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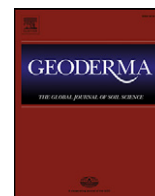


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Belowground carbon allocation patterns as determined by the in-growth soil core ^{13}C technique across different ecosystem types



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ABSTRACT

Belowground carbon inputs, in particular rhizodeposition, are a key component of the global carbon cycle and yet their accurate quantification remains a major challenge. In the present paper, the in-growth soil cores- ^{13}C method was used to quantify net root carbon input (root-derived C). Four different ecosystem types (forest, alpine grassland, apple orchard and vineyard) in northern Italy, characterized by C_3 vegetation with a broad range of aboveground net primary production (ANPP; $155\text{--}770\text{ gC m}^{-2}\text{ y}^{-1}$) were investigated. Cores, filled with soil of a known C_4 isotopic signature were inserted at each site for twelve months. After extraction, root-derived C was quantified by applying a mass balance equation. Gross primary production (GPP) was determined by eddy covariance whereas ANPP was quantified using a biometric approach.

NPP partitioning among sites differed, with fruit production dominating at agricultural sites. At these sites, belowground C inputs were dominated by rhizodeposits, likely due to relatively high root turnover. In natural ecosystems (forest and grassland) fine root production dominated belowground net primary production (BNPP) likely due to higher root growth determined by low phosphorus availability. Root derived C represented a significant contribution to BNPP varying from 40 to 60%. Our results underline the fact that failure to account for rhizodeposits may lead to a significant underestimation of BNPP.

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1. Introduction

The current increase in atmospheric carbon dioxide (CO_2) and the consequent global warming, can be counteracted not only by lowering anthropogenic carbon emissions, but also by finding ways of sequestering CO_2 over extended time periods. Globally, soil organic matter (SOM) contains more than two times (ca 1500 Pg C) as much carbon as the atmosphere (ca 750 Pg C), therefore an overall increase in soil carbon by 10% would imply an approximate decrease (or avoid an increase) in atmospheric carbon of at least 20% (Kell, 2014). The amount of carbon (C) that accumulates in the soil is not only a function of the quantity

and quality of C inputs, but also of environmental and biological factors (Schmidt et al., 2011). An improved understanding of belowground C fluxes and dynamics is therefore a research priority, given the growing interest in storing increasing amounts of C in the soil pool to promote C sequestration (Rumpel and Kögel-Knabner, 2011). Predictions of the responses of ecosystem respiration and soil C sequestration to environmental changes are limited by incomplete understanding of how plant C is allocated to and utilized by different ecosystem components (Carbone et al., 2007).

Belowground Net Primary Production (BNPP) can be defined as the change in root biomass plus any root mortality occurring over a specified period of time or better, in a broader sense, all C allocated belowground by plants and not used for autotrophic respiration (Giardina et al., 2005). Terrestrial plants allocate approximately half of the 120 Pg C fixed annually through photosynthesis belowground (Grace and Rayment, 2000), which is used for the construction and maintenance

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of roots and mycorrhizae. Belowground C allocation may therefore represent the largest sink for gross primary production (Janssens et al., 2001). It is problematic to find reliable estimates of BNPP because some components are usually overlooked, since they are too difficult to estimate (e.g. mycorrhizal fungal biomass, production and turnover, root exudation). As a result BNPP is usually associated with fine root growth, which is the easiest component to be measured (Giardina et al., 2005). Methods for estimating BNPP include a range of approaches that approximate the amount of biomass or C allocated belowground over a certain time period (e.g. biomass change, ingrowth cores, C and nitrogen balances and minirhizotrons), all of which have important strengths but also some limitations (Fahey et al., 1999; Lauenroth, 2000; Hendricks et al., 2006). Given the limitations associated with direct measurements, root to shoot ratios (R:S) are commonly used to indirectly estimate belowground biomass and occasionally its variations when a sufficient time lag is considered (Gill et al., 2002; Scurlock et al., 2002). The relative ease by which above-ground or shoot biomass can be measured compared to below-ground components has led to their widespread use (Mokany et al., 2006) and remains the primary method used by some countries to estimate belowground biomass and C stocks for National Greenhouse Gas Inventories (e.g. Australian Greenhouse Office [AGO]) (Eamus et al., 2000; Keith et al., 2000; Snowdon et al., 2000).

An important but often disregarded component of BNPP is rhizodeposition, although a shared consensus among scientists is lacking on which components should be acknowledged as rhizodeposits (Wichern et al., 2008). Rhizodeposition is the process of release of volatile, non-particular and particular compounds from living plant roots (Wichern et al., 2008). "Rhizodeposits" can be subdivided according to their origin into: i) particular compounds (including root border and root cap cells, sloughed epidermal root cells and root hairs, root fragments and fine roots) and ii) non-particular compounds including passive or controlled diffused root exudates such as: water-soluble exudates; secretions; lysates; gases and mucilage (Grayston et al., 1996). Annual plants transfer between 10 to 46% of the assimilated C (GPP) belowground, whereas perennials can invest up to 70% (Grayston et al., 1996). Although rhizodeposition is a key component of the terrestrial C cycle, it remains one of its most uncertain terms (Pausch et al., 2013). This is largely a result of the difficulty associated with its quantification due to: (a) the amount of organic substances released during rhizodeposition is small compared to other organic substances in the soil; (b) it is restricted to a narrow zone around roots; and (c) fast utilization by micro-organisms due to its high availability (Pausch et al., 2013).

The variation in ^{13}C natural abundance (Balesdent et al., 1987) has been proven to be a sensitive approach for determining net belowground plant inputs or, more broadly, soil C changes in soil-plant systems (Alberti et al., 2015; Cotrufo et al., 2011; Van Kessel et al., 2000). This approach has been widely used in CO_2 enrichment experiments (e.g. Free-Air CO_2 Enrichment, FACE), using of a ^{13}C -depleted CO_2 source for fumigation (e.g. Phillips et al., 2012; Hoosbeek et al., 2004; Nitschelm et al., 1997; Van Kessel et al., 2000). Furthermore, many studies have applied this technique in soil-plant systems where the ^{13}C signal of the C input is different from the native SOM (e.g. C_3 plants grown in C_4 soils or vice versa), providing an in-situ method to monitor and calculate the relative contribution of recent plant inputs to soil organic C (Cotrufo et al., 2011; Del Galdo et al., 2003; Hoosbeek et al., 2004; Martin et al., 1990). The application of C isotopes (^{14}C and ^{13}C) in rhizodeposition studies has led to significant progress, with root-derived C values 3–7 times higher than observed with traditional root washing techniques or root growth estimations (Kuz'yakov and Schneckenberger, 2004). Traditional methods of measuring C and N fluxes into soil fall short, for example, because the variation in root biomass is unable to simultaneously account for root production and mortality, while C isotopes can provide a quantitative estimate of the amount of root-derived C in soil (Scandellari et al., 2007) in particular

the in-growth soil core ^{13}C natural abundance technique (Cotrufo et al., 2011).

The present study had two main aims: i) quantify root-derived C input in different land use types (grassland, vineyard, orchard, forest) using the in-growth soil core ^{13}C natural abundance method; and ii) compare the measured values of root-derived C input with measures of overall or aboveground ecosystem productivity (i.e. GPP and ANPP, respectively) to investigate the C partitioning strategies of the different ecosystems. We conducted this study across the most representative ecosystem types of the Trentino Region, from grasslands to forests, to span a wide range of NPP and root:shoot traits.

