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Pioneer and fibrous root seasonal dynamics of *Vitis vinifera* L. are affected by biochar application to a low fertility soil: a rhizobox approach

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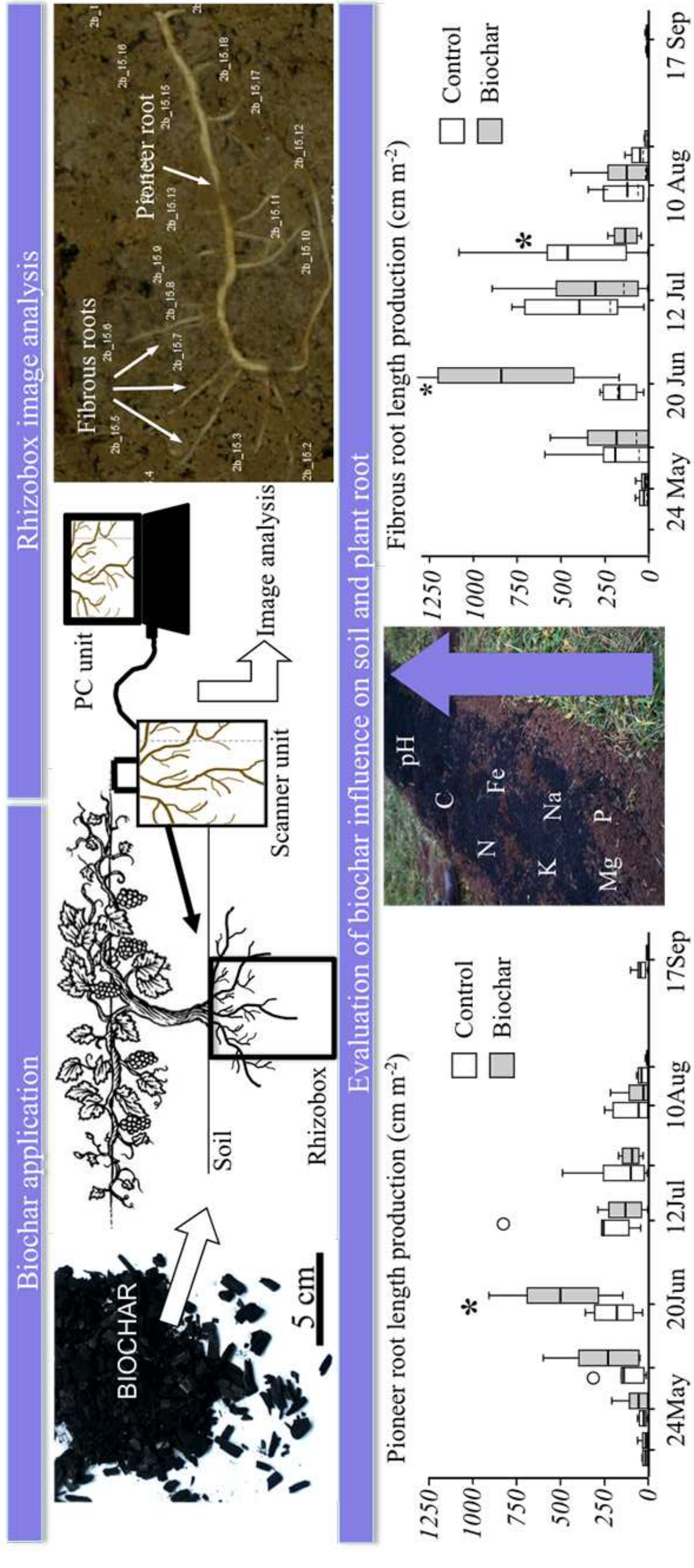
***Graphical Abstract**



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights

- Biochar mitigates greenhouse gas emissions and positively affects soil properties.
- Rhizobox method unveiled unimodal grapevine pioneer and fibrous root seasonality.
- Biochar application enhanced physico-chemical soil properties.
- Treated soil promoted an earlier root production in terms of number and length.
- During the harsh summer, the root systems of treated-plants not need to enlarge.

Abstract

The present work analyzes intra-annual growth dynamics of pioneer and fibrous roots of grapevine in relation to the biochar-induced modification of soil physico-chemical properties. A scanner inserted into a buried rhizobox with a transparent side facing the plant root system was used to acquire images of pioneer and fibrous roots of control and biochar-treated plants throughout the duration of the vegetative season. Images were analyzed with ImageJ software to measure root traits. Physico-chemical analyses of the soil media were performed. Biochar treatment increased soil pH and nutrient concentration, reduced bulk density, and changed water content. Analysis of both pioneer and fibrous root traits highlighted a single peak of growth during the vegetative season. Pioneer roots were thicker and grew faster than fibrous roots which were longer and more numerous. Biochar induced an anticipation of root lengthening and root number production at the onset of the season. High-definition intra-annual monitoring unveiled differences between pioneer and fibrous root traits that could be related to their specific morpho-functionality. Biochar application enhanced physico-chemical soil properties that, in turn, stimulated an earlier root lengthening, and a higher root number in correspondence with the canopy developmental stage, while reducing root growth during the reproductive stage of vine.

1. Introduction

Biochar (BC), similar in appearance to charcoal, is a carbon-rich granular material produced by the pyrolysis of biomass feedstock (Hodgson et al. 2016; Lehmann and Joseph 2015). Depending on the process parameters and feedstock type, BC shows a highly stabilized carbon content, alkaline in nature, a highly porous structure, a large surface area, and a slow decomposition rate (Lehmann and Joseph 2015; Lehmann et al. 2015). BC application has shown positive effects on various soil and crop types, mainly through an increase in both soil nutrient availability and water retention (Major et al. 2010b; Schulz et al. 2013; Vaccari et al. 2011). Indeed, BC soil amendment improves the soil structure by increasing macroaggregates, and chemical fertility (Amendola et al. 2017; Li et al. 2018; Tan et al. 2017; Trupiano et al. 2017), by serving as a direct source of soil carbon and non-N mineral nutrients such as K, P, and Ca (Sackett et al. 2015), and by retaining nutrients from the soil itself (Rawat et al. 2019). In particular, the ash fraction of BC is a significant source of water-soluble P, and enhancements in P availability have been found in field experiments on tropical soils (Parvage et al. 2013). Also, BC affects soil N availability through influencing abiotic (sorption, volatilization, leaching), and biotic (fixation, mineralization, immobilization, denitrification, plant uptake) processes (Baronti et al. 2010; Nguyen et al. 2017). Furthermore, biochar amendment can change the soil pH and cation exchange capacity (CEC) depending on the type of soil and BC used. The application of alkaline BCs to acidic soils can increase soil pH (i.e. liming effect) and in turn enhance the soil quality by improving the availability of basic soil nutrients (Major et al. 2010b; Raboin et al. 2016). This ability of biochar is mainly due to its composition of alkaline substances, including ash and calcium (Ca^{2+}), potassium (K^{+}) and magnesium (K^{+}) carbonates (Yuan et al. 2011), and the reduction of exchangeable acidic aluminum (Al^{3+}) and hydrogen (H^{+}) cations (Masud et al. 2014). Finally, depending on the soil characteristics, its feedstock source and pyrolysis temperature, BC amendment can be considered as a means for mitigating greenhouse gas (GHG) emissions (He et al. 2017).

Recent studies have shown the great benefits of BC application to soil-less substrate (Polzella et al. 2020) and nutrient-poor and degraded soils (El-Naggar et al. 2019). BC application to low fertility soils seems to be a potential best management practice, contributing to their rehabilitation, and improvement of crop productivity (El-Naggar et al. 2019). However, despite improvements in soil nutrient and water status, BC effects on plant growth vary widely, depending on the characteristics of soil, biochar type, and plant species. Several studies demonstrated the lack of significant changes in crop yields 1–4 years after biochar application to fertile mineral soils (Jones et al. 2012; Polzella et al. 2019; Tammeorg et al. 2014), while other studies found BC promoting crop yield, biomass, mineral nutrient absorption, and plant ecophysiology (Ali et al. 2017; Luo et al. 2018). Significant increases in plant growth and yield have mostly been reported, especially when it involved improvements in soil pH and alleviation of Al toxicity (Blackwell et al. 2009). Raboin et al. (2016), in an acidic soil field experiment, found a significant yield increase in maize and common beans, due to the increase of soil pH and the decrease of exchangeable aluminum, although no significant change was observed in rice yields.

Importantly, when BC is applied to the soil it can enhance favorable conditions for plant root growth (Lone et al. 2015), such as an increase of root-associated microorganisms (Brennan et al. 2014; Lone et al. 2015) and soil moisture (Amendola et al. 2017; Joseph et al. 2015; Lehmann and Joseph 2015). Also, BC was shown to enhance soil aeration in both water-based growing systems (e.g. hydroponics; Awad et al. 2017; Kim et al. 2017) and clay-soils (Edeh et al. 2020; Obia et al. 2018), to increase soil drainage and bulk density (Baronti et al. 2014; Hardie et al. 2014), and to modify water field capacity (Peake et al. 2014).

