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

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1	Root morphology, yield and must composition of grapevine subjected
2	to application of urea methods and rates
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17	
18	Abstract
19	Nitrogen (N) is often applied to soil surface during the grapevine cycle, increases N
20	losses. One way to reduce N losses lies on its application method, however how N dose
21	and application mode impact on grapevines remain poorly investigated. The aim of this
22	study is to evaluate the root growth, as well as grape must yield and composition, in a
23	sandy soil and subjected to different N application doses and modes under subtropical

24 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 25

and 100 kg N ha<sup>-1</sup> year<sup>-1</sup>. Leaf N concentration, yield, must quality parameters and root 26 27 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<sup>-1</sup> year<sup>-1</sup>), in the N-Surf and N-Fert 28 29 methods, increased the surface area of root, number of living roots and root production. 30 The application of N rates, in both N application modes, provided adequate N supply to 31 the soil and increased root system development, which contributed to the increase of 32 soil N uptake by plants, higher N concentration in leaves and greater grape yield. 33 However, N supply decreased the quality of the must, as observed in decreased TSS and 34 total anthocyanins values and in increased total titratable acidity values, which are not 35 desirable for red wine making processes.

36

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

39

### 40 Introduction

41 Grapevines are grown worldwide in sandy soils that often have low organic 42 matter (OM) content, a fact that leads to low nitrogen (N) availability in the soil 43 (Gustavo Brunetto et al., 2016, 2014; Lorensini et al., 2017). Thus, applications of N 44 fertilizer are necessary to increase soil mineral N availability, *i.e.* nitrate (NO<sub>3</sub><sup>-</sup>) and 45 ammonium (NH $_4^+$ ) forms, which are the ones uptake by plants. Soil surface (without 46 incorporation) N applications are often recommended, however this approach may 47 increase N losses, mainly due to volatilization (Lorensini et al., 2012; Viero, Bayer, 48 Mara Vieira Fontoura, Paulo de Moraes, & Professor, 2014), and decrease grapevines N 49 uptake (Gustavo Brunetto et al., 2016; Gustavo Brunetto, Kaminski, Melo, Brunning, & 50 Mallmann, 2006). Therefore, it is necessary adopting alternative ways of supplying N to

51 decrease losses and increase N use efficiency. Fertigation is one of these alternatives; it 52 enables urea solubilization and hydrolysis reaction, decreases losses caused by 53 volatilization, since it favors the migration of  $NO_3^-$  and  $NH_4^+$  in soil profile (Comas, 54 Bauerle, & Eissenstat, 2010).

55 Increased mineral N contents in soil subsurface layers can contribute plant root 56 system growth and development (Centinari, Heuvel, Goebel, Smith, & Bauerle, 2016; Comas et al., 2010; Zhou, Yang, Ren, Huang, & An, 2014). Increased NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> 57 58 concentrations in soil solution activates NO<sub>3</sub><sup>-</sup> (NRTs) and NH<sub>4</sub><sup>+</sup> (AMTs) transporters 59 and, consequently, contributes for N uptake (Pii, Aldrighetti, Valentinuzzi, Mimmo, & 60 Cesco, 2019; Tomasi, Monte, Varanini, Cesco, & Pinton, 2015; Xu, Fan, & Miller, 61 2012) and for the increase of grapevine root emission and root surface area (Krouk et 62 al., 2010; Remans et al., 2006). In addition, high  $NO_3^-$  concentrations can inhibit auxin 63 transport in the root system due to competition at the NRT1.1 transporter site; thus, 64 auxin accumulates in plant roots and leads to an increase of lateral root growth in sites 65 presenting high NO<sub>3</sub><sup>-</sup> concentrations (Mounier, Pervent, Ljung, Gojon, & Nacry, 2014). 66 On the other hand, high N doses can decrease soil pH due to NH<sub>4</sub><sup>+</sup> nitrification into NO<sub>3</sub><sup>-</sup>. This phenomenon, which is often seen in vineyards (Lorensini et al., 2017), can 67 solubilize minerals in the soil and release aluminum  $(A1^{+3})$  into soil solution; 68 69 consequently, Al<sup>+3</sup> can be uptake by plant roots and lead to root length shortening and to 70 root diameter increase (Inostroza-Blancheteau, Aquea, Reyes-Díaz, Alberdi, & Arce-71 Johnson, 2011; Riaz et al., 2018; Zhou et al., 2014). This outcome is undesirable 72 because it reduces the volume of soil explored by the root system, as well as the 73 absorption of water and nutrients (Riaz et al., 2018).