2. Methods

2.1. Study sites

The study was performed at four sites located in different land use systems within the Trentino-Alto Adige region (northern Italy): (1) Lavarone silver fir forest (Cescatti and Marcolla, 2004); (2) Viote alpine grassland (Gianelle et al., 2009; Marcolla et al., 2011); (3) Caldaro apple orchard (Zanotelli et al., 2013); and (4) Mezzolombardo vineyard (AA. VV., 2015). All four sites were equipped with an eddy-covariance tower for continuous energy, water and CO_2 ecosystem flux measurements according to the Euroflux methodology (Aubinet et al., 2000, Table 1).

Lavarone (45°57' N, 11°17' E; 1349 m a.s.l.) is a 130 year old Silver fir (*Abies alba* Mill.) forest, with mean annual temperature of 7.2 °C and annual precipitation of 1150 mm. Average canopy height is 28 m, mean tree diameter is 36 cm and stand density is 401 ($\phi > 17.5$ cm) and 1000 trees ha^{-1} ($\phi < 17.5$ cm). Soil is classified as a Humic Umbrisol (FAO-WRB, 1998) with a total C stock of 9.5 kg C m^{-2} (0–30 cm). Ectomycorrhizas are the most common root symbiosis at the site. The eddy covariance tower was built in 2002 and has been continuously recording data since then.

Viole (46°00' N, 11°03' E; 1553 m a.s.l.) is an alpine meadow located on a mountain plateau, characterized by a mean annual temperature of 5.5 °C and annual precipitation of 1244 mm. The site is managed as an extensive meadow with a low annual mineral fertilization (NPK 100 kg ha^{-1}) and is mowed once a year in mid-July. Vegetation at the site is dominated by *Festuca rubra* (L.) (basal cover of 25%), *Nardus stricta* (L.) (basal cover of 13%) and *Trifolium sp.* (L.) (basal cover of 14.5%), with a maximum canopy height of 30 cm. Soil at the site is a Calcaric Phaeozem (FAO-WRB, 1998) with a total C stock of 13.1 kg C m^{-2} (0–30 cm). Arbuscular endomycorrhizae are present at root level. The eddy covariance tower was established in August 2002.

Caldaro (46°21' N, 11°17' E; 240 m a.s.l.) is an apple orchard located in the intensively cultivated valley of the Adige River. Apple trees (*Malus domestica* Borkh var. Fuji grafted on dwarfing M9 rootstock) were planted in 2000 on a regular 3 × 1 m frame (1 m distance between trees, 3 m distance between tree rows). The weed-free tree line is 1.2 m wide and represents the area where most of the apple roots are localized (Zanotelli et al., 2013), while the remnant part (1.8 m wide) is identified as grassed alley. Fertilization is carried out every year by distributing 500 kg ha^{-1} of the commercial formulate AgroBiosol® (Scheier Brennstoffe und Begrünungstechnik, Bürs, Austria) during spring and 400 kg ha^{-1} of the commercial formulate Azacor® 105 (Fomet S.p.A., S. Pietro di Morubio – Verona, Italy) in autumn. The site has been an apple orchard for at least 50 years. Mean annual temperature is 11.6 °C and precipitation is 816 mm. Soil at the site is classified as a Calcaric Cambisol (FAO-WRB, 1998) with a total C stock of 6.2 kg C m^{-2} (0–30 cm). Arbuscular endomycorrhizae are present at root level. The eddy covariance tower was set up at the beginning of 2009.

Mezzolombardo (46°12' N, 11°07' E; 206 m a.s.l.) is representative of a typical vineyard in the Trentino region, and is characterized by a mean annual temperature of 11.8 °C and annual precipitation of

Table 1
Description of main study site characteristics.

	Lavarone (forest)	Viote (grassland)	Caldaro (apple orchard)	Mezzolombardo (vineyard)
Latitude (N)	45°57'23"	46°00'53"	46°21'17"	46°11'49"
Longitude (E)	11°16'52"	11°02'45"	11°16'31"	11°06'49"
Elevation (m)	1349	1553	240	206
Land Use	Forest	Alpine grassland	Apple orchard	Vineyard
Vegetation type	Silver fir (<i>Abies alba</i>)	<i>Nardetum</i> <i>alpigenum</i>	Common apple (<i>Malus domestica</i>)	Common grape vine (<i>Vitis vinifera</i>)
Mean Annual Precipitation (mm yr ⁻¹)	1150	1244	816	822
Mean Annual Air Temperature (°C)	7.2	5.5	11.6	11.8
Soil Type ^a	Humic Umbisol	Calcaric Phaeozem	Calcaric Cambisol	Gleyic/Haplic Fluvisol

^a FAO-WRB (1998).

822 mm. The site is dominated by *Vitis vinifera* (L.) with a mean canopy height of 2 m and has been cultivated as a vineyard for centuries. The vines are planted in a regular frame of 6.5 by 0.6 m. Similarly as for the apple orchard, the weed-free tree-line (1.6 m wide) is the area where most of the vine roots are growing (Morlat and Jacquet, 2003), while the remnant inter-row area (4.9 m wide) represents the grassed alley. Soil at the site is a Gleyic/Haplic Fluvisol (FAO-WRB, 1998) with a total C stock of 5.8 kg C m⁻² (0–30 cm). A mineral (NPK 8–6–18) or organic fertilizer (compost) is applied in spring at a rate of 400–500 kg ha⁻¹. The experimental site was established in 2008, and since then it has been continuously recording eddy covariance measurements of CO₂, water vapor, and sensible heat fluxes. Grapevines are commonly known to form arbuscular mycorrhizae.

Annual average temperature at the sites during the experimental period (2009–2010) were within the long-term annual average ± 1.96 s.d. (1970–2010; source: Meteotrentino [meteorological service of the Autonomous Province of Trento-PAT] and Laimburg Research Centre), thus the productivity values reported in this study can be considered to be representative of the long-term mean.

2.2. Sampling scheme

At each site, six representative sampling points were selected within the fetch area of the eddy tower, at which three replicates were sampled (i.e. total of 18 sampling points per site) according to the “modified” random sample collection method (Stolbovoy et al., 2007). At Mezzolombardo and Caldaro (both agricultural fields) sampling locations were limited to crop rows to avoid problems related to inter-row superficial plowing. Sampling points were augured to a depth of 30 cm (4.8 cm diameter), in preparation for the insertion of soil cores.

2.3. Roots and soil preparation and analysis

C₄ grassland (i.e. Blue Grama (*Bouteloua gracilis* Willd. ex Kunth)) soil ($\delta^{13}\text{C} = -16.7\text{‰}$) sampled at a depth of 0–30 cm (see Cotrufo et al., 2011) was dried at 40 °C, sieved to 2 mm and well mixed to ensure homogeneity. The soil was classified as Zigweid series (fine-loamy, mixed, superactive, mesic Ustic Haplocambids; Soil Survey Staff Classification), with a pH of 7.4 and lower soil nutrient content (C, N, P) compared to the native (C₃) soil at the four study sites (Fig. 1). In-growth cores (2 mm mesh), 30 cm long and 3 cm in diameter were then filled with the C₄ soil resulting in a bulk density of 0.79 g cm⁻³. Cores were then inserted at the 18 sampling points described above and a net was placed to cover the top of each core to avoid the entry of above-ground plant litter.