However, so far, the majority of BC studies have mainly focused on the promotion of aboveground biomass or the increase of crop yield, whereas less attention has been paid to how BC influences root growth (Amendola et al. 2017; Prendergast-Miller et al. 2013; Razaq et al. 2017). This is unfortunate, since roots represent the first interface between BC particles and growing plants, and, thus, BC application to the soil may alter root dynamics and, in turn, affect plant performance (Xiang et al. 2017). In particular, the fine roots fraction of the root system plays a crucial role, since it absorbs and transport nutrients and water from the soil, responding rapidly to environmental changes (Montagnoli et al. 2019; Razaq et al. 2017). These physiological and ecological functions of fine roots are generally linked to their morphological (Ma et al. 2018; McCormack et al. 2015; Montagnoli et al. 2018) and anatomical traits (Hishi and Takeda 2005; Hishi 2007; Zadworny and Eissenstat 2011). Indeed, plants plastically respond to environmental cues, such as resource availability and physical obstacles imposed by high soil bulk density (Ola et al. 2018), through a species-specific modulation of root traits allowing for optimal use of underground resources (Guo et al. 2004; Sattelmacher et al. 1990). Xiang et al. (2017) in a recent metanalysis concluded that BC application, through the modification of soil characteristics, induced increases in root biomass, volume, surface area, length, number of root tips, and diameter. Amendola et al. (2017) found a differentiated response with a preferential radial instead of longitudinal growth type in the case of BC application, and this could be related to the higher soil water and nutrient availability so that the cost-benefit balance tipped in favor of improving transport instead of increasing soil area absorption. In general, this modulation of root traits occurs by producing roots of different forms and functions. Fine roots with a smaller diameter, referred to as fibrous, are lower in carbon costs to be produced (Ostonen et al. 2007), do not typically undergo secondary growth, have a higher absorptive function, and a fast turnover rate (Joslin et al. 2006; Xia et al. 2010). In contrast, fine roots with a larger diameter, referred to as pioneer, represent the root framework, undergo secondary growth, have a longer lifespan, and mainly function in nutrient and water transport (Polverigiani et al. 2011). For example, various authors found that fibrous and pioneers roots differ in their response to variations in soil conditions, such as moisture (Polverigiani et al. 2011), freeze-thaw cycle (Yin et al. 2017), and excavation disturbance (Nakahata 2020). To the authors' knowledge, there is a complete lack of studies relating BC application with roots classification as fibrous and pioneers. To this purpose, detailed root morphological analyses, including descriptions of length, diameter, and temporal changes in fibrous and pioneer root types can improve the comprehension of how the root system of woody plants copes with soil environment changes.

Soil core excavation (e.g. sequential soil coring, ingrowth cores) is a commonly used method for studying fine root dynamics in forest stands (Montagnoli et al. 2012a, b, 2014, 2019) and recently has also been applied in grapevine (Amendola et al. 2017) field experiments. However, this technique causes disturbance of the rooting environment during collection and installation procedures representing a potential drawback (Li and Lange 2015). Other works have used observational non-destructive methods, such as growth in minirhizotrons (Comas et al. 2000; Vamerali et al. 2012) and the use of flatbed scanners (Nakahata 2020; Nakahata and Osawa 2017), which enable direct observation of individual root behavior by recording with a camera or a scanner through a transparent wall pressed to a soil profile (Nakahata 2020). Even in these cases, soil disturbance is unavoidable when the transparent acrylic tube or box is installed into the soil. However, soil disturbance is typically dealt with by delaying the start of root observations (Lukac and Godbold 2010). Among visualization methods, the flatbed scanner facilitates the distinction between pioneers and fibrous roots and the description of their dynamics occurring within a relatively wide observation area (Nakahata 2020; Van Do et al. 2016).

In the present work, we firstly hypothesized that BC application induces a change in soil physico-chemical properties, and secondly that this would lead to different seasonal modulations in pioneer and fibrous roots of grapevine (*Vitis vinifera* var. Chardonnay). To test this hypothesis, control and BC-treated plots were analyzed for physico-chemical soil characteristics, while pioneer and fibrous root traits, such as length, number, diameter size, and growth velocity, were monitored in the upper 30 cm of the soil during an entire growing season. Our objective was to use a rhizobox-flatbed scanner approach to understand how grapevine plants seasonally modify the growth of their pioneer and fibrous roots in an acidic-sandy loam soil to adjust to changes in the rooting environment.

2. Material and methods

2.1 Experimental site and set up

The field activities were carried out on an experimental grapevine site (*Vitis vinifera* L., Chardonnay wine grape variety) near the University of Insubria, Varese, Italy (45°47'52.6"N 8°51'17.5"E; 392 m a.s.l.). The area is characterized by a sub-continental temperate climate with mean annual precipitation of 1500-2000 mm concentrated in two main periods (April–May and August–September), and mean annual temperature of 10–14 °C (Fratianni and Acquaotta, 2017). During our sampling period (March - October 2018) total rainfall was approximately 1010 mm and the average air temperature was 15.9 °C (min -6.3 °C, max 32.0 °C; Figure 2, data from Arpa Lombardia). Precipitations were concentrated in spring (March-May), with an alternation of intense rainfall events and dry days, and less frequent in summer (June-August), with a lower total rainfall and few intense events (Figure 2). Air temperature increased from March reaching maximum values at the beginning of August (Figure 2).

The soil type at the experimental site consists of acidic soils (pH 4.9) classified as Alisols by the World Reference Base (FAO) Soil Group (data from ERSAF-Regione Lombardia). Alisols occur predominantly in humid tropical, humid subtropical and humid temperate regions and are characterized by a high-activity clay-enriched subsoil (Argic horizon) with a low base saturation at 50–100 cm depth, often overlain throughout by loamy sand or coarser textures (Table 1). They are found in China, Japan and the southeast of the United States of America, while minor occurrences have been reported from areas around the Mediterranean Sea (Italy, France and Greece). Alisols only allow cultivation of shallow-rooting and acid-tolerant crops, which suffer from drought stress in the dry season, or low-volume grazing,. Where fully limed and fertilized, crops on Alisols may benefit from its considerable cation exchange capacity (CEC) and good water-holding capacity (IUSS-WRB 2015).

The experimental site, set up in February 2016, consisted of two north-south oriented plant rows (4.8 m spacing), each containing a number of 15 3-year-old plants (80 cm spacing), without irrigation (Figure 1). BC was applied in December 2017, at a rate of 30 t ha^{-1}. To obtain a homogeneous biochar-soil mixture, the biochar was crushed into smaller particles, sieved at 2 mm size and homogeneously broadcast by hand (Major 2010a) between plants. To avoid biochar loss by wind or water erosion, moisture was applied with a Verdigris sprayer immediately after spreading biochar on the soil surface (Karer et al. 2013) and the biochar incorporated into the soil with a hand-powered rotary hoe at low rotation speed (10 cm depth; Karer et al. 2013). Measurements were carried out in ten plots (five control and five biochar-treated) each including three plants displaced on the same row (Figure 1). To prevent weed growth, the two rows were covered with mulch tissue.

2.2 Biochar production and characterization

The biochar used in this study was produced by Romagna Carbone s.n.c. (Italy) from orchard pruning biomass through a slow pyrolysis process with an average residence time of 3 h at 500 °C in a kiln of 2.2 m in diameter and holding around 2 ton of feedstock. Biochar analyses were performed on a subsample obtained by mixing three individual specimens (about 5 g each) withdrawn from different places of the original biochar sample (1 kg) and then thoroughly homogenized by grinding with an agate mortar and pestle, sieved (mesh size: 2 mm), oven-dried at 40 °C for 72 h, and stored at −20 °C prior to analysis. A mixture of biochar and deionized water at a 1:5 v/v ratio was prepared, thoroughly mixed and the pH measured at room temperature with a digital pH meter (HI 98103, Checker®, Hanna Instruments) according to standards methods UNI EN 13037 (2002) and 13040 (2008). The electrical conductivity (EC) value was obtained by direct instrumental determination in 1:5 soil:water (v/v) extracts according to standards methods UNI EN 13038 (2002) and 13040 (2008). Cation exchange capacity (CEC) was determined using a NH4OAc method. In the present work, we referred to IBI standards (2014), which define carbon stability as the molar ratio of hydrogen to organic carbon (maximum 0.7). C_{tot} and N_{tot} contents of biochar were determined using a CHN elemental analyzer (Carlo Erba Instruments, mod 1500 series 2). Samples were sifted by means of a 2 mm sieve and oven dried at 105 °C for 24 h. In the case of C_{org}, combustion was carried out after the complete removal of inorganic C with acid. Available nitrogen (N_{av}) was determined by a modified Kjeldahl procedure using Devarda's alloy (Liao 1981) as reducing agent to convert (NO_3) and (NO_2) into $(NH_4)^+$ followed by Kjeldahl digestion. Total phosphorus (P_{tot}) was determined according to the EPA method 3052 (USEPA 1996) using an ICP-OES spectrophotometer (Varian Inc., Vista MPX). The available phosphorus (P_{av}) was extracted by a $NaHCO_3$ solution at pH 8.5 and evaluated by spectrophotometry according to the Olsen method (Olsen and Sommers 1982). The particle size distribution of biochar was determined by dry sieving as reported in Baronti et al., 2014. Biochar porosity and pore size distribution within the range of 0.003–160 mm were determined with a mercury intrusion porosimeter equipped with a macropore unit (Pascal 140 and 240 series, respectively, ThermoFinnigan, Waltham, MA, USA). Specific surface area (SSA) measurements of biochar samples were performed via the dynamic Brunauer–

Emmett–Teller (BET) method using a MicromeriticsFlowsorb 2309 apparatus (Dunstable, UK) with N_2 as adsorbate. Prior to BET analysis, samples were oven-dried at 250°C for 30 min.

2.3 Soil sampling strategy and chemical analyses.

To assess soil physico-chemical properties and the effects of biochar on these characteristics, at the end of June 2018 a soil sample (12-15 cm depth) was collected for each plot (both BC-treated and control), for a total of 10 samples. Sampling points were located at approximately 40 cm distance from the plants, thus, reasonably far enough to be considered bulk soil even though a few weed roots were found. Soil samples, once freed from roots, were air dried until constant weight, passed through a 2 mm sieve and stored at 4°C temperature until analysis. The pH was determined by a digital pH meter (HI 98103, Checker®, Hanna Instruments in a soil/distilled water solution (1:2.5). Methods used for the characterization of C_{tot}, C_{org}, N_{tot} N_{org} and P_{av} are described in the previous paragraph. Total soil elements (Fe, P, Na, K, Mg) were determined according to the EPA method 3052 (USEPA 1996) using an ICP-OES spectrophotometer (Varian Inc., Vista MPX). Concentrations of exchangeable elements were quantified by extraction with $BaCl_2$-triethanolamine followed by ICP-OES spectrophotometry.

Gravimetric (GWC) and volumetric (VWC) soil water content were measured at field capacity (maximum water holding capacity) on the 1st of August corresponding to the highest air temperature of the season and the lowest precipitation (Figure 2). Ten soil cores (size: 5 cm [diameter] × 5.5 cm [depth]; volume: 108 cm^3) were taken from both BC-treated and control plots, about 2-3 cm below the soil surface. To measure the field capacity, 5 soil cores were transferred to a mesh cylinder (200 µm opening) with the same size as the corer, and gently saturated with distilled water. After free draining until no additional water loss was recorded, the water content was determined by taking the difference in mass of samples before and after oven drying at 105 °C for 72 h (until constant weigh). The same drying procedure was applied to the other 5 samples. The whole-soil bulk density was calculated as: mass of dried soil (g) / soil core volume (cm^3). GWC was defined as the ratio of the water mass (g) to mass of dried soil (g), VWC was calculated as the ratio of the water volume (water mass x water density; cm^3) to soil core volume (cm^3) (Carter and Gregorich 2007).

