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New dual in-growth core isotopic technique to assess the root litter carbon input to the soil

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ABSTRACT

The root-derived carbon (C) input to the soil, whose quantification is often neglected because of methodological difficulties, is considered a crucial C flux for soil C dynamics and net ecosystem productivity (NEP) studies. In the present study two independent methods to quantify this C input to the soil were compared in a young forest plantation in Italy. Specifically, a mass balance approach to the soil C balance and a new dual in-growth core (IGC) isotopic technique that accounts for both root- and non-root-derived C inputs were compared. No statistically significant difference between the results obtained by the two methods was found (3.09 ± 0.50 vs 5.30 ± 2.92 Mg C ha^{-1}). Both estimates matched with the results obtained by other authors for similar ecosystems. The application of this new dual IGC isotopic technique has shown that the traditional IGC isotopic technique can lead to a significant overestimation (60% in the present study) of root C input to the soil as it doesn't take into account changes in the soil C isotopic signature due to factors other than root derived inputs. These include isotopic discrimination that could take place during the decomposition of original soil organic matter (SOM), the contamination due to dissolved organic C (DOC) leaching, or the C input via leaf litter. The proposed dual IGC isotope technique, taking into account the abovementioned non-root derived inputs seems to be a promising and reliable method to estimate root-derived C input to the soil in terrestrial ecosystems

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1. Introduction

Plant roots transfer to the soil a portion of the carbon (C) fixed by photosynthesis which can exceed the aboveground net primary productivity (ANPP) (Alberti et al., 2015; Giardina et al., 2005) and it has been found to be crucial for the C storage potential of different forest ecosystems (Tefs and Gleixner, 2012). This C input is also functionally important in C cycling of terrestrial ecosystems as, depending on its chemical composition, it can stimulate or inhibit the mineralization (priming effect) of original soil organic carbon (SOC), affecting nutrient availability for plant growth with an indirect effect on net primary productivity (NPP) and net ecosystem productivity (NEP) (Finzi et al., 2015; Fontaine et al., 2004; Hill et al., 2015;). The organic substances originating from root turnover and exudation are chemically diverse, ranging from labile exudates to senescent material (Paterson et al., 2009) and their contribution to SOC balance depends on a number of factors, such as root productivity, root turnover rate, mycorrhizal colonization, soil and climate characteristics and species composition (Matamala et al., 2003). The development of accurate methods to quantify the root-derived C input to soil is a challenging issue for both soil C balance and C cycle studies of terrestrial ecosystems (Paterson et al., 2009; Trumbore, 2006). From a methodological point of view, several approaches have been proposed in recent decades. Conventional sequential soil coring, in-growth core methods and minirhizotron techniques (Hendrick and Pregitzer,

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Abbreviations: ANOVA, Analysis of variance; ANPP, aboveground net primary productivity; BNPP, belowground net primary productivity; $C_{litterfall}$, carbon in litterfall; C_{root}, carbon in root standing biomass; $C_{rd\text{-}gross}$, gross carbon input from roots to the soil; $C_{rd\text{-}net}$, net carbon input from roots to the soil; $C_{rd\text{-}net\text{-}nc}$, net carbon input from roots to the soil without control in growth cores correction; C_{soil}, carbon in soil; EC, eddy covariance; F_r, fraction of root derived C in the soil; F_s, soil respiration; F_{soc}, SOM-derived respiration; GPP, gross primary productivity; IGC, in-growth cores; NEP, net ecosystem productivity by eddy covariance; NEP_B, net ecosystem productivity by inventory; NPP, net primary productivity; SOC, soil organic carbon; SOM, soil organic matter; SWC, soil water content; TBCA, total belowground carbon allocation.

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1993) have been used to estimate fine root turnover and longevity and indirectly to calculate the root-derived C input. However, none of these methods consider the root exudates as a component of the root-derived C input, so they underestimate this flux of C (McClaugherty et al., 1982; Persson, 1980). Nitrogen and C budgeting methods have also been adopted to estimate both total belowground carbon allocation (TBCA) and root-derived C input to the soil (Aber et al., 1985; Ehman et al., 2002; Law et al., 2001; Panzacchi et al., 2012). These later methods indirectly estimate the root-derived C input by the direct measurements of several C stocks and fluxes with a potential reduction of precision and accuracy due to the error propagation (Ehman et al., 2002; Law et al., 2001).

Finally, tracer techniques, using C isotopes such as ^{11}C, ^{13}C and ^{14}C, can be used in labelling experiments to estimate the fate of the C fixed by photosynthesis and transferred to the soil via roots (Grayston et al., 1997; Kuzyakov and Domanski, 2000; Meharg, 1994; Nguyen, 2003; Paterson et al., 2009). Among these methods, pulse labelling, continuous labelling and methods based on the difference in ^{13}C natural abundance between C_3 and C_4 plant-derived organic matter, are widely used. Pulse and continuous labelling cannot be easily applied at stand level without the use of special and expensive infrastructure (i.e. Free-air CO_2 enrichment, FACE, experiments) and the availability of a large amount of labelled CO_2. As a consequence these approaches are more frequently used in pot and greenhouse experiments. The difference in ^{13}C natural abundance between C_3 and C_4 plants seems to be a more appropriate and less expensive isotopic approach to be used at stand level. When a natural or human-induced vegetation shift from a C_3- to a C_4-dominated plant community (or vice versa) occurs, the difference in C isotopic signature of SOM derived from $C_{3,}$ with $\delta^{13}C$ values between −21‰ and −30‰ and C_4 plants, with $\delta^{13}C$ values between −10‰ and −15‰ (Ehleringer et al., 2000), can be successfully exploited to estimate the net root-derived carbon input to the soil (Balesdent and Balabane, 1992; Balesdent et al., 1988; Cheng, 1996; Qian and Doran, 1996; Rochette and Flanagan, 1997). Similarly, exogenous C_4 or C_3 soil can be placed in C_3 or C_4-dominated plant community ecosystems, to artificially create the above mentioned difference in isotopic signature and quantify different soil C fluxes (Alberti et al., 2015; Cotrufo et al., 2011; Hoosbeek et al., 2004; Ineson et al., 1996; Martinez et al., 2016).