The sampling depth (30 cm) was considered sufficient to capture the majority of fine growing roots and representative of most of the rooting depth in each investigated ecosystem (Jackson et al., 1996; Zanutelli et al., 2013). Soil cores were extracted one year after their insertion (2009–2010), transported back to the laboratory and cut in half: 0–15 cm (superficial soil) and 15–30 cm (deep soil). Soil was extracted

from the core and carefully washed using water over a 2 mm sieve to separate the root material from fine earth and stones. All material passing through the sieve was collected in a tray positioned below, subsequently the water was evaporated by oven drying at 40 °C and the soil mixed and homogenized with a mortar and pestle. Soils and root samples were oven-dried at 60 °C. Samples from the three cores collected at each of the six replicate points at each site were then bulked, resulting in six replicate samples per site and depth. Soil samples were gently disaggregated and ground with a mortar and pestle to become homogeneous. A representative sub-sample was ground using a ball mill and treated using acidification in silver capsules (“capsule method”) as described in Brodie et al. (2011) to remove carbonates. All soil and root samples were then analyzed for total %C and %N with a Perkin-Elmer (Norwalk, CT, USA) PE2400 CHNS/O elemental analyzer and $\delta^{13}\text{C}$ using an Isotope Ratio Mass Spectrometer (DELTA V, Thermo Scientific, Bremen, Germany) interfaced with an Elemental Analyzer (Flash EA™1112, Thermo Scientific). Available phosphorus (Olsen P) was determined on a homogenized soil subsample (Olsen and Sommers, 1982). Root samples were bulked by replicate (n = 6 per site and depth), ground to a fine powder using a ball mill, and analyzed for $\delta^{13}\text{C}$.

C isotope values are reported in delta notation ($\delta^{13}\text{C}$) against the international standard Vienna-Pee Dee Belemnite (V-PDB).

$$\delta^{13}\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000 \quad (1)$$

where R_{sample} and R_{standard} are molar fractions of ¹³C/¹²C for the sample and standard, respectively. Working in-house standards calibrated against the international reference materials L-glutamic acid USGS 40 (IAEA – International Atomic Energy Agency, Vienna, Austria), fuel oil NBS-22 (IAEA) and sugar IAEA-CH-6 (IAEA) were used. The analytical precision (1 standard deviation) was <0.2‰.

Measured $\delta^{13}\text{C}$ values were then used to calculate the proportion of new C (f_{NEW} , the fraction of root-derived soil C over the study period) and old C (f_{OLD} , the fraction of C coming from the organic matter of the original C₄ soil), by applying a mass balance equation (Del Galdo et al., 2003):

$$f_{\text{NEW}} = (\delta_{\text{SOIL}} - \delta_{\text{OLD}}) / (\delta_{\text{VEG}} - \delta_{\text{OLD}}) \quad (2)$$

$$f_{\text{OLD}} = 1 - f_{\text{NEW}} \quad (3)$$

where $\delta_{\text{SOIL}} = \delta^{13}\text{C}$ of C₄ soil collected from cores following 1 year in the field; $\delta_{\text{OLD}} = \delta^{13}\text{C}$ of original C₄ soil prior to insertion ($-16.7 \pm 0.09\text{‰}$); and $\delta_{\text{VEG}} = \delta^{13}\text{C}$ of roots. Knowing the f values for the new (f_{NEW}) C fraction, in addition to C concentration, soil bulk density (0.79 g cm⁻³) in the C₄ soil core and soil depth (15 or 30 cm), the accumulation of net root-derived C (g C m⁻²) input to the soil during the 12-month experimental period was calculated. Root-derived C represents the fraction of new C entering the core and still remaining in the soil after 12 months, minus losses associated with heterotrophic respiration (Alberti et al., 2015; Cotrufo et al., 2011; Del Galdo et al., 2003), therefore including rhizodeposition (either from roots or mycorrhizal fungi;

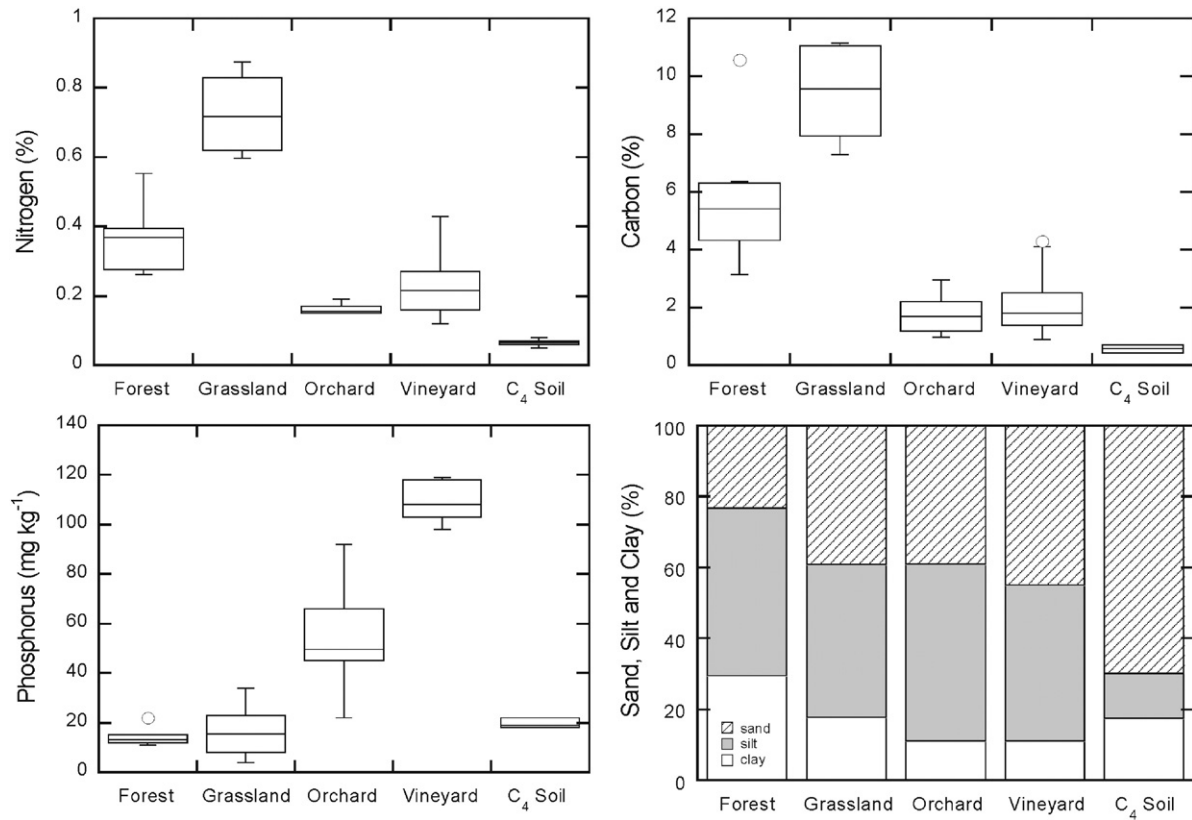


Fig. 1. Box-plots showing differences in nitrogen (%), carbon (%), available phosphorus (Olsen P; mg kg⁻¹) and soil texture (% sand, silt and clay) among the four study sites and C₄ soil placed in the in-growth cores, for a soil depth of 30 cm. Each box encloses 50% of the data (inter-quartile distance, IQD), with the median values of the variable displayed as a line. The top and bottom of the box marks the upper (UQ) and lower (LQ) quartiles. Lines extending from the box mark the minimum and maximum values within the data set that fall within an acceptable range (points whose value is either: less than UQ + 1.5 * IQD or greater than LQ - 1.5 * IQD). Values outside of this range (outliers) are displayed as individual points.