2.4 Rhizobox installation and root measurements

At the experimental site, together with planting operations during the month of February 2016, five plastic boxes (60 cm depth, 37.5 length, 12 cm width) with one transparent acrylic side (29.6 cm depth, 21.6 length, 639.36 cm^2 soil surface area) facing the root system were vertically buried in the topsoil layer for each row at 20 cm distance from the central plant of each plot (Figure 1). Starting from the beginning of March 2018, about each 18 days (±3), a modified charged-coupled device (CCD) scanner (Perfection V600 Photo, Epson; Figure 3) was inserted in the buried box and used for root image acquisition at 800 dpi (Figure 3). Image correction of the scanning position was not necessary before root tracing analysis, because the scanner was strongly embedded in a wooden frame (Figure 3) fitting exactly within the rhizobox avoiding any shifting. Afterward, images were analyzed with ImageJ open source software (www.imaj.org) to measure root traits (Figure 4). Each lateral root newly produced and growing along the transparent acrylic side was counted, labeled, and measured at each time point. Roots were visually distinguished in pioneer and fibrous (Figure 4B, C, and D), according to the appearing morphology as described by Polverigiani et al. (2011). Root traits were analyzed considering cohorts constituted by active roots newly produced or increasing in size (length or diameter) between two consecutive time points. Root traits such as length production (RLP), number (RN), diameter (RD), and growth velocity (RGV) were measured.

2.5 Calculations of root traits

The root length produced between consecutive time points (RLP; mm cm^{-2}) (t_0 and t_1, t_1 and t_2….,t_{n-1} and t_n) was calculated subtracting the value measured at t_n from the value measured at the previous time point (t_{n-1}). The root number (RN; n) was defined as the number of active roots. The mean diameter of a single root was calculated by averaging 10 measurements evenly distributed along the whole root axis starting behind the root tip. At each time point the mean diameter of the active root population (RD; mm) was calculated as the average of the mean diameter of each single root. Root growth velocity (RGV; mm day^{-1} cm^{-2}) was calculated dividing the value of root length production by the number of days characterizing the measured temporal interval between the analyzed time points. Finally, annual mean root traits between t_0 and t_n were calculated as the mean of all values obtained between two time points.

2.6 Statistical analysis

Data of root length production, growth velocity, number of active roots, and mean diameter were not normally distributed nor did they meet the assumption of homoscedasticity. Thus, they were square-rooted or log-transformed to ensure normal distributions and equal variances to allow the use of parametric statistics. A three-way ANOVA was carried out to test the effect of treatment (biochar - control), root type (pioneer - fibrous) and time (15 sampling points) on the dependent variables. Least significant difference (LSD) tests were conducted to detect overall differences between control and BC-treated plants at each time point for both pioneer and fibrous roots. Differences were considered significant at $p<0.05$. Statistical analyses were carried out with SPSS 20.0 software (SPSS Inc, Chicago IL, USA).

3. Results

3.1 Biochar characteristics

The biochar tested was found to meet IBI-Standards (2014) and the Italian legislation regarding amendment and fertilizer requirements with regard to C_{tot} and C_{org} content, and C/H ratio ≥ 0.7, ensuring a good stability to the C_{org} in the soil. Moreover, available phosphorus and nitrogen represented 17.7% and 0.3% of total phosphorus and nitrogen, respectively (Table 2). The pH of biochar was alkaline with a value of 9.7. The total porosity of biochar was 2722 mm^{3}g^{-1} (Table 2) and pore size classes, grouped according to Greenland's (1977) terminology, were as follows: 75% storage pores, 15% residual pores and 10% transmission pores of the over-all porosity. The specific surface area was 410 ± 6 m^{2}g^{-1} and the bulk density 0.33 g cm^{-3}. Particle size distribution was as follows (of total mass): 16% larger than 10 mm, 35% between 10 and 4 mm, and 49% smaller than 2 mm (Table 2).

3.2 Chemical soil properties

During the six months after BC application (December 2017- June 2018), soil pH increased significantly (27%) compared to control plots (pH 5.05 in controls, pH 6.41 in BC-treated soil). The bulk density was significantly lower in BC-treated soil compared to the control. Gravimetric water content (GWC) at field capacity was similar in control and BC-treated soil, while on the hottest day it was higher in BC-treated plots, although not significantly. On the other hand, the volumetric water content (VWC) at field capacity was significantly lower in BC-treated soil compared to the control. Although no significant VWC differences were detected on the hottest day, values were slightly higher in BC-treated plots compared to the control. The total carbon content (C_{tot}) was higher in BC-treated soil with respect to control soil, but no significant differences were detected in

TOC content. A significant increase was found for both total and available N (41.6%, 18.1%, respectively), C (31.9%), total and available P (23.3%, 23.3%, respectively), and total and available K (18.7%, 26.4%, respectively) in BC-treated soil compared to control soil. In addition, both total and available content of Mg and Na significantly increased when BC was added to the soil. Finally, the Fe content was significantly higher in BC-treated soil compared to control soil, but only in the available form (Table 3).

3.3 Root length production

RLP changed significantly during the season, being affected by the interplay of season and BC-treatment (Table 4). Although measurements of root scans were taken from the beginning of March, the first root elongation was observed only from the middle of May for pioneer roots and 10 days later for fibrous roots, independently of treatment (Figure 5A, B). In the case of control plants, pioneer RLP increased significantly at the beginning of the growing season, reaching its maximum value on June 20th and decreasing right after until the end of the growing season (Figure 5A). Fibrous roots of control plants reached the maximum seasonal value on July 12th, slowly decreasing right after until the end of the growing season. RLP of both pioneer and fibrous roots of BC-treated plants increased at the very beginning of the growing season, reaching maximum values on June 20th and decreasing right after until the end of the growing season (Figure 5A, B). Although at a higher magnitude, pioneer RLP of BC-treated plants showed the same pattern as control plants (Figure 5A). On the contrary, fibrous roots of BC-treated plants showed a different timing, reaching a peak in RLP earlier than those of control plants (Figure 5B). Both peak values of BC-treated plants were significantly higher than values measured at the same time point for control plants (Figure 5A, B). In particular, RLP of pioneer and fibrous roots of BC-treated plants were, respectively, almost 2- and 3-fold higher than values measured in control plants. Finally, due to the time shift of fibrous RLP, BC-treated plants had a significantly lower RLP value than control plants on July 26th. Cumulative values showed an exponential RLP pattern with an anticipation of BC-treated plants for both pioneer and fibrous root types (Figure 5, inner panels A and B). The values reached at the top of the growth curve were slightly higher in BC-treated plants compared to control plants, although not significantly (Figure 5 inner panels A and B).

3.4 Root number

The active RN varied significantly according to root type and depending on the interplay of BC-treatment and time (Table 4). RN increase started from the middle of May for both pioneer and fibrous roots (Figure 6A, B). In the case of control plants, RN of both pioneer and fibrous roots reached a maximum value on July 12th. At this time point, RN of fibrous roots was almost 6-fold higher than that of pioneer roots. After reaching a peak, RN of both pioneer and fibrous roots decreased until the end of the growing season. In BC-treated plants, RN of both root types increased rapidly from the beginning of the growing season, reaching its maximum on June 20th. Throughout the season, RN of pioneer roots was higher in BC-treated plants than in control plants, although a significant difference was detected only for the peak value (Figure 6A, B). Furthermore, RN of fibrous roots was almost 5-fold higher than that of pioneer roots. After reaching a peak, RN of both pioneer and fibrous root types in BC-treated plants showed a continuous decrease until the end of the growing season (Figure 6A, B). After the June 20th time point, RN of fibrous roots in BC-treated plants was always of a lower magnitude compared to control plants, although not significantly (Figure 6B). At the maximum time point, RN of fibrous roots was 3-fold higher than that of pioneer roots.

3.5 Root diameter size

The mean RD was significantly influenced by root type and seasonality (Table 4). Pioneer and fibrous roots of both control and BC-treated plants did not show any difference in RD pattern during the growing season (Figure 7A, B). The population of pioneer roots showed a continuous increase in RD, peaking on July 26th and decreasing until the end of the season (Figure 7A). On the contrary, fibrous roots showed a more stable RD throughout the growing season (Figure 7B).

3.6 Root growth velocity

RGV was significantly influenced by root type and seasonality, both alone and in combination (Table 4). Pioneer roots of control plants showed a bimodal RGV pattern, with peaks on June 20th and August 10th, and an intermediate decrease (Figure 8A). The two maximum values were of different magnitude, reaching a velocity of 2.4 and 4.6 mm day^{-1} for the first and second peak, respectively (Figure 8A). Fibrous roots of control plants also showed a bimodal pattern of growth velocity, peaking on June 4th and August 10th and then decreasing until the end of the season (Figure 8B). The first maximum value was of a higher magnitude (1.3 mm day^{-1}) than the second one (1 mm day^{-1}) and these values were, respectively, 2- and 4-fold lower than those of pioneer roots measured at the same time point (Figure 8B). Pioneer roots of BC-treated plants showed a bimodal pattern peaking on June 20th and July 26th (Figure 8A), with the first maximum value being of lower magnitude (2.8 mm day^{-1}) than the second one (3.6 mm day^{-1}) (Figure 8A). Fibrous roots of BC-treated plants had a bimodal growth velocity pattern peaking on June 20th and August 10th, with the first peak being of higher magnitude (0.8 mm day^{-1}) than the second one (0.6 mm day^{-1}). At the two peak time points, pioneer roots of BC-plants reached almost 4- and 6-fold higher growth velocity values, respectively, compared to fibrous roots.

In BC-treated plants, the growth velocity pattern of pioneer roots overlapped during the first half of the season but differed during the second half (Figure 8A). In particular, control values were significantly higher on August 10th due to the earlier peak of BC-treated plants (Figure 8A). On the contrary, fibrous roots of BC-treated and control plants had an asynchronous pattern during the first part of the season, with significantly higher values found for control plants on June 4th, but a mostly overlapping one during the second part (Figure 8B).

