60 cm soil layers, at flowering of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevine (N Surf - Nitrogen surface; N Fert - Nitrogen fertigation; 0, 40 and 100 kg N ha^{-1} year^{-1}). Horizontal bars indicate the standard error ($n = 4$). Different lower-case letters indicate a significant difference among N doses in the same application modes, and different upper-case letters indicate a significant difference among the N application modes in the same N doses by the Scott-Knott test ($p < 0.05$)

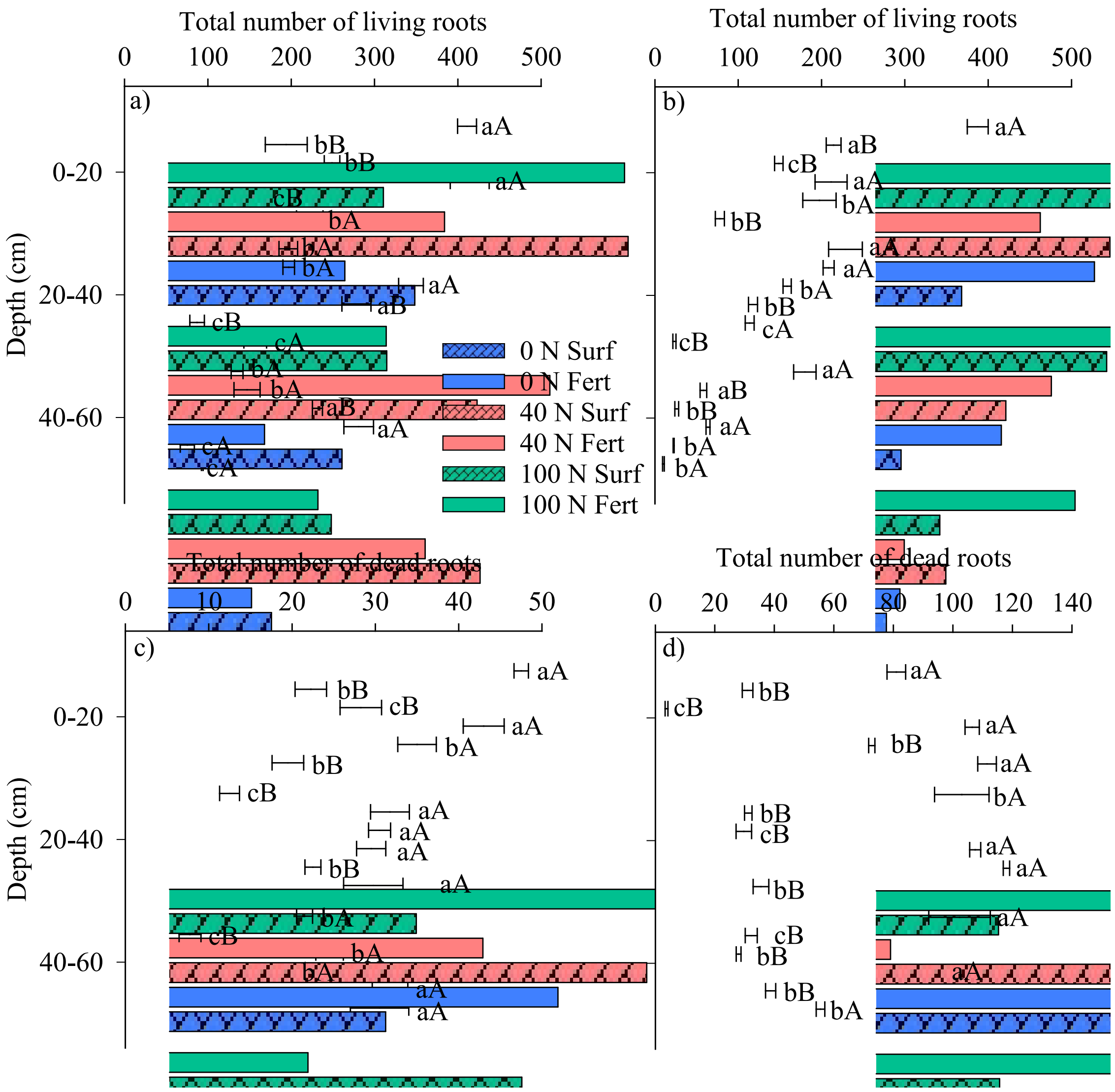


Fig. 3 Effect of N application rate and method on total number of living roots in the 2016/17 (a) and 2017/18 crop season (b); total number of dead roots in the 2016/17 (c) and 2017/18 crop season (d) in 0-20, 20-40 and 40-60 cm soil layers, at flowering of ‘Alicante Bouschet’ (*Vitis vinifera* L.) grapevine (N Surf - Nitrogen surface; N Fert - Nitrogen fertigation; 0, 40 and 100 kg N ha^{-1} $year^{-1}$). Horizontal