Phillips et al., 2012) plus root mortality (fine root and mycorrhizal turnover). Since at the agricultural sites the placement of the sampling points was limited to the rows, root-derived C values calculated for the apple orchard and the vineyard could have been over-estimated due to the higher root density within rows compared to the inter-rows (Morlat and Jacquet, 2003; Smart et al., 2006; Zanotelli et al., 2013). In order to overcome this problem the root-derived C was recalculated as a weighted average between the specific root-derived C obtained from the sampling points placed along the rows and the root-derived C estimated for the grassed inter-rows. We used the relative surface portion of the tree-line and the grassed alley on the total distance between two successive tree lines as weighting factors. Moreover, as the inter-row root-derived C was not measured directly

and the management of the inter-row is comparable to a grassland, a rough estimate was obtained by multiplying the ratio between root-derived C and the C in ANPP of the grassland site (Viote) by the measured (orchard) or derived (vineyard) ANPP C of the grassed inter-rows.

Annual fine root production was determined using the dry weight values of root biomass which grew into the in-growth cores (<2 mm) during the experiment. Measured values of root C content (Table 2) were used for the calculation of annual fine root C accumulation ($\Delta C_{\text{root (fine)}}$). Belowground annual coarse (>2 mm) root growth ($\Delta C_{\text{root (coarse)}}$) was calculated at all sites excluding the grassland where this component is usually minor (Bahn et al., 2013; Table 2). At the forest site, coarse root growth was calculated as a percentage of

Table 2

Site productivity parameters derived from eddy covariance measurements and in-growth soil cores ¹³C method. Values of root-derived carbon and $\Delta C_{\text{root (coarse)}}$ are given for 0–30 cm soil depth. Root carbon content is also reported.

	Lavarone (forest)	Viote (grassland)	Caldaro (apple orchard)	Mezzolombardo (vineyard)
GPP (gC m ⁻² yr ⁻¹)	2400	1086	1263	1145
ANPP (gC m ⁻² yr ⁻¹) ± SE	770 ± 44	155 ± 24	746 ± 74	268 ± 72
BNPP ^a (gC m ⁻² yr ⁻¹)	798	373	329	480
NPP ^b (gC m ⁻² yr ⁻¹)	1568	528	1075	748
BNPP/NPP	0.51	0.71	0.31	0.63
Root-derived C/BNPP	0.38	0.58	0.62	0.63
$\Delta C_{\text{root (coarse)}}$ (gC m ⁻² yr ⁻¹)	154	N/A	13 ± 3	30 ± 19
Root C content (%)	44.5	37.1	44.4	36.6

^a BNPP = $\Delta C_{\text{root (fine)}}$ + $\Delta C_{\text{root (coarse)}}$ + Root-derived C.

^b NPP = BNPP + ANPP.

stem growth given that the root:shoot ratio relates to stand age and tends to be quite constant (average 0.28; Levy et al., 2004; Litton et al., 2007) in mature forests (Giardina et al., 2005). Coarse root growth at the apple orchard was calculated biometrically by Zanotelli et al. (2013), while at the vineyard coarse root production was estimated assuming an average root:shoot ratio of 0.37 (min 0.14, max 0.60; Hunter, 1997) and an average pruning wood production of $81.5 \text{ gC m}^{-2} \text{ yr}^{-1}$.

2.4. Gross primary production (GPP) and aboveground net primary productivity (ANPP)

At all four sites, GPP was calculated as net ecosystem exchange (NEE) plus ecosystem respiration (extrapolated from night time ecosystem respiration; R_{eco}) according to the methodology developed by Reichstein et al. (2005). NEE and R_{eco} were estimated by standard processing of raw eddy-covariance CO_2 flux data (Aubinet et al., 2000). Missing data were gap-filled using a look-up table approach (Reichstein et al., 2005).

Net aboveground primary production (ANPP) of the forest site was determined using an inventory approach, as the sum of the total aboveground biomass production (branches + stems + foliage) during the year of the soil core experiment (Table 2). Annual leaf production for the conifer forest site was calculated using the average of five years of litter trap collection. Total woody biomass production, including stems and branches, was calculated from measurements of stem radial increment and biomass tables developed by Fattorini et al. (2004). For the vineyard, measurements of above-ground biomass components were estimated from grape yield and pruning weight data derived using data typical for the Teroldego cultivar as reported by ACOVIT (Associazione Costitutori Viticoli Italiani www.acovit.it; according to the vine spacing and training system of the local cultivar). Foliage production was derived from the equation of Johnson et al. (2003), using the known number of plants per hectare, the pruning weight, LAI (leaf area index) values and SLA (specific leaf area) measured at the site. Maximum and minimum values for each biomass pool were used to perform a sensitivity analysis and calculate a range of ANPP values for the vineyard (data not shown). Annual hay production of the grassland was determined by cutting and weighing aboveground biomass collected from one to three long cross-stripes (1 m^2) within the footprint of the eddy covariance tower over a number of years (2003, 2004, 2006, 2007, 2011). In the apple orchard, biometric measurements of various aboveground C pools were accurately determined on a monthly basis, including leaves, fruit, aboveground woody tissues (including stems, branches, and shoots) and understory production (Zanotelli et al., 2013).

Belowground net primary productivity (BNPP) was calculated as the sum of $\Delta C_{\text{root (coarse)}}$, $\Delta C_{\text{root (fine)}}$ and root-derived C (Giardina et al., 2005) (Table 2).

2.5. Statistical analyses

Statistical analyses were performed using STATISTICA (StatSoft, 2010, v. 9.1). Kruskal–Wallis ANOVA was adopted to quantify among group differences in median values of the investigated parameters. We decided to analyze the data by means of non-parametric statistical tests because of the intrinsic uncertainty of normality tests (e.g. Shapiro–Wilk test) when applied to limited sample size ($n = 6$ per site in our dataset). In particular, small samples almost always pass a normality test because these tests typically have little power to verify whether or not a small sample originates from a Gaussian distribution (Ghasemi and Zahediasl, 2012). Moreover, soil data are frequently non-normally distributed and often it is not sufficient to use transformations (e.g. logarithmic) to achieve normal distributions (personal observation, data not shown). When comparing two groups a Kolmogorov–Smirnov two sample t -test was applied instead (Zar, 2009). The spatial variability of the soil parameters was

quantified by the coefficient of variation (i.e. $\text{CV}\% = \text{standard deviation} / \text{mean} * 100$; Zar, 2009).