3.7 Annual mean root traits

In both control and BC-treated plants, annual mean RLP of fibrous roots was significantly higher than that of pioneer roots, while no significant effect of BC amendment could be observed (Table 5). Annual mean RN of fibrous roots was significantly higher than that of pioneer roots, in both control and BC-treated plants. BC treatment resulted in a significantly higher annual mean RN of pioneer roots compared to control plants, while in the case of fibrous roots, no significant differences were found between BC-treated and control plants (Table 5). Annual mean RD of both BC-treated and control plants was significantly higher in the case of pioneer roots compared to that of fibrous roots. However, BC treatment did not lead to any significant differences for both root types compared to controls (Table 5). For both BC-treated and control plants, fibrous roots showed a significantly higher annual mean RGV compared to pioneer roots, while no difference was detected between BC-treated and control plants (Table 5).

4. Discussion

In our study, results of soil analysis showed that BC application causes a considerable change in soil physico-chemical properties. Indeed, in line with data reported in the literature (Amendola et al. 2017; Atkinson et al. 2010; Biederman and Harpole 2013; Macdonald et al. 2014), a few months after BC application soil pH increased by approximately 1.3 units. In addition, we measured a significant increment of the main soil elements, such as total carbon, nitrogen, phosphorus, and potassium. BC also significantly increased the plant-available form of nitrogen, phosphorus and potassium (N-P-K) as well as magnesium (Mg) and other microelements (Fe, Na). Indeed, despite its stability, BC can be partially and relatively rapidly mineralized during the first months following incorporation into the soil (Cheng et al. 2006; Nguyen and Lehmann 2009; Kuzyakov et al. 2009). Kuzyakov et al. (2009) reported a differential mineralization of BC in relation to two pyrolysis temperatures (i.e. 350 and 700°C), with the highest one resulting in a more stable BC type. In our case, the temperature of BC production was 500°C allowing us to suppose an intermediate and relatively high value of mineralization in the short term. Other studies pointed out that the alternation of saturated and unsaturated soil water conditions (Nguyen et al. 2010; Nguyen and Lehmann 2009) coupled with a higher mean soil temperature (Ventura et al. 2015) are the most important drivers in natural oxidation of charcoal (Cheng et al. 2008; Glaser and Amelung 2003). This is quite similar to our case, indeed, the high amount of rainfall during the spring season, characteristic of a sub-continental climate, provided an alternation of soil wetting and drying events, which was associated with a rapid increase of the temperature. These conditions boosted BC degradation, thereby affecting the mineral composition of the soil, in terms of both macro and microelements. In our study, physical properties of the soil, such as bulk density, and both volumetric and gravimetric water content, were also influenced by BC application. Soil bulk density was significantly reduced when BC was applied, coherently with findings reported in a recent review (Razzaghi et al. 2020). Moreover, the volume of water content at field capacity was significantly lower in BC-treated plots, in accordance with a decrease in macropore spaces and higher water content at field capacity of dense soils recently reported by Ola et al. (2018). Thus, with the same amount of water input (rainfall), aeration of the soil seemed to be higher in BC-treated plots. Interestingly, this pattern was reversed during the driest period of the season when the soil displayed higher water content, both volumetric and gravimetric, in BC-treated plots, although the difference was not significant.

From a methodological perspective, the rhizobox-flatbed scanner approach revealed to be a suitable method to accurately estimate traits and differentiation of different root types. The method allowed us to draw a well-defined unimodal pattern of seasonal root growth (length production and number of active roots) for both pioneers and fibrous root types. A unimodal pattern has also been observed in northern (Brassard et al. 2009) and temperate forest (Coners and Leuschner 2005; Vanguelova et al. 2005), and grapevine (Comas et al. 2005). However, our pattern was in contrast with the bimodal pattern observed in previous studies on grapevine and tree species (Montagnoli et al. 2012a, b, 2014, 2019; Mullins et al. 1992; Van Zyl 1988). In our study, root length production and number of active roots decline during fruit ripening, resulting in a lack of root flush in the fall, which is probably related to the very quick end of the growing season (Comas et al. 2005).

In control plants, the length production pattern of pioneer roots showed an earlier peak compared to fibrous roots. In addition, on a seasonal basis the mean length production of fibrous roots was almost 30% higher than that of pioneer roots. This discrepancy in root length was probably due to the almost 6-fold higher number of fibrous roots compared to pioneer roots. These observations clearly indicate that pioneer roots are the first to colonize the soil, creating a skeletal root framework from which fibrous roots originate. Indeed, the latter are more numerous and elongate further into the soil in search of water and nutrients (Polverigiani et al. 2011). While fibrous roots diameter remained unchanged throughout the season, our data showed that the diameter of the pioneer root population increased regularly reaching its maximum size at the end of July and decreasing right after until the end of the season. Thus, interestingly, roots that remained active after this peak were only those belonging to the cohort of smaller diameter size. Furthermore, the population of pioneer roots had a significantly higher mean diameter than fibrous roots. Concerning growth velocity, our data showed a bimodal seasonal pattern for both pioneer and fibrous root types. In the case of pioneer roots, the first growth phase was characterized by a slower growth than the second, while the growth velocity of fibrous roots showed the inverse pattern, with the first growth phase being faster than the second. These differences are attributable to the specific morpho-functional characteristics of the two root types. Pioneer roots undergo radial growth, therefore having a relatively coarse diameter and probably a longer life span. In addition, they have a high initial growth rate, extending rapidly into the soil. Pioneer roots primarily function (a) in soil exploration to develop an enduring expanded root system, (b) as conduits for water and nutrient transport to the stem, and (c) as key storage organs for non-structural carbohydrates and mineral nutrients (Polverigiani et al. 2011). The change in root population sizes is also connected to the changing water availability during the transiting from wet to dry season (Comas et al. 2005; Montagnoli et al. 2018). In a recent work Montagnoli et al. (2019) showed that a cohort of fine roots after the spring flush continued their growth in a radial pattern to function in starch storage (Terzaghi et al. 2016), and this seems to be an exclusive feature of the pioneer root type. Vice versa, fibrous roots are those with a shorter life span produced for the seasonal need of water and nutrient uptake, being ephemeral and with a low cost:benefit ratio (Ostonen et al. 2007).

To the best of our knowledge, there are currently no other studies available concerning grapevine pioneer and fibrous root traits and how these traits might be influenced by changes in soil physico-chemical characteristics due to BC application. The effect of N, P, and K addition to the soil on fine root development is still controversial, since both positive and negative trends have been found (Yuan and Chen 2012; Nadelhoffer 2020; Wang et al. 2016; Haynes and Gower 1995; Mei et al. 2008; Wang et al. 2012). Some of the reasons for these incoherent findings are the high species-specificity (Jourdan et al. 2008), and differences in soil type, forest age, land use history, and methodologies used (Wang et al. 2012). In our study, both BC-treated and control plants began their root growth in the middle of May, but in the case of BC-treated plants growth was of a higher magnitude, peaking sharply in the middle of June and decreasing right afterwards. Instead, pioneer roots of control plants showed a lower magnitude of root growth with a broader peak spanning from the end of June to the end of July. Fibrous roots showed an even more pronounced asynchronous timing between BC-treated and control plants. Indeed, BC-treated plants sharply peaked at the end of June, while control plants delayed their peak to the end of July. This shifting in time is more pronounced in fibrous roots probably because these roots are more sensitive to environmental cues. A similar early timing in root growth pattern was found by Comas et al. (2005), who related this shift to quicker root flushing in minimally pruned vines, which developed their canopy earlier and could thus redirect photosynthesis products toward root production. In our case, a higher root number in BC-treated plants was responsible for the shift of the root growth peak in both pioneer and fibrous root types. Growth velocity patterns of BC-treated and control plants were overlapping in the case of pioneer roots, but once more, fibrous roots showed a more pronounced reaction to the change in soil properties by growing faster than control plants. These findings fully support our hypothesis that BC-induced changes in soil characteristics modulate root development as, in fact, BC-treated plants were stimulated earlier to produce more roots, deploying a longer root system, and, in the case of fibrous roots only, to grow faster.

In our study, in spring season, characterized by high water availability, due to abundant precipitation, and by a progressive increment of air temperature, both root length and root number increased earlier and with a higher magnitude in BC-treated plants compared to controls. Root growth patterns and timing are strictly related to seasonal variations in temperature, water and nutrient availability (Montagnoli et al. 2012a, 2014). Since environmental cues are part of the signal for initial root production (Tierney et al. 2003), the higher nutrient availability, the pH increase, and the lowering of both bulk density and volumetric water content observed in BC-treated soil may have interplayed to stimulate earlier root growth at the onset of the season. Thus, our finding are in accordance with Wang et al. (2018) who highlighted that when soil moisture is high and aeration adequate, root growth can be rapid owing to the abundance of water and the lower soil impedance typical at higher soil water contents.

Since moderate soil water stress can also enhance root growth (Montagnoli et al. 2014; Ostonen et al. 2007) during the summer period, our control plants needed to lengthen their root system and exploit as much soil as possible. On the contrary, BC-treated plants, having more water available in the soil, reduced root deployment in terms of length and number in summer. Therefore, in addition to chemically enhancing the soil, BC application in our sandy soils seems to physically enhance root growth through the means of a dual effect: (a) improving soil aeration (i.e. lowering water volume) when soil water levels are highest like during spring rainfall periods, leading to longitudinal growth, and the development of a higher number of roots, and (b) enhancing water content during the harshest period of the season (summer), reducing root growth in comparison with untreated plants.