74 Mineral N provided by N fertilization can be uptake and transported to plant 75 shoot, increasing N content in the leaves. This process occurs mainly in stages of 76 intense root growth, such as at flowering, when the increased root volume contributes to 77 great N uptake (Radville et al., 2016). Increased leaf N content can lead to an increased photosynthetic rate and CO<sub>2</sub> fixation, since RuBisCo corresponds to 50% of soluble 78 79 proteins in the leaves of C3 plants (Stefano, Agyei, Njoku, & Udenigwe, 2018). This 80 outcome favors cell division and elongation conditions in plant tissues and increases 81 vegetative growth (Blank, Tittmann, Ghozlen, & Stoll, 2018; Moriwaki et al., 2019) in 82 grape clusters, resulting in greater crop yield (Gustavo Brunetto et al., 2009a; Yu et al., 83 2012).

In addition to increased crop yield, there may be changes in oenological 84 85 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 86 87 titratable acidity values (Gustavo Brunetto et al., 2009a; Janjanin et al., 2016; Kelly et 88 al., 2017; Spayd, Nagel, & Edwards, 1995). Thus, it is recommended adopting 89 strategies, such as N doses and application modes, to stimulate root system growth in 90 order to increase water and nutrient absorption by plants, as well as to increase crop 91 yield without decreasing the quality of must. The aim of the current study was to 92 investigate whether N rates and modes of application can change root system 93 morphology, yield and the composition of grape must in grapevines (Vitis vinifera L.) 94 grown in sandy soil under subtropical climate conditions.

95

#### 96 Materials and Methods

### 97 Experimental site description

98 The experiment was conducted in a vineyard established in 2011, in Santana do
99 Livramento County, Rio Grande do Sul State (RS), Southern Brazil (30° 48' 31" S; 55°
100 22' 33" W). Alicante Bouschet (*Vitis vinifera* L. grafted onto Paulsen 1103 rootstock)

101 was the adopted cultivar. Plant density was 2,976 hectare<sup>-1</sup> (2.8 m between rows  $\times$  1.2 102 m between plants), based on the espalier system. Soil in the experimental site was 103 classified as Typic Hapludalf (Soil Survey Staff, 2014); the main chemical properties of 104 the 0.0-0.20 m layer before the experiment are shown in Table 1.

105 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 106 107 throughout the year. Mean annual rainfall is 1,600 mm, whereas mean temperature is 108 23.8° C in the warmest month (January) and 12.4° C in the coldest month (July) 109 (Alvares, Stape, Sentelhas, De Moraes Gonçalves, & Sparovek, 2013). Annual 110 insolation reaches approximately 2,500 hours; the site presents gently undulating 111 terrain. Two crop production cycles, 2016/17 and 2017/18, were evaluated. According 112 to data from the National Institute of Meteorology (INMET), the experimental area 113 recorded 513.2 mm and 189.6 mm of rainfall from November 2016 to February 2017 114 and from November 2017 to February 2018 (period between treatment application and 115 harvest), respectively (Fig. 1).

116 Weeds in the grapevine row were desiccated with two to four sequential applications of 2 L ha<sup>-1</sup> y<sup>-1</sup> of non-selective systemic herbicide (*i.e.*, glyphosate), 117 118 depending on cover plant development. Inter-row cover plants were cut near the soil 119 surface, five times throughout each crop season and the residues were deposited on soil 120 surface between rows. Grapevines were subjected to four applications of 45 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-</sup> <sup>1</sup> year<sup>-1</sup> and 45 kg K<sub>2</sub>O ha<sup>-1</sup> year<sup>-1</sup> prior to experiment installation (COFS-RS/SC, 2016). 121 122 Triple superphosphate (42% P) and KCl (60% K) were used as P and K sources, 123 respectively. Nitrogen fertilizers were applied to the crop based on each treatment.

- 124
- 125

### 126 Experimental design and treatments

127 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<sup>-1</sup> year<sup>-1</sup> were evaluated. The 128 129 experiment followed a complete randomized block experimental design with three 130 repetitions (plot) with 2 x 3 factorial arrangement (i.e., two N application modes and 131 three N rates). Each plot consisted of five plants were only the three central plants were 132 used for data collection. In both application methods, urea was employed as N source 133 (45% N); in N-Surf treatment, N was applied on the surface of the soil, within plant 134 canopy projection, without incorporation. Every year, N application was performed 135 during flowering (Gustavo Brunetto et al., 2009a). In N-Fert treatment, N was applied via drip irrigation (Netafim<sup>™</sup> 132 Dripnet PC AS 16250), at a flow rate of 1.6 L h<sup>-1</sup>; 136 drippers were spaced 0.6 m a part along the tree row, within canopy projection. The 137 138 applications were performed at four different times, at 7-day intervals, according to the 139 following procedure: 10 minutes of water; 10 minutes of water + N; and 10 minutes of 140 water. Applications N-Fert provided 6.4 L of water per plant per crop season.