3. Results

3.1. Soil properties and main ecosystem carbon fluxes

The C_4 soil had a higher sand content (sandy loam texture) if compared to the soil of the four investigated sites. The forest soil had the highest clay content (clay loam texture) whereas the texture of the other three sites resulted to be quite similar (loam texture). The soil of the cultivated sites (orchard and vineyard) had a higher P availability (vineyard: 109 mg kg^{-1} ; orchard: 54 mg kg^{-1}) compared to the natural sites (less than 20 mg kg^{-1} ; Fig. 1), whereas for total N the trend was reversed. As a consequence, the N/P ratio resulted to be an order of magnitude lower for the cultivated than the natural sites (i.e. $\text{N/P} = \text{orchard}: 0.03$, vineyard: 0.02 ; forest: 0.19 , grassland: 0.28).

GPP across the four sites ranged between $1086 \text{ gC m}^{-2} \text{ yr}^{-1}$ (grassland) up to $2400 \text{ gC m}^{-2} \text{ yr}^{-1}$ (forest) (Table 2). C in ANPP was highest at the forest site ($x \pm \text{SE}$; $770 \pm 44 \text{ gC m}^{-2} \text{ yr}^{-1}$) and lowest at the grassland site ($155 \pm 23.7 \text{ gC m}^{-2} \text{ yr}^{-1}$); the orchard had quite high values, close to those of the forest ($746 \pm 74 \text{ gC m}^{-2} \text{ yr}^{-1}$) whereas vineyard production was $268 \pm 72 \text{ gC m}^{-2} \text{ yr}^{-1}$. The forest had the highest BNPP C content ($798 \text{ gC m}^{-2} \text{ yr}^{-1}$) while the orchard had the lowest ($329 \text{ gC m}^{-2} \text{ yr}^{-1}$). The C in NPP was allocated differently above and belowground according to ecosystem type: 71% of the NPP C was allocated belowground in the grassland, whereas in the forest, it was equally allocated above and below ground (Table 2). A considerable investment in belowground compartments was also evident in the vineyard (63% of C as ANPP C) whereas in the orchard a higher C quantity was invested in the aboveground organs (Table 2).

3.2. Rhizodeposition and belowground carbon partitioning

No statistically significant differences were found for either soil or root $\delta^{13}\text{C}$ in the in-growth cores among sites (according to the median values to a depth of 30 cm; Fig. 2). SOC concentration within the in-growth cores at the end of the twelve-month experiment was highest in the grassland and lowest at the vineyard (Kruskal–Wallis H-value = 18.43; $p = 0.0004$) with statistically significant differences (multiple comparisons p values) found between the grassland and both agricultural sites, vineyard ($p < 0.01$) and apple orchard ($p < 0.05$), and between the forest and vineyard ($p < 0.05$). The fraction of new C (f_{NEW}) ranged between 0.16 ± 0.12 of the grassland and 0.26 ± 0.12 at the orchard, although no statistically significant differences were observed between sites (Fig. 2). Accordingly, root-derived C over the entire soil profile (0–30 cm) ranged from $204 \pm 47 \text{ gC m}^{-2} \text{ yr}^{-1}$ (orchard) up to $302 \pm 22 \text{ gC m}^{-2} \text{ yr}^{-1}$ (vineyard). The median annual fine root C accumulation [$\Delta C_{\text{root(fine)}}$] (0–30 cm) was highest at the forest site ($344 \pm 27 \text{ g m}^{-2}$) and lowest at the apple orchard site ($112 \pm 22 \text{ g m}^{-2}$), with a statistically significant difference between the values (Kruskal–Wallis H-value = 13.18; $p < 0.01$; Fig. 2). Both the grassland and orchard had a higher root density in the superficial soil layer, whereas the forest and vineyard had a similar distribution in the two layers (Fig. 3). The spatial variability of both root-derived C and root biomass C was quite high, as can be seen from the standard errors (Fig. 2) and confidence intervals of the means (Fig. 3).

The fraction of C in BNPP apportioned to fine root biomass (i.e., $\Delta C_{\text{root (fine)}}/\text{BNPP}$) was similar at the forest (43%) and grassland (42%) sites, while relatively lower amounts of BNPP C accumulated in fine roots at the orchard (34%) and vineyard (32%) sites (Fig. 4). As a result, a much higher proportion of BNPP was invested into rhizodeposition (i.e. root-derived C/BNPP) at the cultivated sites, with 63% and 62% at the vineyard and apple orchard sites respectively, while the natural sites were relatively lower: 38% at the forest and 58% at the grassland (Table 2; Fig. 4). Overall, the C transferred belowground as rhizodeposition was a relevant part of BNPP ranging from 38 to 63%,