Interestingly, although we found substantial differences in the seasonal pattern of root traits between BC-treated and control plants, when values were averaged on a seasonal basis, BC alone did not induce a significant alteration of root length production, diameter, and growth velocity. We therefore believe that direct observation at a high-definition intra-annual timescale may unveil fine root responses to BC application, which could instead remain hidden in a lower definition study in terms of sampling timescale. This hypothesis is further supported by the three-way ANOVA test, which showed that BC treatment did not explain the data variation of root traits when the variable of time was excluded from the analysis. Comas et al (2005) suggested that, in contrast to the optimization theory that suggests a selective resource allocation to best acquire limiting resources, shifts in allocation may only occur at times of the year, such as the early season, when strong competition from reproductive sinks are not present. In line with this observation, our analysis revealed that BC application modified the intra-annual root dynamics allocating more resources to root lengthening and root number production in the early developmental stage of the vine, from canopy development to bloom (Comas et al. 2005). Furthermore, in our case, later in summer when reproductive development reaches stages of high carbon demand, with a higher soil water content due to the BC application, an additional shift in resource allocation seems to lower new root production and lengthening.

In conclusion, our study illustrates the seasonality of root length production, number, diameter size and growth velocity in grapevine active roots, highlighting differences between pioneer and fibrous root types, which are connected to their specific functionality. A rhizobox-flatbed scanner approach allowed defining a unimodal pattern of root production, which is probably jointly regulated by exogenous and endogenous factors. BC-application enhanced physico-chemical soil properties by increasing pH and plant available NPK, reducing soil bulk density and water content at field capacity, and increasing soil water content during the harsh summer period. The interplay between these modified factors, overlaid to the normal seasonality of root dynamics, promoted an early spring root production in terms of lengthening and number, which coincided with an earlier canopy development. During the harsh summer period, BC-treated plants, meeting higher water availability in the soil, did not need to enlarge the root system as in the case of control plants. Such an understanding of grapevine root dynamics provides useful information in terms of functionality of different root types, the effect of BC on acidic sandy soils, and intra-annual adaptation strategies to modified environmental characteristics.

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Figure captions

Figure 1. Schematic design of the sampling plots. Black dots indicate a grapevine plant, with three plants composing one plot. Plots were divided in two rows, control and biochar-treated. White rectangles indicate the positioning of the rhizobox with respect to plots.

Figure 2. Monthly average air temperature (solid line) and total rainfall (black bars) for the entire sampling period (March-October 2018); data obtaintd from ARPA Lombardia (www.arpalombardia.it). Dashed lines indicate root and soil sampling points.

Figure 3. Above, from left to right, (a) buried rhizobox, (b) flatbed scanner embedded in a woody frame, (c) close-up of a buried rhizobox with the transparent acrylic side facing the soil. Below, (d) setup sketch of the rhizobox-flatbed scanner system. The scanner connected to the laptop is inserted into the rhizobox for root image acquisition.

Figure 4. (A) A whole scanned image (size 20.6 x 21.6 cm) with a subsample indicated by the black rectangle. From B to D, a temporal series of the image subsample showing pioneer (PR) and fibrous (FB) roots.

Figure 5. Seasonal pattern of (A) pioneer and (B) fibrous root length production (cm m^{-2}) for each time point. White and dark-gray bars indicate data of control and biochar-treated plants, respectively. Inner panels show the cumulative values for control (solid line) and biochar-treated (broken line) plants. Data refer to 0–30 cm soil depth. Each time point is represented as mean (n=5). Asterisks indicate statistically significant differences ($p < 0.05$) between control and biochar-treated plants within the same time point. Vertical boxes represent approximately 50% of the observations and lines extending from each box are the upper and lower 25% of the distribution. Within each box, the solid horizontal line is the mean value and the broken line is the median.

Figure 6. Seasonal pattern of (A) pioneer and (B) fibrous root number (n) for each time point. White and dark-gray bars indicate data of control and biochar-treated plants, respectively. Data refer to 0–30 cm soil depth. Each time point is represented as mean (n=5). Asterisks indicate statistically significant differences ($p < 0.05$) between control and biochar-treated plants within the same time point. Vertical boxes represent approximately 50% of the observations and lines extending from each box are the upper and lower 25% of the distribution. Within each box, the solid horizontal line is the mean value and the broken line is the median.

Figure 7. Seasonal pattern of (A) pioneer and (B) fibrous root diameter size (mm) for each time point. White and dark-gray bars indicate data of control and biochar-treated plants, respectively. Data refer to 0–30 cm soil depth. Each time point is represented as mean (n=5). Asterisks indicate statistically significant differences ($p < 0.05$) between control and biochar-treated plants within the same time point. Vertical boxes represent approximately 50% of the observations and lines extending from each box are the upper and lower 25% of the distribution. Within each box, the solid horizontal line is the mean value and the broken line is the median.

Figure 8. Seasonal pattern of (A) pioneer and (B) fibrous root growth velocity (mm day^{-1}) for each time point. Solid and broken line indicate data of control and biochar-treated plants, respectively. Data refer to 0–30 cm soil depth. Each time point is represented as mean (n=5) ±1SE. Asterisks indicate statistically significant differences ($p < 0.05$) between control and biochar-treated plants within the same time point.

Table 1

Table 1. Soil texture characteristics of the experimental vineyard

	Size range (metric)	Values (%)
Boulder	> 256 mm	0.00
Cobble	64 - 256 mm	0.00
Very coarse gravel	32 - 64 mm	0.00
Coarse gravel	16 - 32 mm	1.91
Medium gravel	8 - 16 mm	3.19
Fine gravel	4 - 8 mm	3.39
Very fine gravel	2 - 4 mm	2.19
Very coarse sand	1 - 2 mm	21.96
Coarse sand	0.5 - 1 mm	16.82
Medium sand	0.25 - 0.5 mm	15.38
Fine sand	125 - 250 µm	14.86
Very fine sand	62.5 - 125 µm	14.50
Silt	3.9 - 62.5 µm	5.50
Clay	0.98 - 3.9 µm	0.30

Table 2. Chemical and physical characteristics of pure biochar applied in the field experiment. Each value represents the mean (n = 8) ±1 SE.

PARAMETER	UNIT	VALUE
pH	-	9.7 ±0.1
EC	dS m^{-1}	7.5 ±0.4
CEC	cmol kg^{-1}	21.3 ±0.3
Moisture	g kg^{-1}	62.4 ±1.2
N_{tot}	g kg^{-1}	9.1 ±0.2
N_{av}	mg kg^{-1}	30.0 ±0.4
P_{tot}	mg kg^{-1}	1221 ±21
P_{av}	mg kg^{-1}	217 ±3
C_{tot}	g kg^{-1}	778 ±0.1
C_{org}	g kg^{-1}	705 ±0.1
H/C_{org}	-	0.76
Alkalinity	% $CaCO_3$	18.2 ±0.6
BET	m^2 g^{-1}	410 ± 6
Total porosity	mm^3 g^{-1}	2722
Transmission pores	mm^3 g^{-1}	318
Storage pores	mm^3 g^{-1}	1997
Residual pores	mm^3 g^{-1}	406
Particle size distribution	mm g^{-1}	
50-20	%	4
20-10	%	12
10-8	%	19
8-4	%	16
≤ 2	%	49

EC: Electrical conductivity; CEC: Cation exchange capacity

Table 3. Chemical-physical analysis performed on soil samples (0-10 cm depth) of control and biochar-treated plots. Each value represents the mean (*n* = 5) ± 1 SE. Bold values are significantly different ($p<0.05$).

PARAMETER		UNIT	CONTROL	BIOCHAR
pH			**5.05 ±0.04**	**6.41 ±0.02**
Bulk density		g cm^{-3}	**0.83 ±0.05**	**0.69 ±0.02**
GWC	Field capacity	g g^{-1}	0.56 ±0.02	0.57 ±0.02
	1st August	g g^{-1}	0.19 ±0.05	0.25 ±0.04
VWC	Field capacity	cm^3 cm^{-3}	**0.46 ±0.02**	**0.39 ±0.01**
	1st August	cm^3 cm^{-3}	0.18 ±0.03	0.22 ±0.02
C_{tot}		%	**2.63 ±0.08**	**3.47 ±0.1**
N_{tot}		%	**0.24 ±0.01**	**0.34 ±0.01**
H_{tot}		%	0.68 ±0.02	0.69 ±0.02
TOC		%	2.34 ±0.03	2.57 ±0.2
N_{av}		%	**0.22 ±0.01**	**0.26 ±0.01**
P_{tot}		ppm	**760 ±43**	**937 ±4**
P_{av}		ppm	**76.0 ±4.3**	**93.7 ±0.4**
Mg_{tot}		ppm	**4591 ±325**	**5647 ±147**
Mg_{av}		ppm	**56.9 ±1.9**	**76.8 ±1.5**
Na_{tot}		ppm	**63.1 ±2.1**	**75.3 ±1.8**
Na_{av}		ppm	**60.0 ±5.5**	**72.9 ±4.0**
K_{tot}		ppm	**517 ±37**	**614 ±8**
K_{av}		ppm	**66.7 ±1.9**	**84.3 ±1.6**
Fe_{tot}		ppm	19031 ±1242	18947 ±345
Fe_{av}		ppm	**77.9 ±2.2**	**93.2 ±1.2**

GWV = gravimetric water content; VWC= volumetric water content

Table 4 Three-way ANOVA to test the effects of BC-treatment (biochar vs. control), root type (pioneer vs. fibrous) and time (15 sampling points) and their interaction on root length production, growth velocity, number, and mean diameter.

Independent variables	df	Dependent variables							
		RLP		RN		Mean Diameter		Growth velocity	
		F	*p*	F	*p*	F	*p*	F	*p*
BC-treatment (BT)	1	0.080	.779	0.36	.851	0.184	0.670	7.781	0.117
Root type (RT)	1	1.138	0.290	10.033	**0.002**	53.361	**0.000**	27.856	**0.000**
Sampling points (Time)	12	3.847	**0.002**	0.945	0.494	2.678	**0.011**	3.974	**0.000**
BT x RT	1	0.223	0.638	0.961	0.331	0.223	0.639	5.008	0.343
BT x Time	11	3.288	**0.004**	2.505	**0.021**	0.370	0.932	1.712	0.323
Time x RT	11	0.349	0.943	0.846	0.566	1.908	0.076	2.299	**0.022**

Root length production (RLP); Root number (RN); Root growth velocity (RGV); Root diameter (RD).