141

## 142 Minirhizotron installation

143 Minirhizotron tubes (Supplementary material 1) were installed in the ground of 144 four plants per treatment in May 2016 to enable scanning the grapevine root system in 145 situ. For each assessed grapevine plant, a hole was made with a spiral drill (70 mm 146 diameter and 1,500 mm length), pulled by motorized ground driller (Branco, Bps 52), 147 0.5 m away from the trunk, inside the canopy projection. Each individual hole was 148 made at an angle of 45° to soil surface and a clear acrylic tube (70 mm x 1050 mm) was 149 inserted. Three images were taken along each tube, referring to the soil layers: 0-20; 20-150 40; and 40-60 cm deep. Each image had fixed dimensions of 216 mm × 196 mm. A

151 flexible, lightweight, waterproof and non-toxic plastic polyethylene rod (CID Bio-152 Science, Inc. Camas, WA, USA) internally protected the tubes. PVC pipes (100 mm 153 diameter) were used to protect the external part of the tubes from damage caused by 154 weather, handling or pest attacks. Soil disturbance during the hole drilling process made 155 it necessary waiting eight months for soil-plant-tube system stabilization, before the 156 beginning of the scanning activities.

157

### 158 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<sup>™</sup> cylindrical scanner (CID Bio-Science, Inc. Camas, WA, USA).

165 Subsequently, images were captured at different soil layers (0-20, 20-40 and 40-166 60 cm deep) and individually analyzed. The CI-600 scanner was connected to a 167 computer by USB cable. Images captured in TIFF format (600 dpi resolution) were analyzed in the RootSnap!<sup>®</sup> CI-690 software version 1.3.2.25 (CID Bio-Science, Inc. 168 169 Camas, WA, USA), which enables seeing the fine roots ( $\leq 2$  mm). Parameters evaluated 170 in each tube based on generated images were (i) root surface area (mm<sup>2</sup> tube<sup>-1</sup>); (ii) 171 mean root diameter (mm tube<sup>-1</sup>); (iii) number of living roots; (iv) number of dead roots; 172 and (v) root production as the difference between the total length of living and dead 173 roots (mm tube<sup>-1</sup>).

174

### 175 Leaf collection for nitrogen analysis

176 Six fully expanded leaves located in the opposite direction of the grape clusters, 177 in the middle third of the annual branches, were collected at flowering (October 2016 178 and 2017) and at berry veraison (December 2016 and 2017). According to CQFS-179 RS/SC (2016), these leaves are the diagnostic organ used to assess the nutritional status 180 of grapevines. Leaves were washed in distilled water and dried in forced-air-circulation 181 oven at 65 °C, until they reached constant weight. Then, they were milled in Wiley mill 182 and sieved through 2-mm mesh. Subsequently, the leaf tissue was prepared and 183 subjected to sulfuric digestion (Tedesco, Gianello, Bissani, Bohnen, & Volkweiss, 184 1995). Total N concentration was determined in Kjeldahl semi-micro steam distiller 185 (Tecnal, TE-0363, Brazil).

186

### 187 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<sup>-1</sup>). 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.

195

## 196 Chemical analysis of the must

197 The stored berries were peeled and only the pulp and seeds were analyzed. A 198 benchtop refractometer (Cosmo, Type K - 32) was used to determine the total soluble 199 solids (TSS) based on standards set by AOAC (2005). Total titratable acidity was 200 determined by transferring 10 mL of extract to a 100 mL volumetric flask, whose

1(

volume was completed with distilled water. For the titration of the resulting solution 0.1
mol L<sup>-1</sup> of sodium hydroxide (NaOH) was used (IAL, 2008).

