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Short-term effects of biochar on grapevine fine root dynamics and arbuscular mycorrhizae production

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ABSTRACT

Application of biochar to the soil is globally recognised as a means to improve soil structure and fertility, increase carbon sequestration, enhance crop production and mitigate climate change. However, although the fine root system is fundamental for plant growth, crop productivity, carbon and nutrient cycling, little is known about the effect of biochar on plant fine roots. This study, conducted in a Montepulciano (*Vitis vinifera* L.) vineyard, was aimed at investigating the impact of biochar application (at the rate of 10 t ha^{-1}) on soil chemical and physical properties, fine root dynamics and arbuscular mycorrhizal fungi (AMF) production during a one-year sampling period. To this aim, seasonal variation of fine root mass, length and diameter was measured by the sequential coring technique, whereas fine root annual production was calculated by minimum-maximum procedure and turnover rate of live roots by maximum standing biomass. For AMF annual production, in-growth mesh bags were used to measure glomalin as quantitative indicator of mycorrhizae presence. Results showed that biochar significantly increased organic carbon (20.7%), available ammonium (84.4%), and available water content of the soil (11.8%), while it also promoted the formation of the large fraction of macro aggregates ($ø > 2$ mm; 3.1% control; 5.5% treated). Cation exchange capacity, pH, total nitrogen content, and total and available phosphorus content remained unaffected. Immediately after biochar soil amendment, while fine root length remained unchanged, a significant increase in fine root biomass was measured resulting in a higher mean annual biomass (8.56 g m^{-2} control; 13.34 g m^{-2} treated), annual production (8.71 g m^{-2} control; 12.7 g m^{-2} treated) and lifespan (as evidenced by a lower turnover rate; 1.02 yr^{-1} control; 0.95 yr^{-1} treated). Moreover, the increase of fine root biomass resulted to be associated with radial growth since mean fine root diameter was significantly higher in biochar-treated plants (0.56 mm) than in control plants (0.46 mm). Biochar had no significant effect on the annual production of AMF. The results of the present study show that the improvements of soil chemical and physical features due to biochar application have an immediate effect on fine root dynamics and morphology. Furthermore, the increase of fine root biomass is mainly due to radial growth and occurs during the water shortage period, supporting fruit setting and ripening in grapevine plants.

1. Introduction

Biochar, a charcoal produced by controlled pyrolysis, has been widely recognised for its potential use to improve soil fertility, sequester carbon (C), mitigate climate change (Lehmann et al., 2006; Lehmann, 2007; Laird, 2008; Sohi et al., 2010) and enhance phytostabilisation of contaminated soils (Brennan et al., 2014; Lomaglio et al., 2016). Indeed, the positive effects of biochar on agricultural productivity have been attributed to: i) the reduction of soil acidity (Yuan et al., 2011; Pereira et al., 2015); ii) the improvement of cation-exchange capacity (CEC) and nutrient availability; iii) the dissolution of organic carbon in low-pH acidic soils (Mukherjee and Zimmerman, 2013); iv) the increase of water

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retention capacity (Downie, 2011; Baronti et al., 2014); and v) the availability of plant water content (Tammeorg et al., 2014). Despite the growing amount of data reported in the literature on the positive effects of biochar on agricultural productivity, plant responses to biochar soil amendments have largely focused on above-ground biomass and crop yields. Biochar has been reported to increase rooting during germination (Vookova and Kormutak, 2001), and to enhance root biomass (Lehmann et al., 2003; Joseph et al., 2010; Makoto et al., 2010) and length (Noguera et al., 2010). However, in most of these cases the analysis of root response to biochar amendment was limited to biomass measurements (Lehmann et al., 2003; Noguera et al., 2010; Prendergast-Miller et al., 2011) and, therefore, the mechanisms controlling root–biochar interactions still remain poorly understood (Lehmann et al., 2011). Roots have important functions in plants, including nutrient and water uptake, anchorage, and mechanical support, and are the first organs to be affected by biochar (Prendergast-Miller et al., 2013). Furthermore, within the plant root system, fine roots are the principal structures involved in water and nutrient acquisition (Mainero et al., 2009; Montagnoli et al., 2012a,b, 2014; McCormack et al., 2015; Terzaghi et al., 2016). Fine root lifespan has important implications for individual plant growth, crop productivity, plant-environment interactions, and belowground carbon (C) and nutrient cycling (Godbold et al., 2003; Montagnoli et al., 2010; Di Iorio et al., 2013; Madhu and Hatfield 2013a,b; Terzaghi et al., 2013; McCormack and Guo, 2014). Indeed, Jackson et al. (1997) estimated that as much as 33% of global annual net primary productivity in terrestrial ecosystems is devoted to fine root production, and the growth and maintenance of fine roots may use up to 50% of the daily-produced photosynthate in crop plants (Lambers, 1987).

It has been proposed that biochar may affect root growth and plant performance through two mechanisms: i) as a direct nutrient source and ii) by enhancing nutrient availability (Lehmann et al., 2011). In a recent investigation, Prendergast-Miller et al. (2013) showed that biochar controls plant root nutrient acquisition in rhizobox-grown spring barley (*Hordeum vulgare* L.), both directly as a nutrient source and indirectly by altering soil nutrient content. Similarly, again in a rhizobox experiment, Reibe et al. (2015) found that nutrients released from different kinds of biochar might affect root morphology of spring wheat. Furthermore, different types of chars had different effects on root and shoot growth and soil changes, depending on the feedstock, the production process and the amount of biochar applied (Bhattacharjya et al., 2015).