bars indicate the standard error (n = 4). Different lower-case letters indicate a significant difference among N doses in the same application modes, and different upper-case letters indicate a significant difference among the N application modes in the same N doses by the Scott-Knott test (p < 0.05)

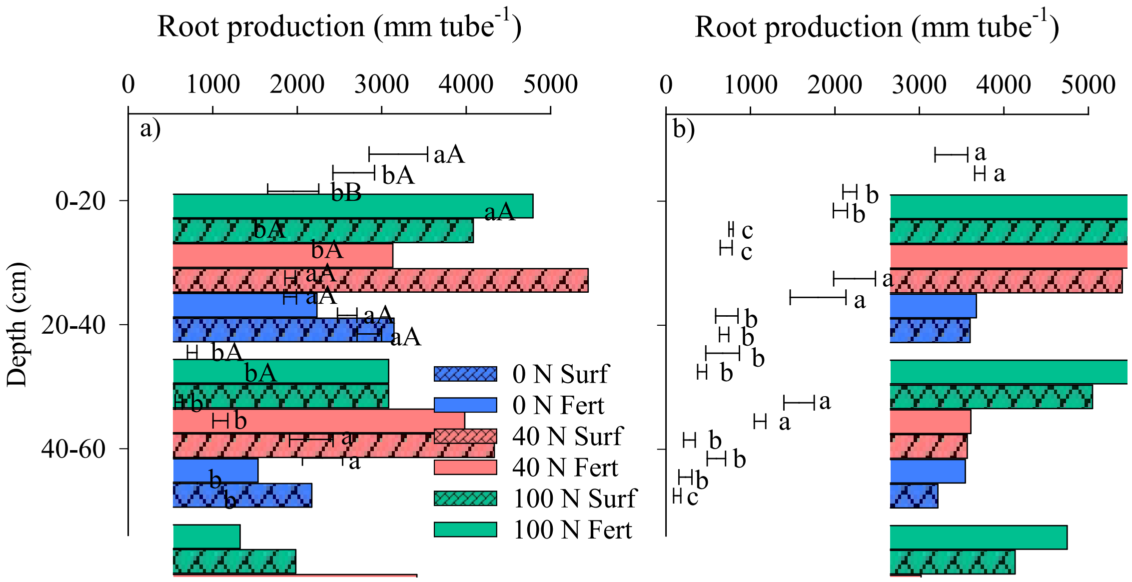


Fig. 4 Effect of N application rate and method on root production (mm tube^{-1}) in the 2016/17 (a) and 2017/18 crop season (b) at flowering of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevine (N Surf - Nitrogen surface; N Fert - Nitrogen fertigation; 0, 40 and 100 kg N ha^{-1} year^{-1}). Horizontal bars indicate the standard error (n = 4). Different lower-case letters indicate a significant difference among N doses in the same application modes, and different upper-case letters indicate a significant difference among the N application modes in the same N doses by the Scott-Knott test ($p < 0.05$)