203 Berry peel was used to determine total anthocyanin contents through a contact 204 with a 70:30 acidified (1% HCl) ethanolic solution (Ju and Howard 2003) at 205 peel/solution ratio of 1:3 (m/v) and crushed in a blender (Arno, Clic Lav Top, Brazil) at 206 low (1500 RPM) and high speed (3500 RPM) for 40 seconds each. The solution 207 resulting from the aforementioned extraction was placed in a 250 mL beaker and left to 208 rest for 30 minutes at room temperature ( $20 \pm 1$  °C) before it was centrifuged at 3500 209 RPM for 5 minutes. Extract supernatant was removed and stored in amber bottle, which 210 was placed in refrigerator (4-10°C). Subsequently, triplicate readings of these extracts 211 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<sup>-1</sup> potassium chloride at pH 1.0 and 0.4 mol L<sup>-1</sup> 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):

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

219 Pigment concentration in the extract was calculated and represented in cyanidine-3-220 glycoside through equation (2).

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

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

224

#### 226 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.

233

### 234 **Results**

# 235

# 5 Root morphological parameters

236 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<sup>-1</sup> year<sup>-1</sup> 237 <sup>1</sup> as N-Surf and of 100 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Fert (Fig. 2a). As for the 2017/18 crop 238 239 season, the highest root surface areas were observed in grapevines subjected to the application of 40 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Surf and of 100 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Surf and 240 241 N-Fert (Fig. 2b). In the 2016/17 crop season, the highest root surface area, recorded in 242 the 20-40 cm and 40-60 cm layers was observed in grapevines subjected to the application of 40 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Surf (Fig. 2a); whereas in the 2017/18 crop 243 244 season, the highest values recorded were observed in grapevines subjected to application of 100 kg N ha<sup>-1</sup> year<sup>-1</sup> and 40 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Fert and N-Surf, 245 246 respectively (Fig. 2b).

In the 2016/17 crop season, the highest mean root diameters, recorded in the 0-248 20 cm layer were observed at rate of 0 kg N ha<sup>-1</sup> year<sup>-1</sup> and 100 kg N ha<sup>-1</sup> year<sup>-1</sup>, as N-249 Surf and N-Fert, respectively (Fig. 2c). On the other hand, the highest root diameters, 250 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<sup>-1</sup> year<sup>-1</sup>) 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<sup>-1</sup> year<sup>-1</sup> in the N-Surf mode and to 100
kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Fert mode (Fig. 2d).

255

# 256 Root production and mortality

257 In 2016/17 and 2017/18 crop seasons, the highest number of living roots, 258 recorded in the 0-20 cm layer, were observed in plants subjected to 40 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Surf and to 100 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Fert mode (Fig. 3a, b). In the 2016/17 crop 259 260 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<sup>-1</sup> year<sup>-1</sup> as N-Surf and N-261 262 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<sup>-1</sup> 263 year<sup>-1</sup> as N-Surf and N-Fert (Fig. 3b). In 40-60 cm layer, the highest number of living 264 265 roots was in the 100 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Fert (Fig. 3b).

266 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<sup>-1</sup> year<sup>-1</sup> as N-Surf 267 mode and to 100 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Fert mode (Fig. 3c). In the 2017/18 crop season, 268 269 the highest number of dead roots, recorded in the 0-20 cm layer, were observed in grapevines subjected to 100 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Fert and to 0 kg N ha<sup>-1</sup> year<sup>-1</sup> and 40 270 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Surf (Fig. 3d). In the 2016/17 crop season, the highest number of 271 272 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-273 274 Surf mode (Fig. 3c). In the 2017/18 crop season, the highest number of dead roots, 275 recorded in the 20-40 cm layer, were observed in grapevines grown in control soil (0 kg N ha<sup>-1</sup> year<sup>-1</sup>) N-Fert and in soil subjected to the application of 40 kg N ha<sup>-1</sup> year<sup>-1</sup> as NSurf 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 NFert 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<sup>-1</sup> year<sup>-1</sup> as N-Fert and to
40 kg N ha<sup>-1</sup> year<sup>-1</sup> 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<sup>-1</sup> year<sup>-1</sup> as N-Surf and to 100 kg N ha<sup>-1</sup> year<sup>-1</sup> 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<sup>-1</sup> year<sup>-1</sup> as N-Surf and N-Fert (Fig. 4b).

288

### 289 Leaf N concentration

290 In the 2016/17 crop season, the highest N concentrations, in leaves collected at 291 flowering and at berry veraison were observed in grapevines subjected to application of 40 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Surf and N-Fert (Fig. 5a, c). In the 2016/17 crop season, N 292 293 concentration in leaves, collected at flowering were significant difference between N 294 supply methods, except for rate of 100 kg N ha<sup>-1</sup> year<sup>-1</sup>, and at veraison there was a significant difference between the N supply methods for 0 kg N ha<sup>-1</sup> year<sup>-1</sup>. In the 295 296 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<sup>-1</sup> year<sup>-1</sup> as 297 298 N-Fert (Fig. 5b, d). In 2017/18 crop season, N concentrations in leaves collected at 299 flowering and at berries veraison in grapevines subjected to N-Surf did not show 300 significant difference among N rates (Fig. 5b, d).