Table 5. Seasonal mean root traits for pioneer and fibrous roots

PARAMETER	UNIT	PIONEER		FIBROUS	
		Control	Biochar	Control	Biochar
RLP	mm day^{-1} m^{-2}	1440 ± 27 b	1310 ± 53 b	2000 ± 48 a	2200 ± 69 a
RN	n m^{-2}	34.4 ± 4.7 c	56.3 ± 9.4 b	222 ± 36 a	195 ± 39 a
RD	mm	0.55 ± 0.03 a	0.54 ± 0.04 a	0.26 ± 0.01 b	0.27 ± 0.01 b
RGV	mm day^{-1}	2.27 ±0.22 a	2.17 ±0.28 a	0.84 ±0.06 b	0.65 ±0.05 b

Values represent the mean (n = 5) ± 1SE. Letters indicate significant differences ($p<0.05$) for each parameter among root types and treatments. Root length production (RLP); Root number (RN); Root growth velocity (RGV); Root diameter (RD).

Figure 1

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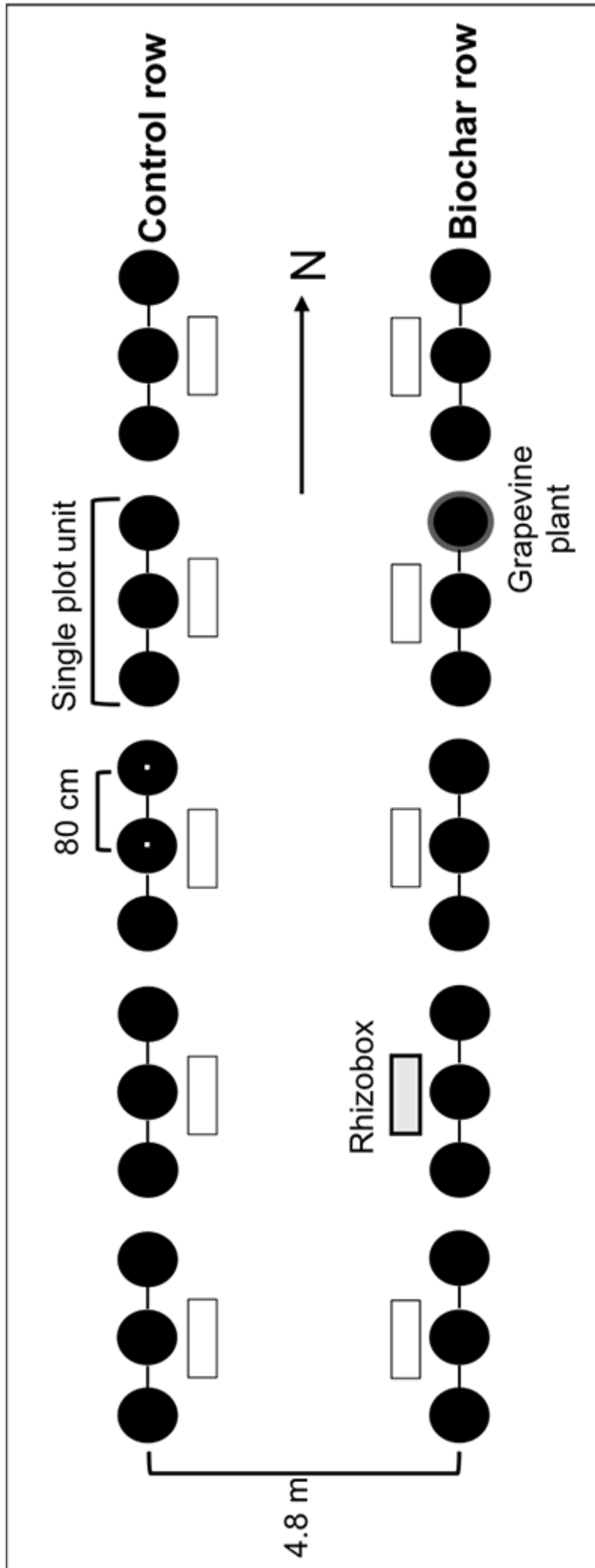

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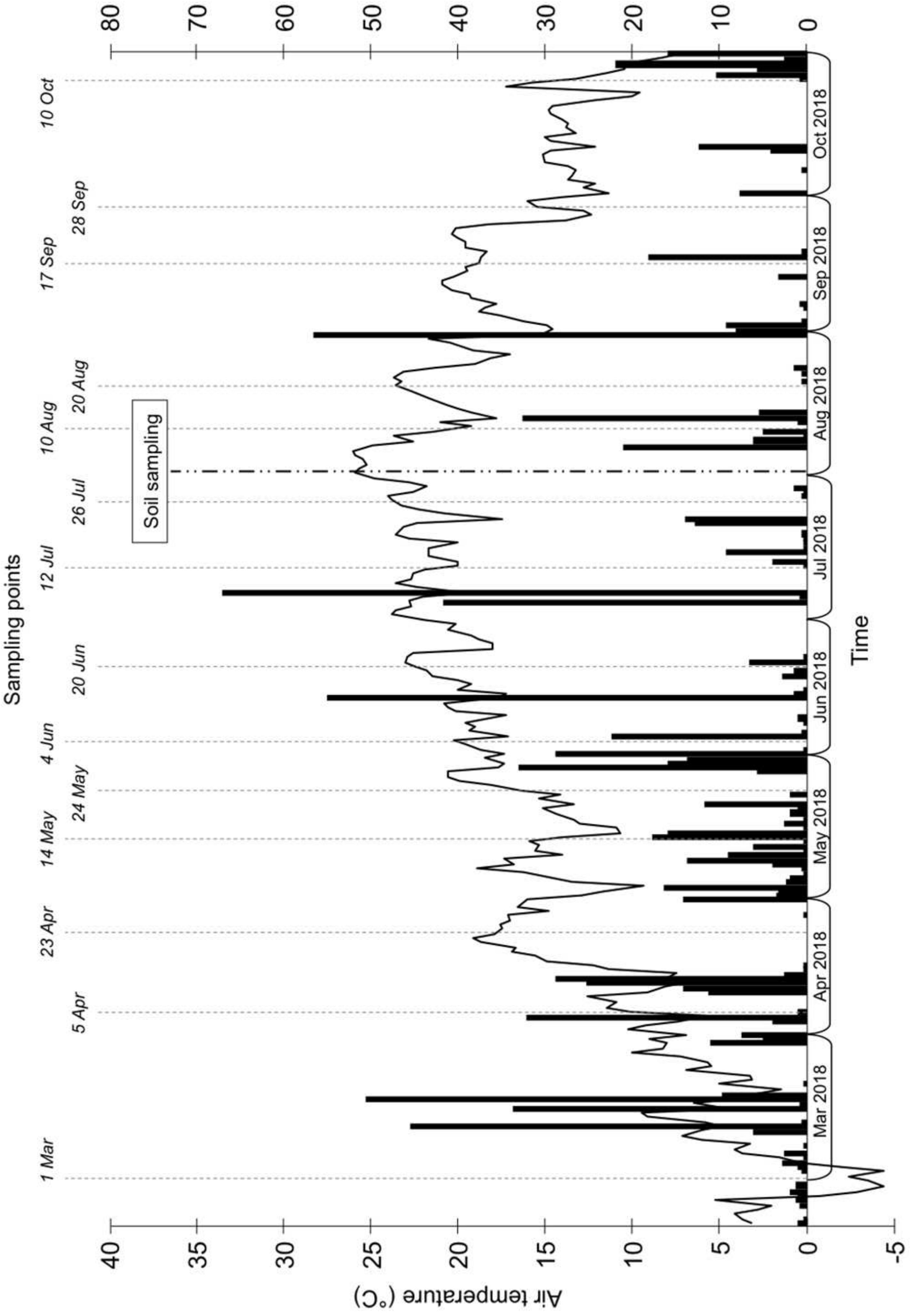

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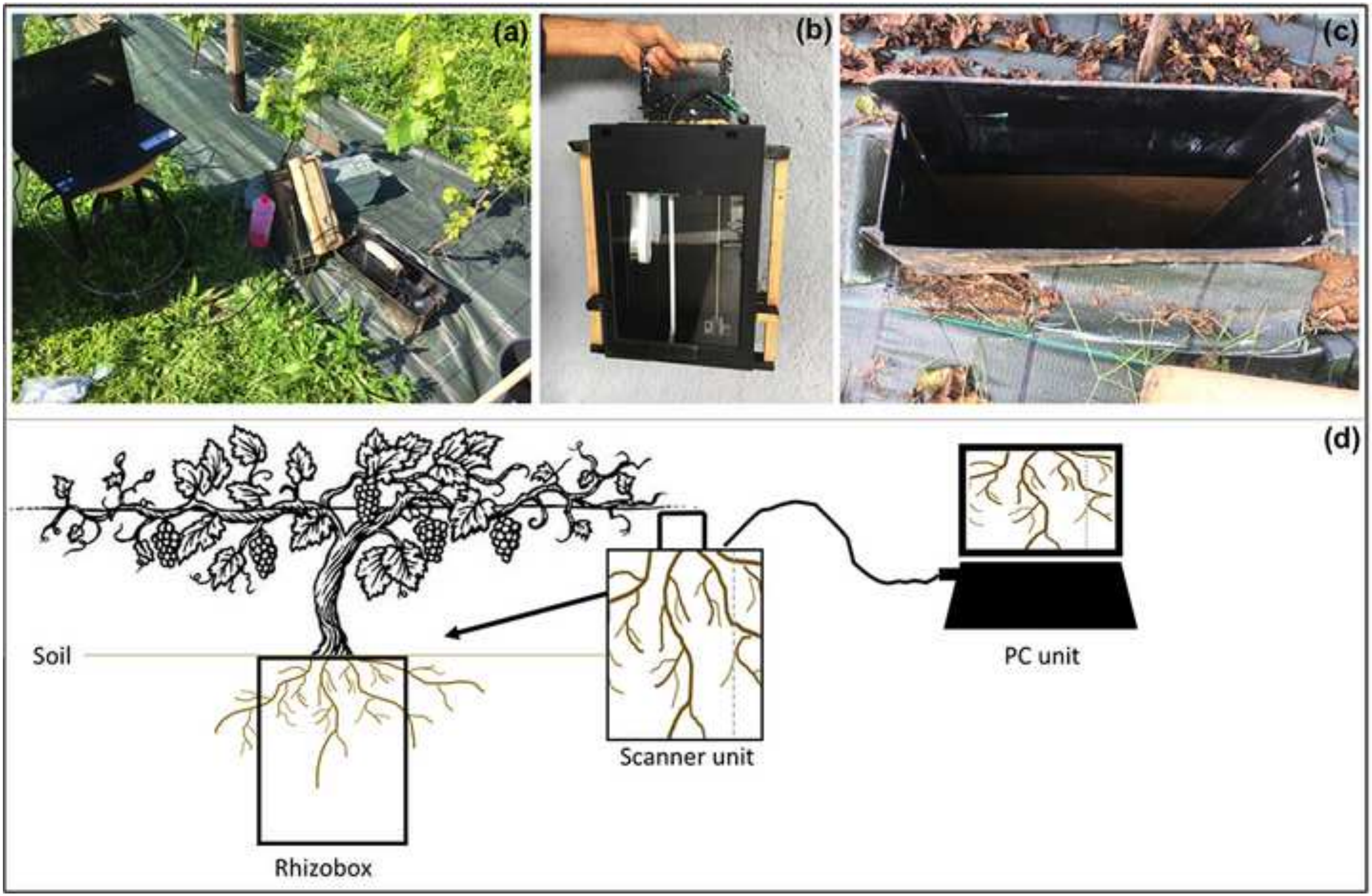