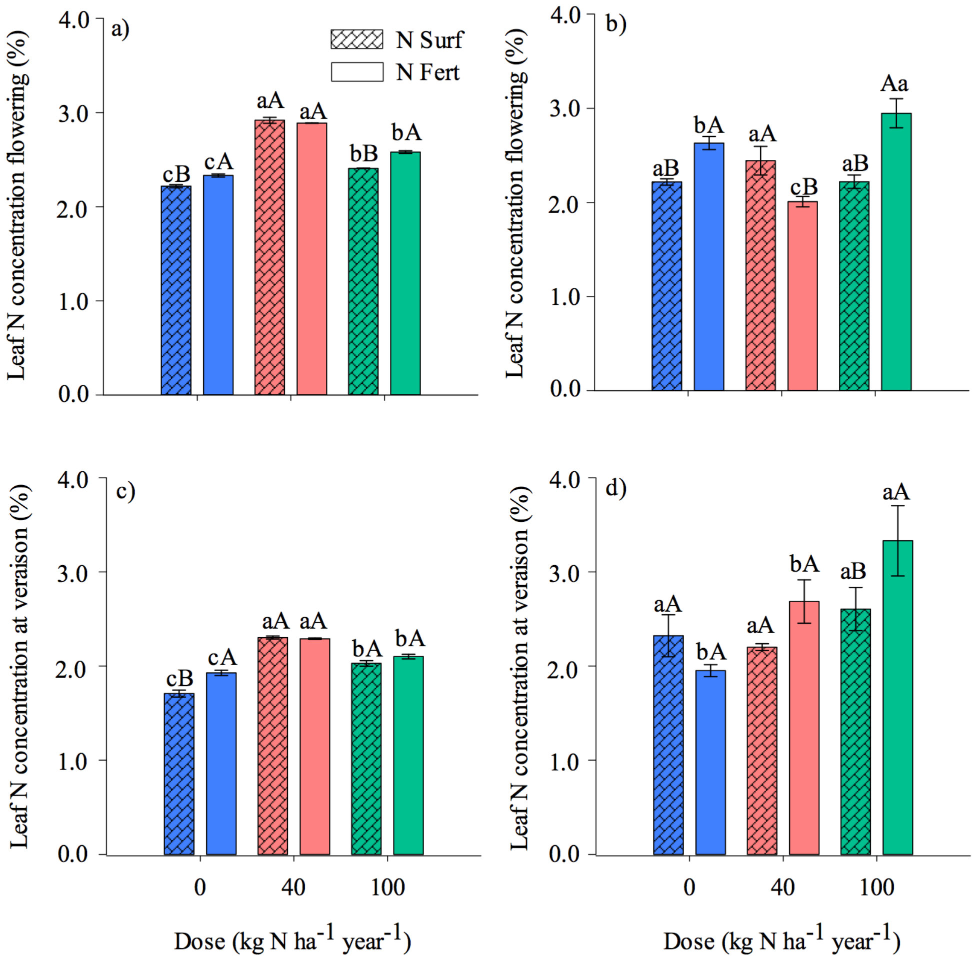


Fig. 5 Effect of N application rate and method on leaf N concentration at flowering in 2016/17 (a) and 2017/18 crop season (b); leaf N concentration at veraison in 2016/17 (c) and 2017/18 crop season (d) of ‘Alicante Bouschet’ (*Vitis vinifera* L.) grapevine (N Surf - Nitrogen surface; N Fert - Nitrogen fertigation; 0, 40 and 100 kg N ha^{-1} year^{-1}). The vertical bars indicate the standard error ($n = 4$). Different lower-case letters indicate a significant difference among N doses in the same application modes, and different upper-case letters indicate a significant difference among the N application modes in the same N doses by the Scott-Knott test ($p < 0.05$)