### 302 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<sup>-1</sup> year<sup>-1</sup>, than 40 kg N ha<sup>-1</sup> year<sup>-1</sup>, 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<sup>-1</sup> year<sup>-1</sup> (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<sup>-1</sup>) (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<sup>-1</sup> year<sup>-1</sup> as N-Surf and N-Fert (29.77 and 27.86 Mg ha<sup>-1</sup>, respectively) (Fig. 6f). In the 2016/17 and 2017/18 seasons, crop yield was not statistically affected by N application modes (Supplementary material 2).

324

# 325 Chemical parameters of the must

326 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<sup>-1</sup> year<sup>-1</sup> as N-Fert (Fig. 7a), 327 328 whereas in the 2017/18 crop season, the highest total titratable acidity was recorded in 329 grape musts subjected to the application of the same N dose, in both N-Fert and N-Surf 330 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<sup>-1</sup> year<sup>-1</sup>) (Fig. 7c, 331 332 d). In the 2016/17 crop season, the highest total anthocyanin were observed in grape 333 that were not fertilized (Fig. 7e), whereas in the 2017/18 crop season, the highest total 334 anthocyanin values recorded were observed in grapevines subjected to the application of 0 kg N ha<sup>-1</sup> year<sup>-1</sup> and 40 kg N ha<sup>-1</sup> year<sup>-1</sup>, for both N application modes (N-Surf and N-335 336 Fert modes) (Fig. 7f).

- 337
- 338

### Principal Component Analysis (PCA)

339 The sum of components PC1 and PC2 explained 54.18% of original data 340 variability (Fig. 8). The PC1 explained 32.22% of data variability and enabled 341 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<sup>-1</sup> year<sup>-1</sup> dose (at the right 342 343 side) in the spatial distribution. These results differed from those recorded for N doses 344 of 40 kg N ha<sup>-1</sup> year<sup>-1</sup> and 100 kg N ha<sup>-1</sup> year<sup>-1</sup>, which alternated in the most productive 345 position between crop seasons and always headed left in the spatial distribution.

346 The PC2 explained 21.96% data variability and it was efficient in separating 347 grapevines' productive behavior in each crop season, which was delimited by different ellipses. Thus, plants subjected to the application of 40 kg N ha<sup>-1</sup> year<sup>-1</sup> and 100 kg N 348 ha-1 year-1 were mostly influenced by production variables such as yield, number of 349 350 grape clusters and weight of 100 berries; as well as by several root parameters such as

351 surface area, diameter, number of living roots and root production, which were 352 positively correlated to each other. On the other hand, grapevines that were not 353 subjected to N application mainly showed the influence of variables on quality 354 parameters such as total anthocyanins and TSS. This variable of quality parameters also 355 showed negative linear correlation to grape yield.

356

# 357 **Discussion**

358 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 359 360 the soil which derived from the applied urea (Radville et al., 2016; Yan et al., 2017). 361 Nitrogen application as fertigation probably promoted the movement of NO<sub>3</sub>, a form of 362 N common in subtropical soils (Barlow, Bond, Holzapfel, Smith, & Hutton, 2009; G. 363 Brunetto et al., 2017), through to deeper soil layers and, consequently, reduced N losses 364 due to volatilization. This outcome most likely resulted from the downward flow of 365 water deriving from fertigation (Castellanosa et al., 2013), considering that the 366 investigated soil was sandy with low OM content, a fact that facilitates the ion 367 approximation through mass flow until the outer surface of the root system (Comas et 368 al., 2010).

Increased NO<sub>3</sub><sup>-</sup> concentration in soil solution stimulates the development of plant root system components, such as root diameter and surface area, two components sensitive to NO<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> uptake by plants (Krouk et al., 2010; Pii et al., 2013; Remans et al., 2006). This NO<sub>3</sub><sup>-</sup> root transporter is also capable of transporting auxin, under the control of NO<sub>3</sub><sup>-</sup> in root-growth medium. Roots grown at high NO<sub>3</sub><sup>-</sup> concentrations shows the 376 preference for transporting  $NO_3^-$  over auxin, which accumulates in the roots and favors 377 root growth (Mounier et al., 2014). Increased  $NO_3^-$  concentrations in root tissues also 378 increases cytokinins metabolism rates (Silva and Delatorre 2009); cytokinines are 379 important cell division regulators that lead to increased root system surface area 380 (Centinari et al., 2016; Comas et al., 2010).