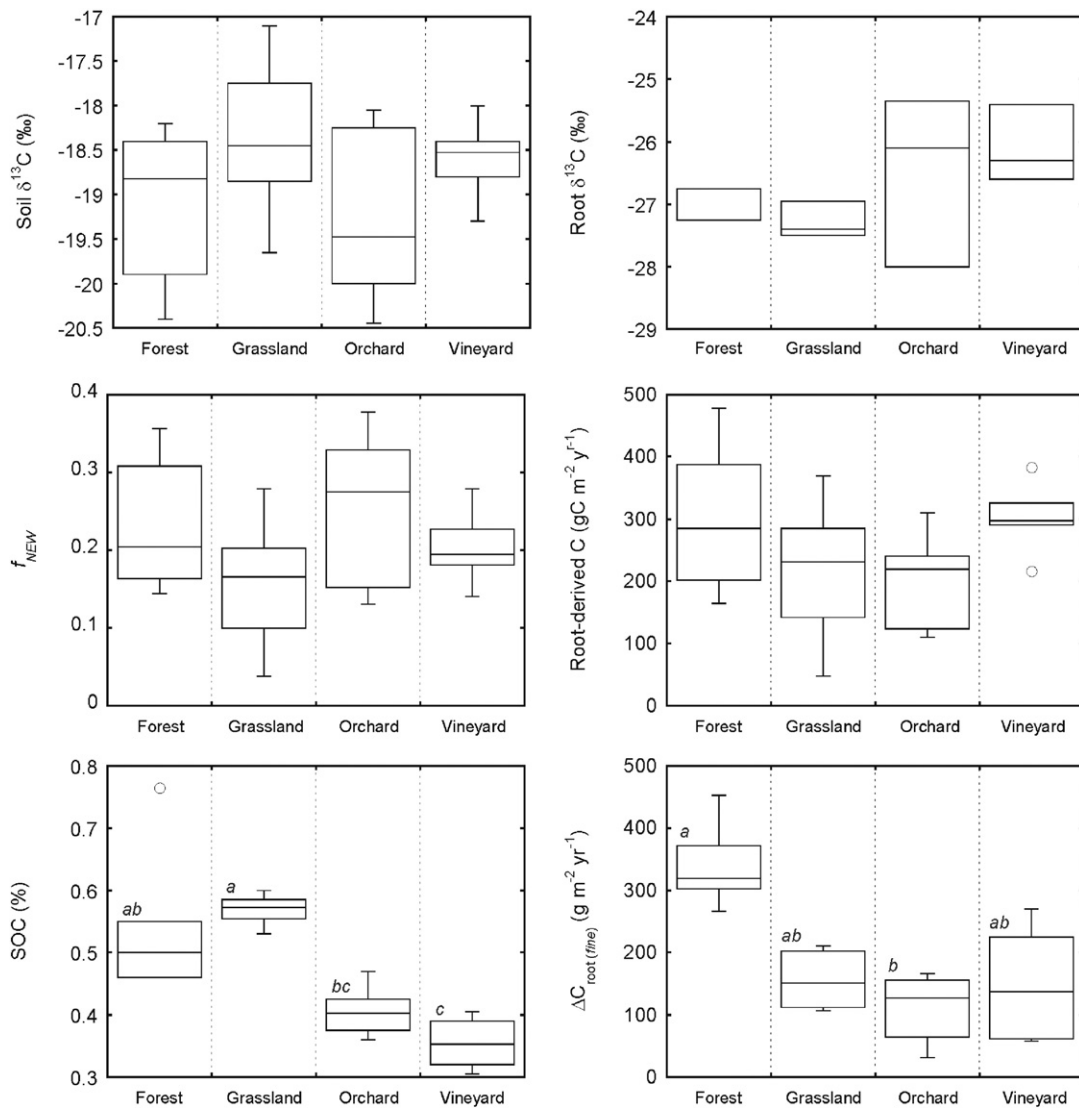


Fig. 2. Box-plots showing differences among the four study sites for the various soil properties (0–30 cm) within the in-growth soil cores. Each box encloses 50% of the data (inter-quartile distance, IQD), with the median values of the variable displayed as a line. The top and bottom of the box marks the upper (UQ) and lower (LQ) quartiles. Lines extending from the box mark the minimum and maximum values within the data set that fall within an acceptable range (points whose value is either: less than $UQ + 1.5 * IQD$ or greater than $LQ - 1.5 * IQD$). Values outside of this range (outliers) are displayed as individual points. Different letters indicate a significant difference among sites ($p < 0.05$; Kruskal–Wallis ANOVA). No letters indicate no statistical differences.

therefore leading to a substantial change in the estimated value of NPP in all ecosystems (Fig. 5).

4. Discussion and conclusions

4.1. Ecosystem strategies of carbon allocation

Ecosystems belowground functioning have received increasing attention in the last few years as a consequence of the acquired awareness that soil can sequester a great amount of C. Soil itself is far from saturated and most of the C is derived from roots rather than from shoots and leaf litter (Kell, 2014). C allocation has been estimated for decades with C mass-balance approaches, which combine measurements of standing biomass with measurements of respiratory CO_2 efflux (Epron et al., 2012). Although these approaches have been demonstrated to give good results in quantifying the whole ecosystem C budget, large uncertainties remain about the contribution of different above- and belowground C fluxes to ecosystem respiration (Epron et al., 2012). Concerning ecosystem-scale fluxes, the C in ANPP calculated for the

different sites is within the range of values reported previously: forest (this study: $770 \text{ gC m}^{-2} \text{ yr}^{-1}$; literature: $610\text{--}2170 \text{ gC m}^{-2} \text{ yr}^{-1}$, Wang et al., 2011; Grier, 1979); vineyard (this study: $268 \text{ gC m}^{-2} \text{ yr}^{-1}$; literature: $300 \text{ gC m}^{-2} \text{ yr}^{-1}$, Williams et al., 2011); and grassland (this study: $155 \text{ gC m}^{-2} \text{ yr}^{-1}$; literature: $157\text{--}699 \text{ gC m}^{-2} \text{ yr}^{-1}$; Bahn et al., 2008). ANPP at the apple orchard site ($746 \text{ gC m}^{-2} \text{ yr}^{-1}$) is slightly higher than other analogous sites ($513\text{--}657 \text{ gC m}^{-2} \text{ yr}^{-1}$, Faqi et al., 2008; Panzacchi et al., 2012; Papale et al., 2005), however it is known to represent an area of intense apple production (60 t ha^{-1}), which is much higher than the average Italian (37.2 t ha^{-1}) and EU-25 (18.8 t ha^{-1}) apple production rates (European Commission, 2012). Furthermore, fruit production accounts for approximately 52% of total NPP, thus explaining the relatively higher ANPP value at this site (Zanotelli et al., 2013). The grassland showed a NPP which is close to the lowest value reported in literature, however, such a value (average of 5 years, see Methods section) is considered representative of ANPP at the site during the study period and falls well within the range of values measured during the last decade (Marecolla et al., 2011). ANPP was shown to be highest at the forest and orchard

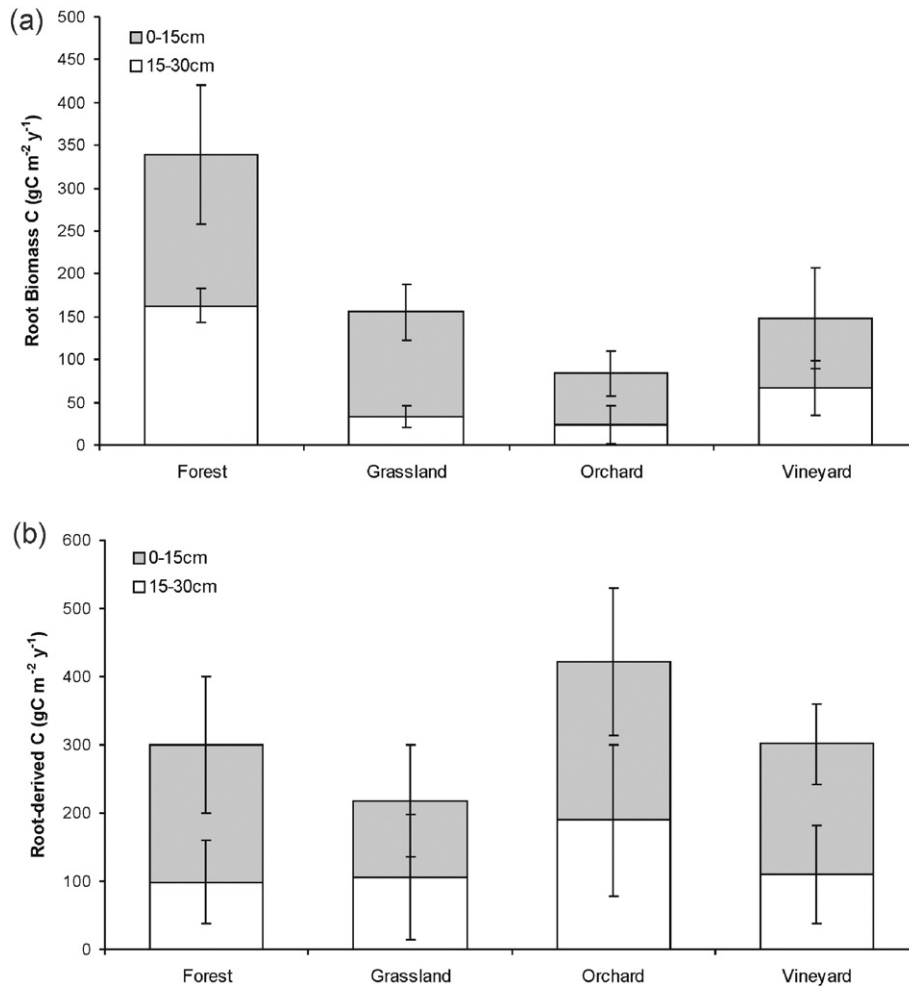


Fig. 3. Average root biomass carbon and root-derived carbon across the four study sites: forest, grassland, apple orchard and vineyard. The values are reported according to the sampling depth: superficial soil (0–15 cm) and deep soil (15–30 cm). Vertical bars are \pm confidence interval (CI) of the mean (calculated at 0.05 critical value of the t distribution; $n = 6$).

sites (Table 2). These two sites are also those with the lowest proportion of C in NPP allocated belowground (51% and 31% respectively), while the grassland, had the highest BNPP C allocation (71%). Grasslands are known to contain about one third of the global terrestrial C pool with the majority of their C and N stocks located belowground (White et al., 2000). Furthermore, BNPP has been reported to contribute

between 20 and 90% of NPP in grassland ecosystems (Gill and Jackson, 2000; Lauenroth and Gill, 2003; Stanton, 1988).