Through its effect on nutrient cycles (Steiner et al., 2008) or soil structure (Mummey and Rillig, 2006), biochar has also been shown to create a habitat for beneficial soil microorganisms (Rillig and Thies, 2009), which in turn may improve plant growth (Warnock et al., 2007). However, the effects of biochar on soil biota abundance and composition may differ for different groups of microorganisms (reviewed in Lehmann et al., 2011). Living in symbiosis with plant roots, arbuscular mycorrhizal fungi (AMF) develop an extensive extraradical hyphal network, which plays an important role in plant nutrient uptake (Harrison and van Buuren, 1995; Read and Smith, 1997; Avio and Giovannetti, 2002 promoting plant growth (Schwartz et al., 2006; Compant et al., 2010). In fact, AMF provide their host plants with mineral nutrients receiving photosynthetically-derived carbohydrates in return (Read and Smith, 2008). Thus, the presence of AMF is particularly important in marginal soils, where their contribution to nutrient uptake may be more critical to the plant (Bücking et al., 2014). Glomalin is a wall protein of the AMF mycelium with concentrations in the soil generally ranging from 2 to 14 mg g^{-1} (Pikul et al., 2002) and, therefore, commonly used as a quantitative indicator (Upadhyaya and Wright, 1996; Lovelock et al., 2004). The presence of biochar in the soil seems to have a general positive effect on mycorrhizal fungi (reviewed in Warnock et al., 2007), although negative results have also been reported (Birk et al., 2010; Warnock et al., 2010).

Due to the economic importance of grapevine, over the last years much attention has been paid to the effect of biochar on amended groves. Recent studies revealed that the positive effects of biochar on grape yield and quality are mainly due to: i) the attenuation of water stress (Baronti et al., 2014; Genesio et al., 2015); ii) the improvement of soil chemical and biological fertility and nutrient supply to plants (Glaser et al., 2002; Sohi et al., 2010; Vaccari et al., 2011; Schulz et al., 2013); iii) the enhancement of plant growth and yield (Lehmann and Rondon, 2005; Chan et al., 2007; Major et al., 2010b); and iv) the reduction of greenhouse gas emissions through C sequestration (Van Zwieten et al., 2010; Ippolito et al., 2012; Zhang et al., 2012). Once again, in these studies, the effects of biochar were investigated mainly in terms of changes in soil physical and chemical characteristics, plant yield and biomass production. Indeed, to our knowledge, the influence of biochar on fine root lifespan and on the mutualistic interaction between grapevine roots and AMF (Groot-Obbink and Possingham, 1971; Deal et al., 1972; Menge et al., 1983; Nappi et al., 1985) has not been investigated yet. Studying the impact of biochar on fine root dynamics of perennials plants such as grapevine is fundamental for understanding plant-soil interactions and their consequences for plant growth. Given the above-mentioned effects that biochar can have on soil nutrient and water availability, we hypothesised that changes in resource supply play an immediate role in root dynamics and AMF colonization, thereby further affecting crop production and yield. To test this hypothesis, after assessing the effects of biochar on soil physical-chemical properties, fine root dynamics and AMF production in a vineyard were investigated in a short-term (one-year) time course experiment. The identification of possible relationships between any alterations of soil physical-chemical properties, fine root dynamics and AMF production may further contribute to elucidating the mechanisms of biochar actions.

2. Material and methods

2.1. Experimental site and set up

The field experiment was carried out in a vineyard of the *Valerio Vini* estate (41°32′19.8″N 14°09′34.9″E; 270 m a.s.l.) in the municipality of Monteroduni (Molise, Central Italy). The vineyard (Montepulciano wine grape variety) consists of 24 north-west oriented plant rows (2.5 m spacing), each containing 15-year-old plants (80 cm spacing), not irrigated. During the study period, from May 2014 to May 2015, total rainfall was approximately 1310 mm with an average air temperature of 14 °C (data from the Fornelli (IS) weather station, supplied by the Regione Molise). Soil-milling operations (20 cm depth) were carried out at the beginning of April 2014 as usual management practice. At the beginning of May 2014, biochar was applied at a rate of 10 t ha^{-1} (Van Zwieten et al., 2008; Brandstaka et al., 2010; Ndor et al., 2015). In order to obtain a homogeneous soil application, the biochar was crushed into smaller particles, sieved at 2 mm size and homogeneously broadcasted by hand (Major, 2010a), between plants and within the whole plot area (4 m^2). To avoid biochar loss by wind or water erosion, immediately after spreading biochar on the soil surface, moisture was applied with a Verdigris sprayer (Karer et al., 2013) and biochar was incorporated into the soil with a hand-powered rotary hoe at low rotation speed (10 cm depth; Karer et al., 2013). Finally, another inter-row soil milling was carried out one year later (April 2015), before the last sampling point. Measurements were carried out in eight plots (four control and four biochar-treated) of 4 m^2 in size, each including three plants displaced on the same row (Fig. 1).