381 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 382 383 application rate of 40 and 100 kg N ha<sup>-1</sup> year<sup>-1</sup>. It may have happened due to decreased 384 soil pH values, which reduced cell division and elongation (Barlow et al., 2009; Yan et 385 al., 2017). Such process often takes place because H<sup>+</sup> is released during nitrification and stimulates the solubilization of Al<sup>+3</sup> from soil native minerals (Miotto et al., 2019). 386 Released A1<sup>+3</sup> can bind to carboxylic groups and to pectic materials in the cell wall. 387 388 This process increases the stiffness and decreases the extensibility of root cell walls; 389 consequently, it can lead to disorganized cell division, which impairs root elongation 390 and enables larger root diameter (Riaz et al., 2018; Zhou et al., 2014).

391 Nitrogen applications increased mineral N contents in the soil, a fact that 392 stimulated root emission, as observed in the total number of living roots in grapevines 393 subjected to N addition in both application modes. Roots preferentially grow in soil 394 regions presenting the highest nutrient contents (Centinari et al., 2016), including 395 mineral N (Lima, Kojima, Takahashi, & von Wirén, 2010). This because NO<sub>3</sub><sup>-</sup> available 396 in the soil acts as a signaling molecule that can regulate the expression of genes 397 involved in plant hormonal balance (Lee et al., 2016; Xuan, Beeckman, & Xu, 2017). 398 This process contributes to the production and development of new roots, mainly in soil 399 regions where  $NO_3^-$  is available (Krouk et al., 2010; Remans et al., 2006).

400 The increased production and number of living roots in grapevines subjected to the highest N dose application (100 kg N ha<sup>-1</sup> year<sup>-1</sup>) can also be associated with 401 402 strategies adopted by plants to uptake and accumulate N, such as amino acids and 403 proteins, in perennial organs (Ortiz-Lopez, Chang, & Bush, 2000). The increased N 404 availability in the soil increases the likelihood of nutrient uptake; however, in addition 405 to N supply, root emission and renewal processes also depend on the internal carbon (C) 406 balance of grapevines (Jagodzinski and Kalucka 2011; Yan et al. 2017). In periods of 407 higher metabolic activity, such as intense annual plant growth, part of N can be 408 redistributed to shoots and leaves, in order to enable biochemical and physiological 409 processes in grapevines (Gustavo Brunetto et al., 2016; Zambrosi et al., 2012). 410 However, senescence is expected to happen in part of the roots when most of the C 411 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 412 grapevines subjected to 40 kg N ha<sup>-1</sup> year<sup>-1</sup> as N-Surf mode and to 100 kg N ha<sup>-1</sup> year<sup>-1</sup> 413 414 as N-Fert mode, in the two evaluated crop seasons.

415 Increased N concentrations in leaves collected at flowering and at berry veraison 416 can be attributed to increased mineral N content in the soil, in grapevines subjected to N 417 applications. Part of N uptake by plants can be incorporated into C skeletons, such as 418 amino acids, amines and proteins, which can be transported to leaves, which undergo 419 intense cell division and elongation processes (Radville et al., 2016; Yu et al., 2012). 420 This phenomenon, in association with the application of higher N rates such as 100 kg N ha<sup>-1</sup> year<sup>-1</sup>, led to increase of N concentrations in leaves of plants subjected to this 421 422 treatment. In addition, large rainfall volumes recorded in concomitance of N 423 applications, as observed in the 2016/17 crop season (Fig. 1), have stimulated NO<sub>3</sub><sup>-</sup> 424 movement in the soil solution, since NO<sub>3</sub><sup>-</sup> links itself to reactive particles in the soil by

428 The increased number of grape clusters per plant and yield of grapevines subjected to the highest N application (100 kg N ha<sup>-1</sup> year<sup>-1</sup>) might be associated to a 429 430 higher mineral N availability in the soil (Brunetto et al., 2016; Steenwerth and Belina, 431 2010), a fact that stimulated root growth to 60 cm. On the one hand, increased N 432 concentrations in plant leaves stimulated photosynthetic processes, since higher 433 concentrations of chlorophylls and chloroplast proteins, such as RuBisCO, enabled increased CO<sub>2</sub> fixation in leaf tissues, as well as increased plant growth (Blank et al., 434 435 2018; Moriwaki et al., 2019). On the other hand, plants needed to be photosynthetically 436 active in order to enable NO3<sup>-</sup> uptake and reduction. Therefore, higher rates of 437 photosynthesis can also favor the uptake of N to be increased, since enzymes 438 responsible for reducing N were regulated by light, whereas the energy used in this 439 process derived from chloroplasts (Krouk et al., 2010).