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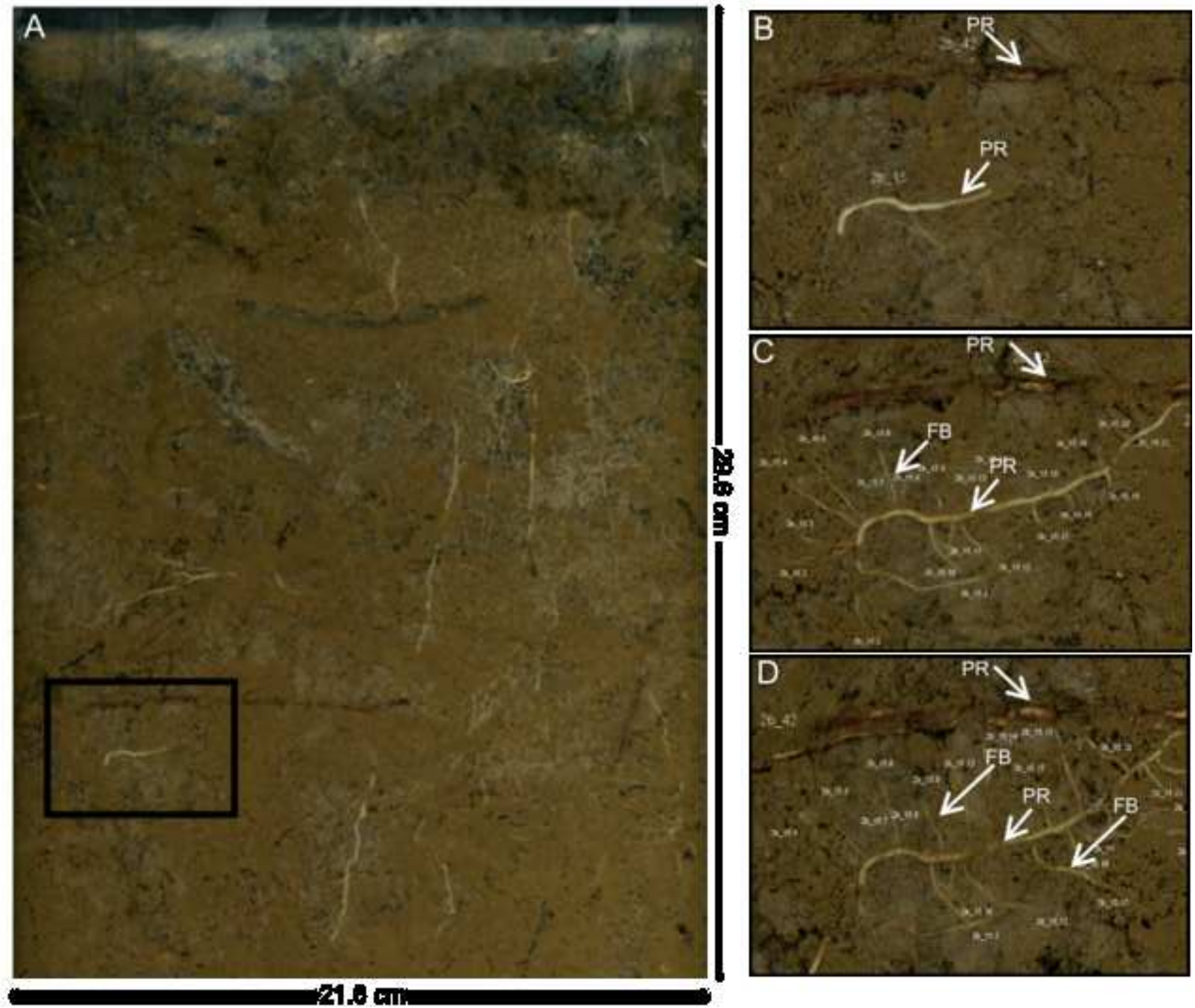

Figure 5

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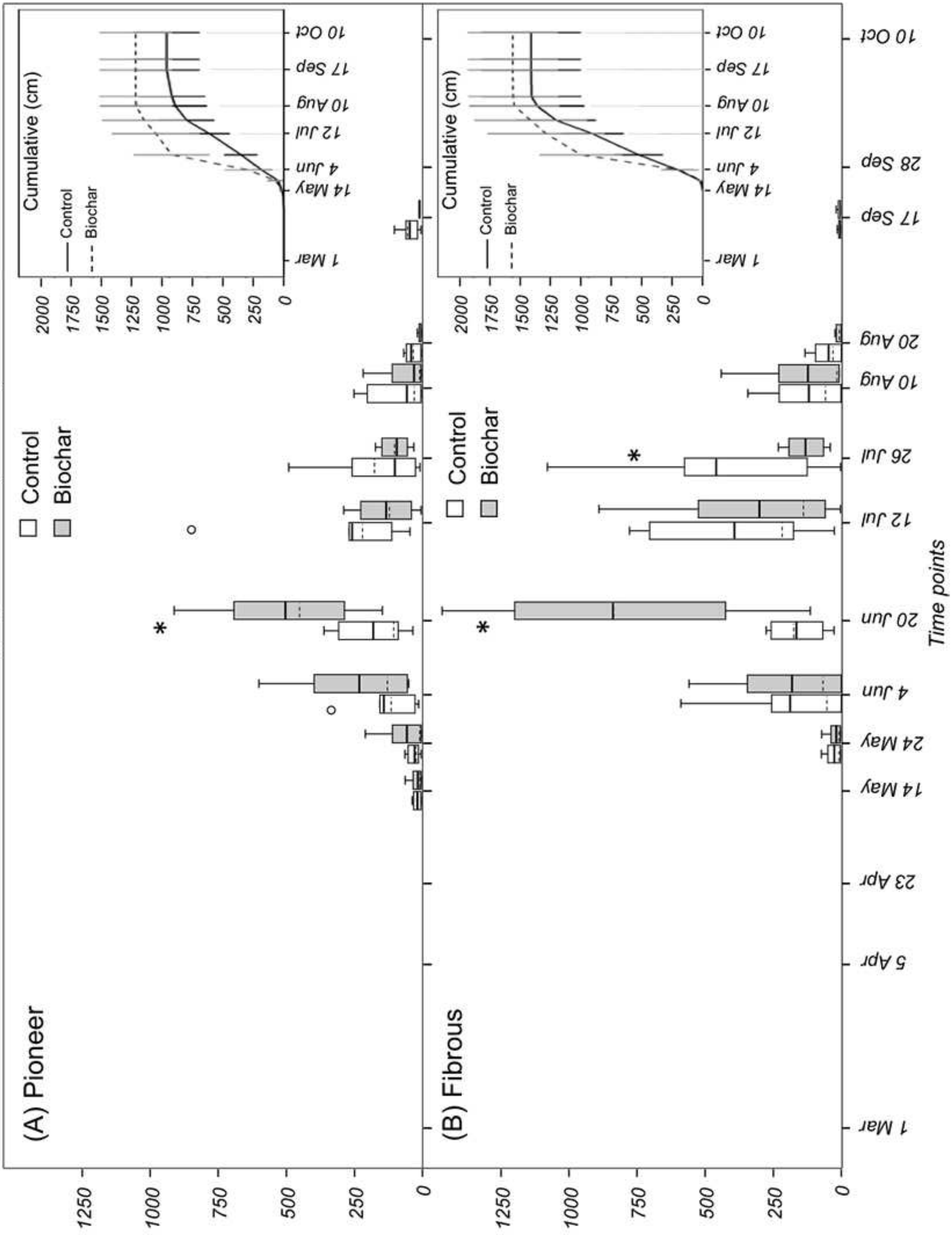