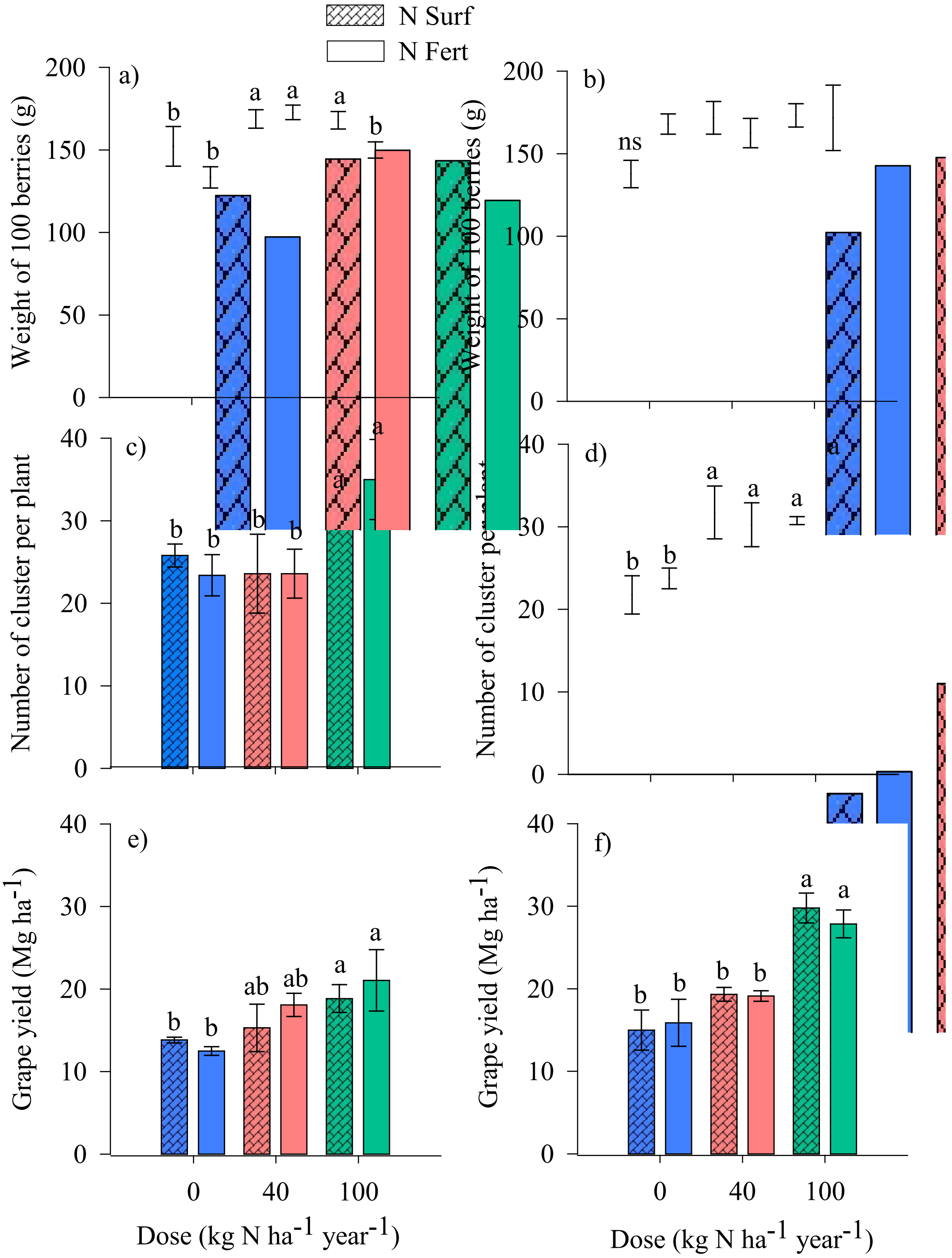


Fig. 6 Effect of N application rate and method on weight of 100 berries in 2016/17 (a) and 2017/18 crop season (b); number of cluster per plant in the 2016/17 (c) and 2017/18 crop season (d); grape yield in the 2016/17 (e) and 2017/18 crop season (f) of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevine (N Surf - Nitrogen surface; N Fert - Nitrogen fertigation; 0, 40 and 100 kg N ha^{-1} year^{-1}). The vertical bars indicate the standard error (n = 4). Different lower-case letters indicate a significant difference among treatments by the Tukey test ($p < 0.05$)