4.2. Soil root-derived carbon input

For practical reasons, soil sampling in this study was standardized to a depth of 30 cm. If, and to what degree, this could have conditioned the results obtained is difficult to quantify. We are aware that grasslands have shallow rooting systems (Jackson et al., 1996) and that orchards apportion a large fraction of their roots to the upper soil horizons (Gong et al., 2006; Kadayifci et al., 2010). Furthermore, Jackson et al. (1996) reported that approximately 52% of root biomass is found in the upper 30 cm of the soil in coniferous forests, 70% in crops, and 83% in grasslands. Thus root biomass and root-derived C may be somewhat underestimated at the forest site. On the other hand, the main purpose of this study was the analysis of the C partitioning strategies within the ecosystems and not the direct comparison between ecosystems. This kind of comparison would be quite difficult due to the fact that different plant species have different shoot and root turnover times, therefore influencing the final amount of C allocated in above and belowground compartments. Solly et al. (2014) reported fine root decomposition rates in grasslands that were two times faster than in forests due to a lower Ca content and higher lignin:N ratio in tree roots. A consistent between-sites comparison should also consider the effects of temperature on SOM decomposition which would affect the quantity of C remaining in the soil at the end of the experiment and the amount of

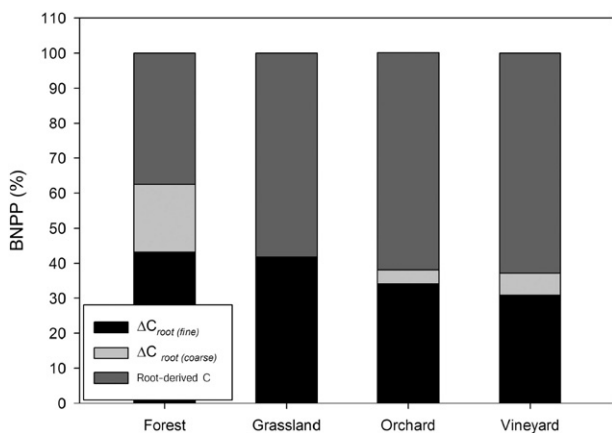


Fig. 4. Partitioning of the belowground net primary production (BNPP) into three components: coarse and fine root growth and rhizodeposition across four land use types: forest, grassland, apple orchard and vineyard.

SOM leaving the ecosystem as dissolved organic C DOC. Therefore, a controlled condition experiment involving different vegetation types but on the same soil type would be advisable but was out of our scope. The root-derived C values measured in this study are not immediately and easily comparable with rhizodeposition data reported in the literature. The most common differences consist in sampling methods, reference soil depth, units used to express quantities, measuring period, and vegetation types. However, there are at least two studies in which the same technique was applied. In a Mediterranean evergreen woodland, Cotrufo et al. (2011) found root-derived C input ranging from $\sim 140 \text{ gC m}^{-2} \text{ yr}^{-1}$ to $\sim 453 \text{ gC m}^{-2} \text{ yr}^{-1}$ depending on the selected treatment, whereas Alberti et al. (2015) considered six high fertility oak and beech forests in central and northern Italy and quantified net- C_{root} input in the range $420\text{--}818 \text{ gC m}^{-2} \text{ yr}^{-1}$. The values in the current study ($204\text{--}302 \text{ gC m}^{-2} \text{ yr}^{-1}$) are closer to those reported by Cotrufo et al. (2011) whereas those observed by Alberti et al. (2015) are much higher and probably reflect the high productivity/fertility of the considered sites. Contrary to other measurement approaches (e.g. isotopic labeling methods), which can be used to obtain estimates of root exudates over a short period of time, the adopted approach enables long term (annual) evaluation of net root C inputs remaining in the soil, including both exudates and root mortality, thus making comparisons quite difficult.

According to Jones et al. (2009) approximately 40% of net fixed C is allocated belowground, however they also point out that there is a lack of knowledge regarding rhizodeposition in forests. Grayston et al. (1996) highlighted that the percentage of C in GPP allocated to root exudation can range from less than 1% up to 4%. We found much higher values (i.e. from 12.0 to 26% of GPP C content) due to the fact that root mortality is also included in our measurements. A total of $60 \text{ gC m}^{-2} \text{ yr}^{-1}$ including exudation and fungi allocation was observed by Drake et al. (2011) for a loblolly pine forest North Carolina. Considering an average NPP C of $980 \text{ gC m}^{-2} \text{ yr}^{-1}$ (McCarthy et al., 2010) for the same forest, the percentage of NPP C allocated to root exudates is equal to 6.1%. Also in this case the presence of root mortality leads to higher estimates in the current study, i.e. 19–41% of NPP C content. Tierney and Fahey (2007) reported BNPP estimates for major terrestrial biomes in relation to total NPP with grasslands having the highest BNPP/NPP ratio (up to 52%) and forests being in the range 18–40%. Essentially, there is a problem of comparability due to the fact that different components are measured in different studies. The lack of a direct link between fine root density and root-derived C may be interpreted as evidence that root productivity is not a suitable proxy of BNPP as already pointed out by Tierney and Fahey (2007).

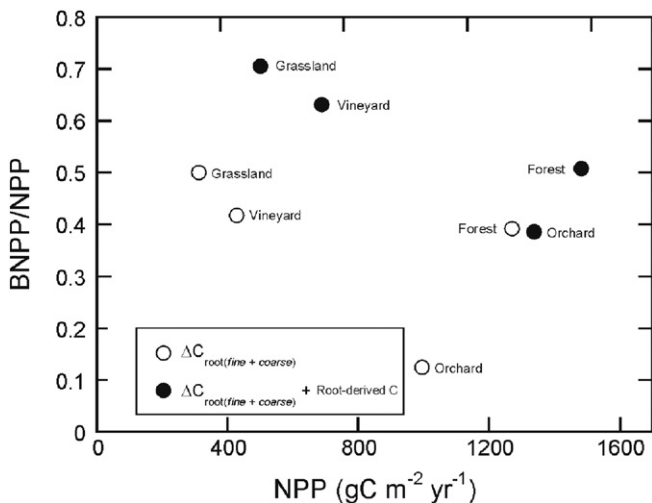


Fig. 5. BNPP/NPP versus NPP, calculated with (closed symbols) and without (open symbols) the contribution of root-derived carbon.