Figure 6

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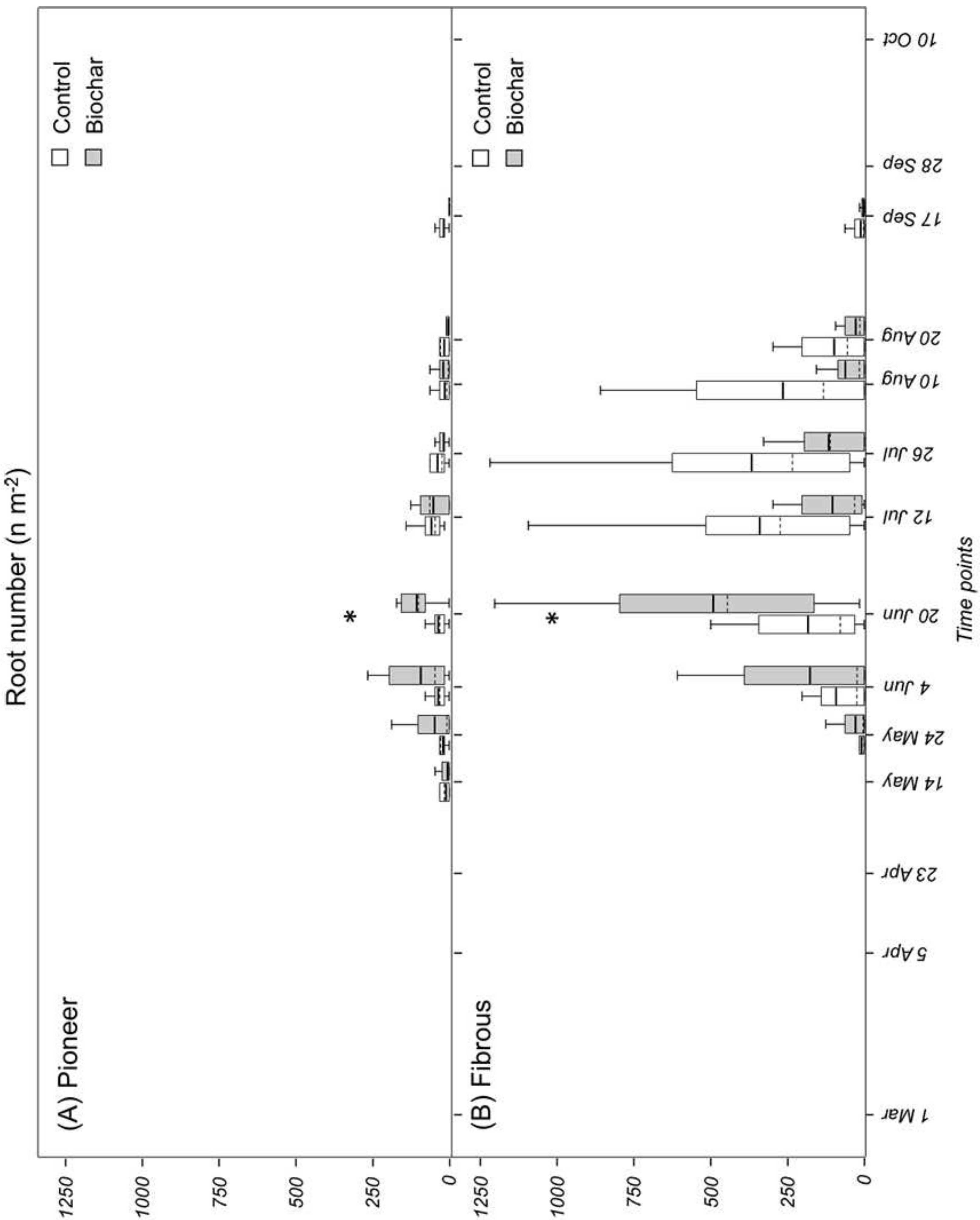

Figure 7

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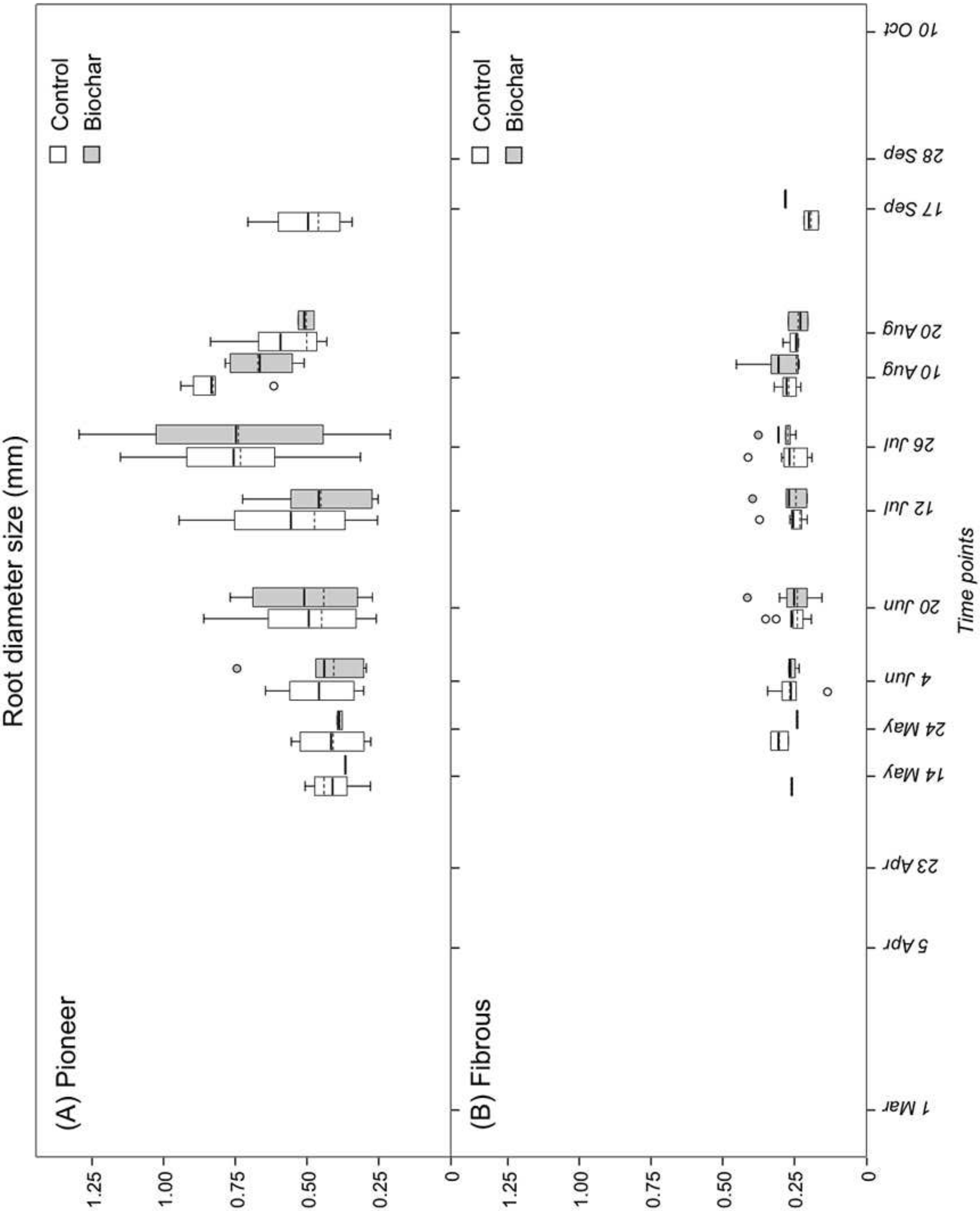

Figure 8

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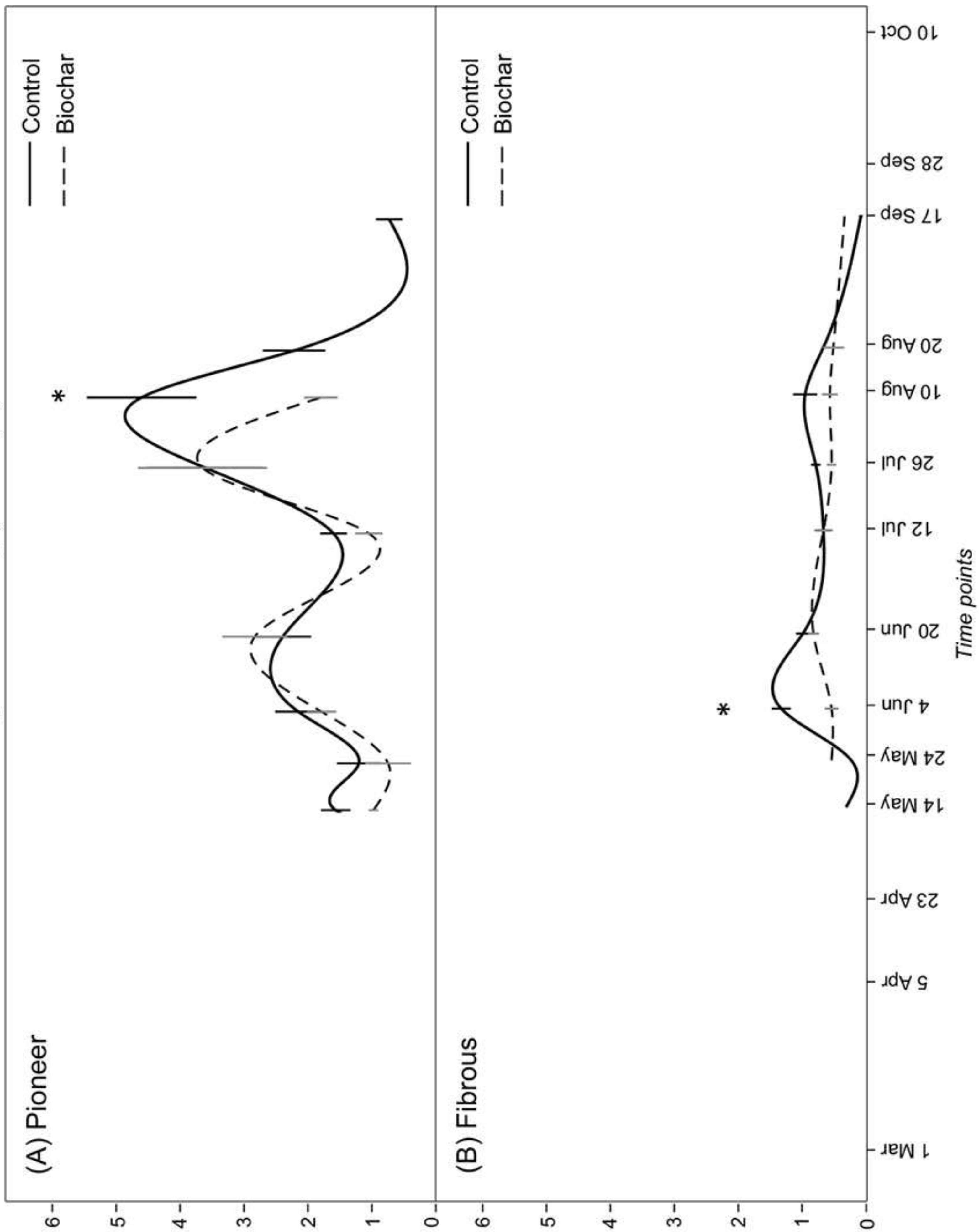

Conflicts of Interest Statement

Manuscript title: **Pioneer and fibrous root seasonal dynamics of *Vitis vinifera* L. are affected by biochar application to a low fertility soil: a rhizobox approach**

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