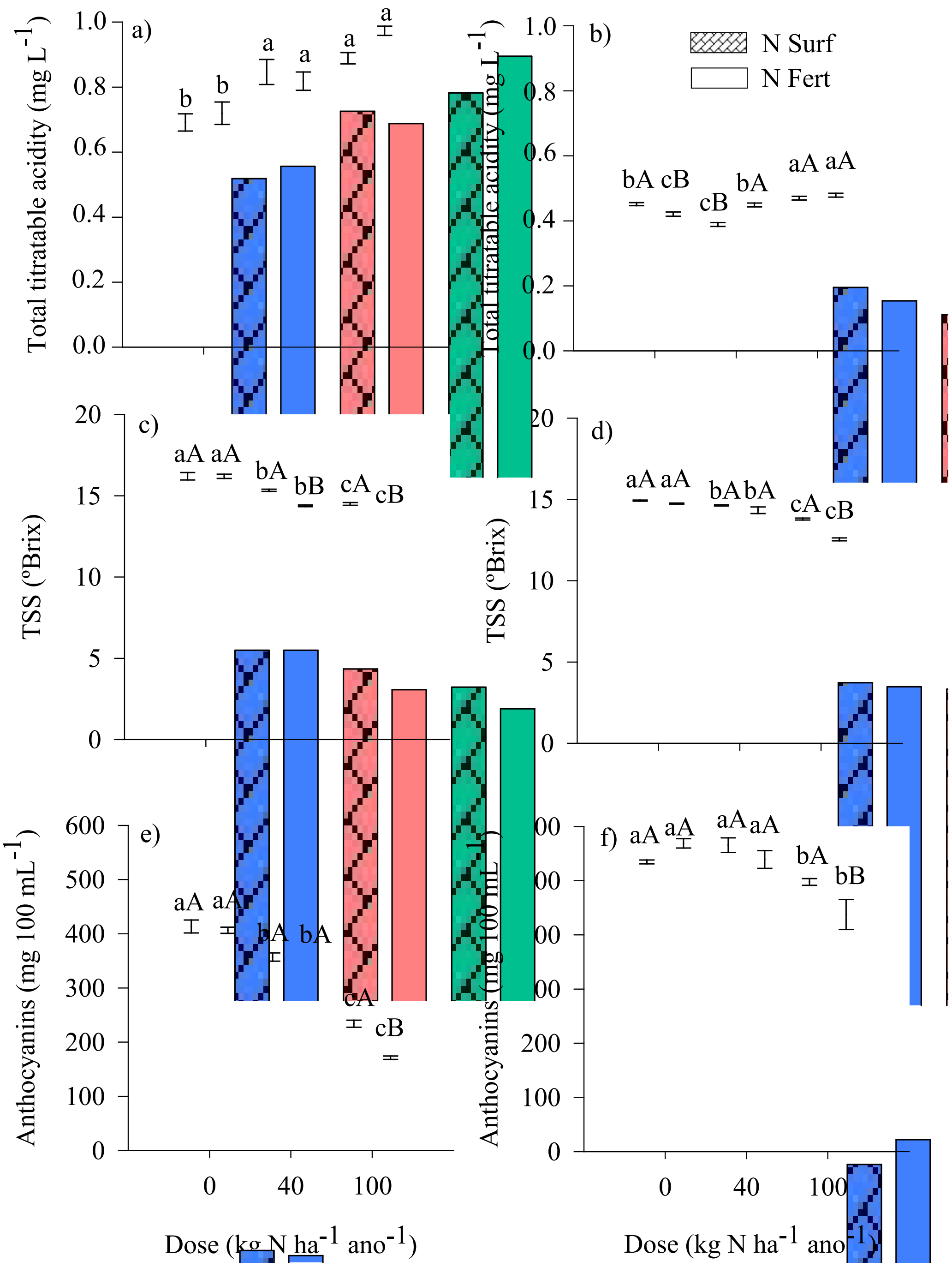


Fig. 7 Effect of N application rate and method on total titratable acidity in 2016/17 (a) and 2017/18 crop season (b); total soluble solids (TSS) in the 2016/17 (c) and 2017/18 crop season (d); total anthocyanins (Anthocyanins) in the 2016/17 (e) and 2017/18 crop season (f) of the must of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevine (N Surf - Nitrogen surface; N Fert - Nitrogen fertigation; 0, 40 and 100 kg N ha^{-1} year^{-1}). The vertical bars indicate the standard error (n = 4). Different lower-case letters indicate a significant difference among N doses in the same application modes, and different upper-case letters indicate a significant difference among the N application modes in the same N doses by the Scott-Knott test ($p < 0.05$)

