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Research paper

Characterization of fine root system and potential contribution to soil organic carbon of six perennial bioenergy crops

Carlo Chimento, Stefano Amaducci*

Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122, Piacenza, Italy

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ABSTRACT

Perennial bioenergy crops provide biomass for renewable energy production, but also sequester atmospheric carbon (C) in the soil. Roots represent one of the most important soil C inputs-root length density (RLD, cm cm^{-3}), root diameter and fine root biomass (FRB, Mg ha^{-1}) in the top 1 m of soil were characterized for three woody (poplar, black locust, willow) and three herbaceous (giant reed, miscanthus, switchgrass) perennial crops in the same location. The vertical distribution of FRB and RLD was described by fitting the "beta" (β) model to the experimental data. The herbaceous species had higher β values for FRB and RLD than woody crops, suggesting that the former explore the deeper soil layers with a greater proportion of roots. In particular, 3.7 Mg ha^{-1}, or 43% of the whole root mass, was found below the ploughing soil layer (0.3 m) for the herbaceous species, while only 1.2 Mg ha^{-1}, or 26% of the whole root mass, was allocated by woody crops to the same soil layer. In all the species, the majority of the sampled roots (99.1%) had a diameter lower than 2 mm, and in the first 10 cm of the soil the woody species tended to produce roots with a smaller diameter than those of the herbaceous species. Overall, the herbaceous crops have a higher potential to contribute to C storage in the deep soil layers, while the woody species, have a greater potential to affect soil organic carbon in the top soil layer.

1. Introduction

Cultivation of perennial bioenergy crops combines the provision of biomass for renewable energy production with the sequestration of atmospheric C in the soil and biomass [1–3]. Considering that on a global scale soils contain more C than the vegetation and the atmosphere [4] and that the C in the soil is mainly root derived [5–7], the root systems represent a fundamental component of the global C cycle [8]. At the soil level, it was demonstrated that roots play an important role in macroaggregate stabilization [9], and that through their C inputs they have an important effect on microbial activity [7] and the soil priming effect [8,10]. Roots with a diameter of less than 2 mm are very dynamic and play a key role in the ecosystem C cycling [11,12] and they perform important physiological functions, such as uptake (very fine root 0.0–0.5 mm diameter) and transport (fine root; 0.5–2.0 mm diameter) of water and nutrients. The production and turnover of roots with a diameter lower than 2 mm are controlled by environmental factors, such as drought condition [13], bulk density, soil fertility [14], and by the genetic background [15]. Root diameter and soil depth at which roots develop are important factors controlling the root C input in the soil, and many studies have reported on the link between these factors and the rate of root mortality. Roots with a smaller diameter have a faster turnover rate than roots with a larger diameter, while the mortality of the roots with a diameter lower than 2 mm decreases with the increase in soil depth [16–18].

The root system represents an important C input in the upper soil layers but, together with dissolved organic matter it is the main input in deeper soil layers [19]. Jobbagy & Jackson [20] compared the soil profiles of a wide range of natural environments and ecotypes and concluded that the distribution of organic carbon (OC) along the soil profile is affected by the type of vegetation. In particular, they pointed out that the aboveground C input accumulation on the soil surface was responsible for the shallow soil organic carbon (SOC) accumulation in tree dominated ecosystems, while the vertical root distribution was responsible for the SOC accumulation at deep soil layers in shrub dominate ecosystems. The accumulation of C at deep soil layers, where oxygen concentrations is limited and organic matter mineralization is reduced [19,21] is considered an important C sequestration strategy [22]. A thorough characterization of fine roots is therefore an essential step in

* Corresponding author.
E-mail address: stefano.amaducci@unicatt.it (S. Amaducci).

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understanding the C sequestration potential of different bioenergy species. Main root traits are genetically controlled [23,24] but environmental and soil conditions also play an important role in their determination [25]. To limit the environmental effect the study of root traits of contrasting species should be carried out in the same location in a completely randomized experimental design. In the literature, there are very few studies that have compared the root systems of perennial bioenergy crops. Monti & Zatta [26] compared the root systems of three of the most common herbaceous bioenergy crops (switchgrass, miscanthus and giant reed) and found that switchgrass and giant reed developed a high proportion of their root biomass in the deeper soil layers. Zan et al. [2] found that switchgrass allocated more root biomass at deeper soil layers compared to willow.

The main objective of this work was to compare the main traits of the root system in the top 1 m of soil for six perennial bioenergy crops: three woody crops *Populus* spp. (poplar), *Robinia pseudoacacia* (black locust), and *Salix* spp. (willow) – and three herbaceous crops – *Arundo donax* L. (giant reed), *Miscanthus* × *giganteus* (miscanthus), and *Panicum virgatum* (switchgrass) at the sixth year from plantation in a field trial in northern Italy.

2. Materials and methods

2.1. Study site and soil sampling

The field trial was established in April 2006 in the Po valley, at Gariga di Podenzano, Italy (44° 58′ 47.5″ N, 9° 41′ 08.9″ E), on a silt loam soil classified as Chromic Luvisols (FAO) with low carbonates content and neutral pH. Prior to planting, the experimental site had hosted a maize monoculture for 30 years. Mean annual temperature and precipitation are 12.2 °C and 890 mm respectively.