440 The highest total titratable acidity in the must of grapevines grown in soil 441 subjected to the highest N applications may be explained by the inverse relationship 442 between the total titratable acidity in the must and the vegetative growth of grapevine 443 shoots. Increased leaf yield also increases the shading of clusters inside plants, which, in 444 its turn, delays the ripening of grapes and decreases organic acid degradation in berries 445 (Centinari et al., 2016; Vilanova, Fandiño, Frutos-Puerto, & Cancela, 2019). Decreased 446 TSS values observed in the must of grapevines grown in soil subjected to the 447 application of higher N doses resulted from the increased number of grape clusters per 448 plant and, mainly, from the increased grape yield, which contributed to dilute the sugars 449 in the berries (Guilpart, Metay, & Gary, 2014). In addition, decreased TSS levels in the

450 must may have resulted from increased leaf area in grapevines subjected to higher N 451 applications, since excessive leaf growth reduces direct sunlight incidence on berries, a 452 fact that leads to lower sugars concentration (Sadras and Moran 2012). Nitrogen applications at the highest dose (100 kg N ha<sup>-1</sup> year<sup>-1</sup>) and N supply modes (N-Surf and 453 454 N-Fert) led to TSS values lower than 14 °Brix, which are not appropriate for 455 winemaking processes (Chiarotti, Guerios, Cuquel, & Biasi, 2011). The application of 456 40 and 100 kg N ha<sup>-1</sup> year<sup>-1</sup>, in both N supply methods, also reduced total anthocyanin 457 values in grape must. This outcome may be associated with increased number of grape 458 clusters per plant and with grape yield, which is often associated with increased berry 459 size and pulp/peel ratio. Thus, the concentration of important compounds, such as total 460 anthocyanins in the must, which account for the reddish color of the must and, 461 consequently, of the wine, gets diluted (Brunetto et al. 2009b; Sadras and Moran, 2012; 462 Sofo et al. 2012). In addition, as plants' vegetative growth increases, part of the 463 anthocyanins found in the clusters may have been redistributed to other growth sites and 464 had their contents decreased in berries (Terrier, Poncet-Legrand, & Cheynier, 2009).

465 Results of the principal component analysis showed intrinsic climate variation in 466 each crop season, which resulted in plants' productive response to the application of 467 different treatments. It also showed annual variations in fruit yield and nutritional status. 468 It happened because fruit plant species have a cyclical dynamics of C allocation to 469 different organs that can favor yield in a given year and species maintenance in the 470 following year, due to reserve accumulation in organs that require more energy for 471 protein synthesis, mainly in plants subjected to N applications (Ceccon et al. 2016; 472 Artacho and Bonomelli 2017). The PCA enabled a better understanding of the positive 473 relationship among root system, plants yield, N application rates and modes. These 474 results showed that as photoassimilates accumulated in grapevines roots, water and 475 nutrient intake increased, a fact that improved crop yield (Antolín et al., 2003; Barlow et 476 al., 2009; Comas et al., 2010). In addition to this process, there was a decrease in the 477 oenological quality of the must, which was diagnosed based on decreased total 478 anthocyanins and total soluble solids values, as well as on increased total acidity values.

479

# 480 **Conclusion**

The application of 40 kg N ha<sup>-1</sup> year<sup>-1</sup>, 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

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

493

## 494 **Conflicts of interest**

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

496

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

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



Fig. 1 Monthly accumulated rainfall (mm) and average monthly temperature (°C) 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



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**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<sup>-1</sup> year<sup>-1</sup>). 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<sup>-1</sup> year<sup>-1</sup>). 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<sup>-1</sup>) 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<sup>-1</sup> year<sup>-1</sup>). 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<sup>-1</sup> year<sup>-1</sup>). 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<sup>-1</sup> year<sup>-1</sup>). 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)