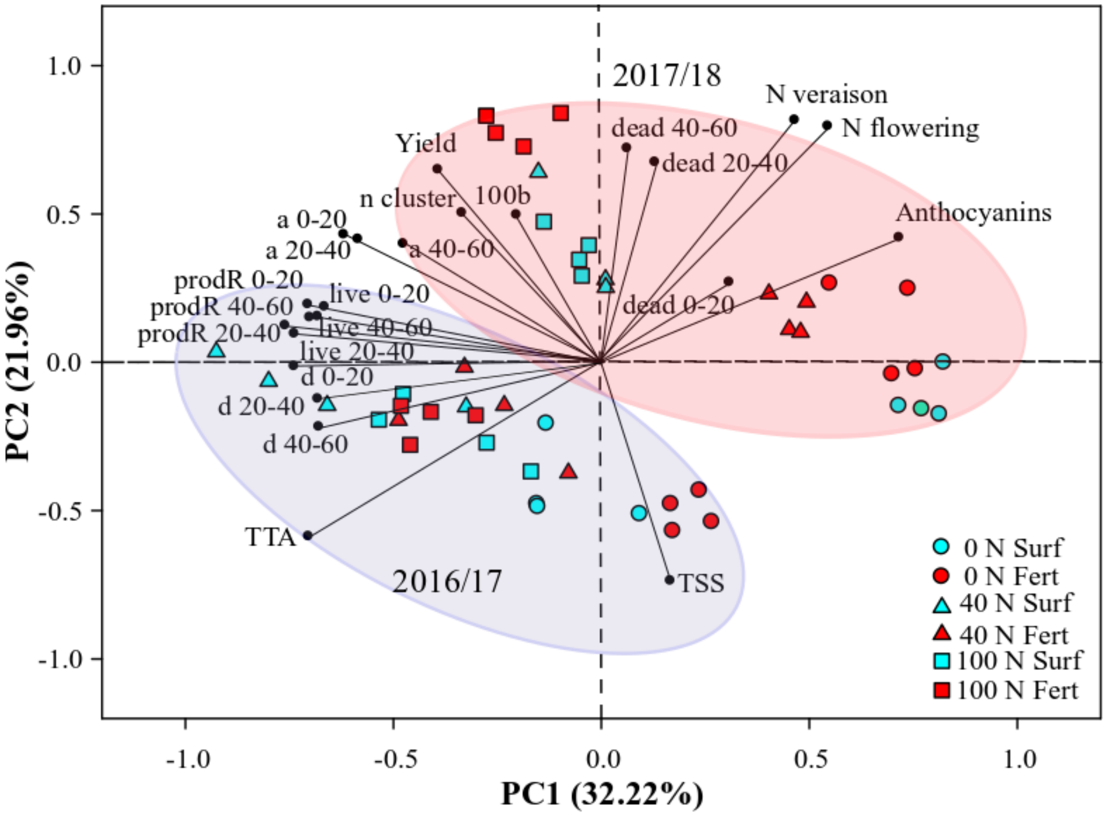
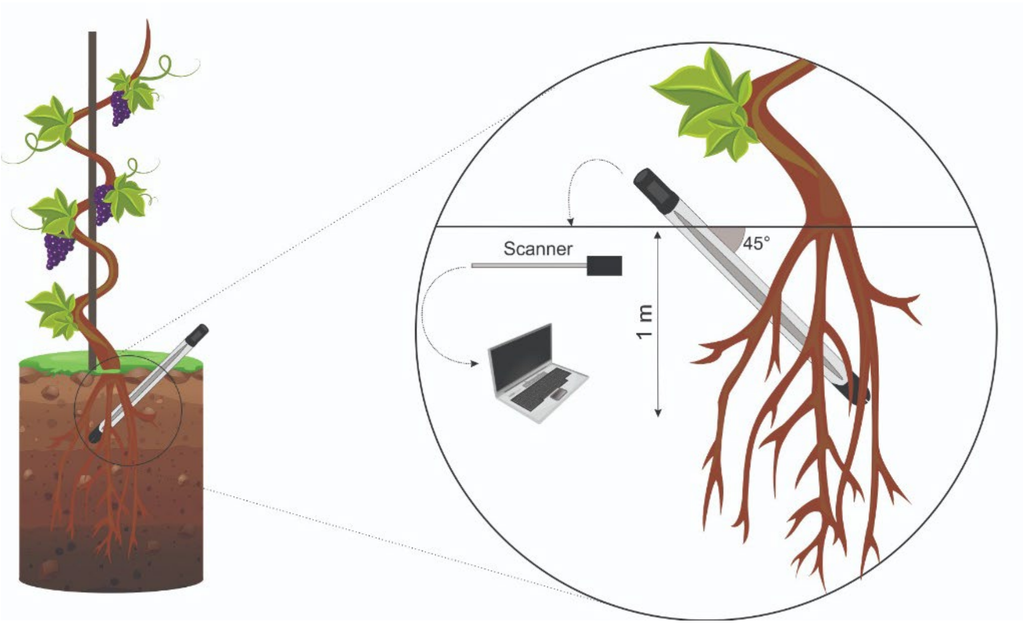