The experimental layout is a randomized complete block design with three blocks and a single plot size of 450 m^2 (15 m × 30 m), to compare six biomass crops: three herbaceous (*A. donax* L., *P. virgatum,* and *Miscanthus* × *giganteus*) and three woody bioenergy crops (*Populus* spp., *Salix spp.*, and *R. pseudoacacia*). Planting density was 1 plant m^{-2} for miscanthus and giant reed, 0.7 plant m^{-2} for all the woody species while switchgrass was planted with a pure live seed rate of 10 kg ha^{-1}. Inter-row distance was 0.4 m for switchgrass, 1.4 m for miscanthus and giant reed, and 2.5 m for all the woody species.

On September 2012, a self-constructed "Shelby" tube sampler of known volume (7 cm diameter and 120 cm length) was pressed with the hydraulic arm of a digger [27] in the inter-row of each crop to collect, on equal spacing, soil samples for fine root characterization, avoiding rhizomes in the herbaceous species and the stump in the woody species. A variable number of soil samples was taken to account for the different width of the inter-row space, in particular in each plot, and for each block, 5 samples were taken for woody species and 3 samples for miscanthus, giant reed and switchgrass. Whole soil cores were divided in portions representative of seven soil depths: 0–10; 10–30; 30–45; 45–60; 60–75; 75–90; 90–105 cm. The samples collected in the same plot were combined so to have one sample per each soil depth.

2.2. Root characterization

Soil samples were stored at −20 °C before root separation and analysis. In order to separate roots from soil, samples were first kept in a solution of oxalic acid (2%) for 2 h, and then washed in a hydraulic sieving-centrifuge device [26]. Once cleaned, roots were hand recovered from the water using a 2 mm mesh sieve.

Determination of root length density (RLD, cm cm^{-3}) and root diameters was performed with the software winRHIZO *Reg* 2012. The images were acquired using the TWAIN interface at 600 dpi and with a scanner (model: Epson Expression 10000xl) equipped with a double light source to avoid roots overlapping. Fine roots dry biomass weight (FRB, Mg ha^{-1}) was determined gravimetrically, after taking scanned images, drying the roots at 60 °C until constant weight.

The vertical distribution of FRB and RLD was described fitting the "beta" (β) model to the 7 pairs of data collected in the 3 blocks of the experimental design. The β model is a non-linear asymptotic function used to study root distribution in several previous works [26,28–31]:

$$y = 1 - \beta^x$$

where y represents the cumulative root content (%) from the soil surface to a certain depth (x) expressed in centimeter. The β value describes the shape of the vertical distribution of RLD or FRB within the whole sampled soil profile. High β values indicate that a large proportion of RLD or FRB is present in deep soil layers, whereas low β values indicate that a greater proportion of FRB or RLD is in the upper soil layer.

The diameter class length (DCL, mm cm^{-3}) was calculated for very fine (0.0–0.5 mm), fine (0.5–2.0 mm) and coarse (>2 mm) diameters for the whole sampled soil profile [24]. For the first two soil layers (0–10 and 10–30 cm) the DCL was calculated for seven diameter classes from 0 to 2 mm (0.0–0.25; 0.25–0.50: 0.5–0.75; 0.75–1.0; 1.0–1.25; 1.25–1.5; 1.5–1.75 and 1.75–2.0 mm). To calculate the root diameter with the higher DCL among crops, the root DCL of the first two soil layers were fitted with the non-linear regression Extreme value model (Systat TableCurve 2D) as suggested by Zobel et al. [32]:

$$y = a + bEXP\left[- EXP\left[-\frac{x - c}{d} \right] - \frac{x - c}{d} + 1 \right]$$

where y is the DCL, x is the root diameter, the coefficient *a* is the base line, coefficient *b* is the peak (DCL) value for y, *c* is the diameter class at peak value and the coefficient d is the amplitude of the curve.

2.3. Statistical analysis

The data were analyzed with the mixed effect model using the "nlme" package [33] of RStudio3.1.1. Species and depth were included in the mixed effect model as fixed factors while block effect was considered as random. Mean values were separated using the "Post-Hoc Interaction Analysis" package [34] ($\alpha = 0.05$). The differences of the β and c values were tested with the ANOVA and the mean were separated with a HSD Tukey test ($\alpha = 0.05$). The statistical significance of the regression coefficient was tested by analyzing the variance of the regression (F-statistic for $P < 0.05$). The statistical significance of the curve parameters β and c were assessed through testing their standard errors using the t-statistics at $P < 0.05$.

3. Results

3.1. Root length density (RLD) & fine root biomass weight (FRB)

Both RLD and FRB were significantly affected by species, soil depths and by the interaction of both factors. In the top soil layer (0–10 cm), poplar, willow and switchgrass showed the highest RLD values, while giant reed and black locust the lowest (Table 1).

Moving to the sub-surface soil layer (10–30 cm of soil) RLD values decreased by over 60% in woody crops, while, among the