801 Fig. 7 Effect of N application rate and method on total titratable acidity in 2016/17 (a) and 2017/18 crop 802 season (b); total soluble solids (TSS) in the 2016/17 (c) and 2017/18 crop season (d); total anthocyanins 803 (Anthocyanins) in the 2016/17 (e) and 2017/18 crop season (f) of the must of 'Alicante Bouschet' (Vitis 804 vinifera L.) grapevine (N Surf - Nitrogen surface; N Fert - Nitrogen fertigation; 0, 40 and 100 kg N ha<sup>-1</sup> 805 year<sup>-1</sup>). The vertical bars indicate the standard error (n = 4). Different lower-case letters indicate a 806 significant difference among N doses in the same application modes, and different upper-case letters 807 indicate a significant difference among the N application modes in the same N doses by the Scott-Knott 808 test (p < 0.05)



810 Fig. 8 Relationship between principal component 1 (PC1) and 2 (PC2) of the morphological parameters 811 of the root system (total surface area of roots (a), average diameter of roots (d), total number of living 812 roots (living), total number of dead roots (dead) and root production (prodR) in 0-20, 20-40 and 40-60 cm 813 soil layers), leaf N concentration (leaf N concentration at flowering (N flowering) and leaf N 814 concentration at veraison (N veraison)), grape yield parameters (grape yield (Yield), number of cluster 815 per plant (n cluster), weight of 100 berries (100b)) and chemical parameters of the must (total 816 anthocyanins (Anthocyanins), total soluble solids (TSS), total titratable acidity (TTA)) of 'Alicante 817 Bouschet' (Vitis vinifera L.) grapevine subjected to different N doses and application modes





820 Supplementary material 1. Schematic representation of the imaging system from minirizhotron tubes

821 installed in the vineyard of the experimental area; *in situ* image capture high resolution (600 dpi) from a

822 cylindrical scanner (Root Imager – CI600®).

823 Supplementary material 2. Analysis of variance of the roots parameters, leaf N concentration, grape

824 yield and chemical of the must of 'Alicante Bouschet' (Vitis vinifera L.) grapevine subjected to different

825 N doses and application modes.

Variables	Effect				
v ariables	Soil layer (cm)	<b>Crop season</b>	Mode	Dose	Interaction
	0.20	2016/17	***	***	***
	0-20	2017/18	***	***	***
Total surface area of roots (mm <sup>2</sup> tube-1)	20.40	2016/17	***	***	
Total surface area of roots (mm <sup>-</sup> tube <sup>-</sup> )	20-40	2017/18	***	***	***
	40.00	2016/17	***	***	***
	40-00	2017/18	***	***	***
	0-20 -	2016/17	***	**	***
		2017/18	***	***	***
Average diameter of reats (mm)	20-40	2016/17	***	**	***
Average diameter of roots (mm)		2017/18	***	***	***
	40.60	2016/17	***	***	
	40-00	2017/18	***	***	***
	0-20 -	2016/17		***	***
		2017/18	***	***	***
Total number of living roots	20.40	2016/17		***	***
Total number of fiving roots	20-40	2017/18	***	***	**
	40.60	2016/17	**	***	
	40-00	2017/18	***	***	***
	0.20	2016/17	***	**	***
	0-20	2017/18	***	***	***
Total number of dead roots	20.40	2016/17	***	**	***
Total number of dead roots	20-40	2017/18	***		***
	40.60	2016/17	***	***	*
	40-00	2017/18	*	***	***
	0-20 -	2016/17	*	***	**
		2017/18		***	
Doot production (mm tube <sup>-1</sup> )	20-40	2016/17	**	***	
Koot production (inin tube )		2017/18		***	
	40.60	2016/17		***	
	40-00	2017/18		***	**
$\mathbf{I}$ as $\mathbf{f}$ N concentration at flowering (9/)	_	2016/17	***	***	***
Lear N concentration at nowering (78)		2017/18	*	**	***
Last N concentration at varaison $(%)$	_	2016/17	***	***	***
Leaf IV concentration at veraison (76)		2017/18		**	*
Crops yield (Mg hs <sup>-1</sup> )	<u> </u>	2016/17		***	
Grape yield (Nig lia )		2017/18		***	
Number of cluster per plant	_	2016/17		**	
Number of cluster per plant		2017/18		***	
Waight of 100 harries $(a)$	_	2016/17		***	
weight of too bei ties (g)		2017/18			
Total anthocyaning (mg 100mI -1)		2016/17	***	***	**
		2017/18		***	*
Total soluble solids (PDwix)		2016/17	***	***	***
i otal soluble sollus ( DFIX)		2017/18	***	***	***
Total titratable asidity (mg I -1)		2016/17		***	
$\frac{1}{2} \operatorname{Oral ultratable acidity} (\operatorname{mg L}^2)$	-	2017/18	*	***	***

826 \*0.05; \*\*0.01; \*\*\*<0.001