The aim of this study was to estimate the annual root-derived C input to the soil by two independent methods in a young deciduous forest plantation. The first method was based on the soil C balance approach as previously described by Panzacchi et al. (2012) and the second one was the in-growth core isotope technique as proposed by Hoosbeek et al. (2004) and Cotrufo et al. (2011) and here modified to increase the accuracy of the estimation. Finally, TBCA and its partitioning into several components was estimated with the mass balance approach originally proposed by Raich and Nadelhoffer (1989) and then modified by Giardina and Ryan (2002) and the results compared and discussed.

2. Material and methods

2.1. Study site

The study was carried out in a fifteen-year-old mixed hardwood plantation established in northern Italy on land previously occupied by agricultural crops. The stand covers an area of 38 ha and is located in a flat rural area of the Po Valley in northern Italy near the town of Nonantola (44°41 N, 11°02 E; 15 m a.s.l.). The climate is sub-Mediterranean with dry summers and two peaks of precipitation in spring and fall. The mean annual temperature is 14 °C and the mean annual precipitation is 700 mm (Magnani et al., 2005). The soil is classified as Eutric Vertisol according to the FAO classification (IUSS Working Group WRB, 2006) and as fine, mixed, mesic, Entic Chromusterts according to the Soil Taxonomy(Soil Survey Staff, 1999); the clay content ranges between 58 and 62% and the drainage of surplus water is generally slow. During the rainy seasons water may easily stagnate in the upper soil layer, while in the summer the level of the water table may decrease considerably, resulting in drought conditions (Ponti et al., 2004). Soil bulk density in the 0–60 cm layer was 1.33 ± 0.09 g cm^{-3}, and SOC content was $1.49 \pm 0.04\%$ (data are means ± standard error; $n = 29$), leading to 11.8 kg m^{-2} of total SOC in the upper 60 cm layer. Chemical and physical characteristics of the soil are reported in Table 1.

The dominant tree species were pedunculate oak (*Quercus robur* L.) and narrow-leaved ash (*Fraxinus oxyphylla* Bieb.) representing 35.2% and 25.4% of the total number of trees, respectively. Canopy height averaged 11.0 m, and the mean diameter at breast height (DBH) of the living trees (DBH > 3 cm) was 10.5 cm. Planted with an original density of 1111 plants ha^{-1}, the stand in 2006 had a density of 830 plants ha^{-1} and a mean Leaf Area Index (LAI) of 3.4 m^2 m^{-2}.

The ANPP of this site was measured by inventory methods every year since 1999 (Magnani et al., 2005). In 2001 the site became part of the CARBOEUROFLUX network and both net ecosystems exchange (NEE) by eddy covariance (EC) and soil respiration by direct chamber measurement of CO_2 fluxes were monitored. Gross primary productivity (GPP) and NEE data for 2006 are available from the European Fluxes Database Cluster (EFDC) (Papale et al., 2006; Reichstein et al., 2003).

2.2. Estimation of TBCA

In 2006, the TBCA, (i.e. the C allocated belowground by plants to produce new roots, root exudates and to maintain root respiration) was estimated by the mass balance approach originally proposed by Raich and Nadelhoffer (1989) and then modified by Giardina and Ryan (2002) and Davidson et al. (2002) as follows

$$\mathrm{TBCA} = \Delta C_{\mathrm{root}} + \Delta C_{\mathrm{soil}} + F_s + \Delta C_{\mathrm{litter}} - C_{\mathrm{litterfall}} \tag{1}$$

where F_s is the annual total soil CO_2 efflux, C_{lf} is the annual C input from aboveground litter, ΔC_{soil} is the annual change in SOC, ΔC_{rr} is the annual C change in root biomass, and ΔC_{litter} is the annual C change in leaf litter.

We assumed that ΔC_{soil} was negligible at an annual basis compared to other fluxes (Forrester et al., 2006; Giardina and Ryan, 2002; Newman et al., 2006) and difficult to quantify due to the high spatial variability (Ruark and Zarnoch, 1992; Smith et al., 2007), while ΔC_{litter} was considered negligible for the period of interest based on direct measurements in the period 2005–2007. Consequently, Eq. (1) was simplified as follows:

$$\mathrm{TBCA} = F_s - C_{\mathrm{litterfall}} + \Delta C_{\mathrm{root}}. \tag{2}$$

The total soil CO_2 efflux (F_s) was measured from January to December 2006 with an infrared gas analyser (EGM 4, PP Systems, UK) connected to a closed dynamic chamber (SRC 1, PP Systems, UK) fitted to PVC collars (10 cm internal diameter, 12 cm long) that were inserted 8 cm deep into the soil. In the lower part of each collar, two rows of 8 holes (diameter 0.8 cm) were previously drilled in order to minimize soil disturbance. Twelve collars in six

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