Fig. 8 Relationship between principal component 1 (PC1) and 2 (PC2) of the morphological parameters of the root system (total surface area of roots (a), average diameter of roots (d), total number of living roots (living), total number of dead roots (dead) and root production (prodR) in 0-20, 20-40 and 40-60 cm soil layers), leaf N concentration (leaf N concentration at flowering (N flowering) and leaf N concentration at veraison (N veraison)), grape yield parameters (grape yield (Yield), number of cluster per plant (n cluster), weight of 100 berries (100b)) and chemical parameters of the must (total anthocyanins (Anthocyanins), total soluble solids (TSS), total titratable acidity (TTA)) of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevine subjected to different N doses and application modes



Supplementary material 1. Schematic representation of the imaging system from minirizhotron tubes installed in the vineyard of the experimental area; *in situ* image capture high resolution (600 dpi) from a cylindrical scanner (Root Imager – CI600®).

Supplementary material 2. Analysis of variance of the roots parameters, leaf N concentration, grape yield and chemical of the must of 'Alicante Bouschet' (*Vitis vinifera* L.) grapevine subjected to different N doses and application modes.

Variables			**Effect**		
	Soil layer (cm)	**Crop season**	**Mode**	**Dose**	**Interaction**
Total surface area of roots (mm^2 $tube^{-1}$)	0-20	2016/17	***	***	***
		2017/18	***	***	***
	20-40	2016/17	***	***	
		2017/18	***	***	***
	40-60	2016/17	***	***	***
		2017/18	***	***	***
Average diameter of roots (mm)	0-20	2016/17	***	**	***
		2017/18	***	***	***
	20-40	2016/17	***	**	***
		2017/18	***	***	***
	40-60	2016/17	***	***	
		2017/18	***	***	***
Total number of living roots	0-20	2016/17		***	***
		2017/18	***	***	***
	20-40	2016/17		***	***
		2017/18	***	***	**
	40-60	2016/17	**	***	
		2017/18	***	***	***
Total number of dead roots	0-20	2016/17	***	**	***
		2017/18	***	***	***
	20-40	2016/17	***	**	***
		2017/18	***		***
	40-60	2016/17	***	***	*
		2017/18	*	***	***
Root production (mm $tube^{-1}$)	0-20	2016/17	*	***	**
		2017/18		***	
	20-40	2016/17	**	***	
		2017/18		***	
	40-60	2016/17		***	
		2017/18		***	**
Leaf N concentration at flowering (%)		2016/17	***	***	***
		2017/18	*	**	***
Leaf N concentration at veraison (%)		2016/17	***	***	***
		2017/18		**	*
Grape yield (Mg ha^{-1})		2016/17		***	
		2017/18		***	
Number of cluster per plant		2016/17		**	
		2017/18		***	
Weight of 100 berries (g)		2016/17		***	
		2017/18			
Total anthocyanins (mg $100mL^{-1}$)		2016/17	***	***	**
		2017/18		***	*
Total soluble solids (°Brix)		2016/17	***	***	***
		2017/18	***	***	***
Total titratable acidity (mg L^{-1})		2016/17		***	
		2017/18	*	***	***

*0.05; **0.01; ***<0.001