4.3. Belowground carbon partitioning

According to the “functional equilibrium hypothesis” (Poorter et al., 2012) plant C allocation would be strongly sink driven, with photosynthates being preferentially transferred to tissues with the highest demand. That is, under light limitation plants tend to allocate a higher proportion of assimilated C to aboveground organs whereas, under reduced nutrient and/or water supply they invest more C into the root system. The latter pattern is reversed as GPP increases and belowground resources (e.g. water and nutrients) are no longer limiting (Litton et al., 2007). As a matter of fact, Scandellari et al. (2007) found that both C and N rhizodeposition was positively correlated with NPP in apple trees and suggested that the increased availability of photosynthates (i.e. NPP) generates a higher flux of C and N from roots to the soil. Meanwhile, Amos and Walter (2006), in a review paper of 12 maize studies found net rhizodeposited C as a percentage of total net root derived belowground C to be highly correlated with an index combining irradiance level, photoperiod, and ambient temperature, leading to the conclusion that net rhizodeposited C is strongly dependent upon photosynthesis and soil respiration rates. The present study is the first to our knowledge to examine net rhizodeposition in a range of ecosystem types under natural and managed “non-stressed” conditions. When considering C allocation between above- and belowground pools, some interesting trends emerged: at the forest site the C in BNPP was partitioned approximately equally between fine root biomass C and rhizodeposition (43% and 38% respectively). Root-derived C at the grassland and two agricultural sites, on the other hand, accounted for a much greater fraction of BNPP, with 58%, 62% and 63% respectively. We believe this to be the result of a higher level of root turnover (i.e. mortality) due to the higher maintenance costs associated with fruit production (particularly in the orchard, which represents intense apple production rates). While rhizodeposition can occur as a result of several processes (e.g. sloughing-off of root border cells, mucilage secretion, etc.), root exudation and root death are considered the most quantitatively important (Nguyen, 2003; Scandellari et al., 2007). Furthermore, minirhizotron data from apple trees have shown that approximately 50% of roots die within 4–6 weeks of their appearance during summer (i.e. there is a fast turnover of roots; Scandellari et al., 2007). The need for such a high root turnover rates is probably due to the fact that in young apple trees, respiration associated with their maintenance represents a substantial cost (Scandellari et al., 2007). Another contributing factor could be tree morphology: vines (i.e. lianas) are known to lack the requirement for large diameter structural roots (Putz, 1991), and thus invest little in belowground woody compartments and roots. Furthermore, the apple trees in the orchard were grafted on dwarfing M9 rootstock, a widely used practice in intensive orchards to restrict tree volume and thus reducing operational costs (e.g. harvest and pruning) (Cohen and Naor, 2002; Costes and Garcia-Villanueva, 2007), and thus have a relatively small tree framework and root system (Zanotelli et al., 2013).

4.4. Strengths and limitations of the approach

The ¹³C natural abundance method (Balesdent et al., 1987) has been proven to be a sensitive approach for determining soil C changes in soil-plant systems (Alberti et al., 2015; Cotrufo et al., 2011; Van Kessel et al., 2000). This method combined with in-growth cores is one way of overcoming the issue of soil-plant pairs (i.e. it is unnatural to find C₃ plants growing in C₄ soils and vice versa), however it is important to note that it too has some potential drawbacks, such as achieving the same soil properties inside the core as outside (i.e. in the bulk soil), which could affect root growth patterns inside the in-growth core (Steingrobe et al., 2000). In particular, the N content and the soil bulk density inside the mesh bags should be comparable to the bulk soil outside (Steingrobe et al., 2000). These are probably the most difficult conditions to be satisfied since it is not even easy to find a C₄ soil with a

strong and well-defined isotopic signature. In terms of soil nutrient content the agricultural sites seem to be well differentiated from the natural sites, the higher phosphorus content likely being a consequence of periodical fertilization. This could account for the different C partitioning strategies in the various ecosystems. In particular, in natural ecosystems (forest and grassland), a low concentration of soil nutrients (in particular available P; Fig. 1) could have favored the allocation of C resources to root tips as suggested by the “functional equilibrium hypothesis” (Poorter et al., 2012). At the agricultural sites (orchard and vineyard) the contrary would be the case. Since the C₄ cores were randomly positioned within each site and we presume that root growth was not conditioned by the presence of the core, we can expect a higher root biomass inside the core in places where soil root density is higher, therefore increasing the chances for a root to enter the core. This hypothesis holds even if the root growth is not random but driven by nutrient, water, texture, or even bioacoustics stimuli (Gagliano et al., 2012) due to the small diameter of the C₄ core which is unlikely to represent an obstacle to root growth dynamics. The absence of between-site statistically significant differences in root-derived C values was likely due to the high within-site variability of the recorded values, therefore a higher sampling intensity would be required in future studies to better characterize the C₃ C input. Another possible drawback of the method is the potential input from DOC leaching of decomposing C₃ litter that could be incorrectly accounted for as root growth. In fact, even if the cores were protected in the upper part to avoid litter C input, lateral infiltration of DOC via water percolation cannot be excluded, and could potentially be more problematic in irrigated or drip irrigated sites. The apple orchard and the vineyard are both irrigated during the summer season, therefore the root-derived C estimation may have been slightly overestimated at these sites. How much DOC contributed to the C input values at the end of the experiment is difficult to predict, since DOC concentration and transport depends on a series of factors including vegetation type, ecosystem productivity, soil type and texture (Camino-Serrano et al., 2014). This problem could however be restrained if necessary by covering the core with a small shelter, without, however, altering the soil water regime and therefore affecting root growth.

The N, P and C content of the C₄ soil used in the current study was lower than the native soil at the sites (Fig. 1), this fact together with the coarse texture (sandy loam) should have avoided a nutrient induced attraction of the root tips towards the soil core therefore preventing an overestimation of rhizodeposition. However in order to test this hypothesis, additional soil cores filled with native, root free sieved soil should be installed in experimental sites close to the C₄ cores and root biomass compared. Soil texture has been shown to significantly influence rhizodeposition rates. Scandellari et al. (2007, 2010) found a higher rhizodeposition in apple trees growing on fine textured soils compared to coarse textured soils as a result of (1) higher root mortality associated with the higher mechanical impedance (i.e. friction), therefore promoting sloughing-off of root cap cells; and (2) uneven distribution of nutrients due to slow diffusion typically of fine-textured soil, leading to the formation of nutrient patches which may stimulate the intensive root colonization in particular spots followed by root decay (Scandellari et al., 2007). It is difficult to determine whether soil texture influenced rhizodeposition rates in this study. The C₄ soil in the in-growth cores was relatively coarse compared to the native soil at each of the sites (Fig. 1), suggesting that mechanical impedance would have been quite low and thus rhizodeposition rates were conservative. However the lower levels of rhizodeposition observed in the forest site, where the soil had the highest clay content (clay loam texture), is in agreement with the hypothesis of Scandellari et al. (2007).

Exudates are easily decomposed, however, as a result, a high fraction of them can also be used for microbial products and thus persist in the soil for longer periods of time. As a result, the root-derived C input as measured in this study is a function of microbial C use efficiency (MCUE). That is, the higher the MCUE of exudates, the higher the fraction of net root C input (Cotrufo et al., 2013).

Since most studies assume BNPP = root production, this study demonstrated that failure to account for rhizodeposits leads to a significant underestimation of BNPP (Fig. 5). Within the agricultural sites examined in this study, root-derived C was shown to represent a considerable portion of BNPP C content (62% in the orchard and 63% in the vineyard).

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