Soil C:N stoichiometry controls carbon sink partitioning between above-ground tree biomass and soil organic matter in high fertility forests

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The release of organic compounds from roots is a key process influencing soil carbon (C) dynamics and nutrient availability in terrestrial ecosystems. Through this process, plants stimulate microbial activity and soil organic matter (SOM) mineralization thus releasing nitrogen (N) that sustains gross and net primary production (GPP and NPP, respectively). Root inputs also contribute to SOM formation. In this study, we quantified the annual net root-derived C input to soil (Net-Croot) across six high fertility forests using an in-growth core isotope technique. On the basis of Net-Croot, wood and coarse root biomass changes, and eddy covariance data, we quantified net belowground C sequestration. Belowground C accumulation and GPP were inversely related to soil C:N, but not to climatic or stand age. Soil C content and C:N were also related to soil texture. At these high fertility sites, biomass growth did not change with soil C:N; however, biomass growth-to-GPP ratio significantly increased with increasing soil C:N. This was true for both our six forests sites and for another 23 high fertility sites selected at a global scale. We suggest that, at high fertility sites, plant N demand interacts with soil C:N stoichiometry and microbial activity, resulting in higher allocation of C to above ground tree biomass with increasing soil C:N ratio. When C:N is high, microbes have a low C use efficiency, respire more of the fresh C inputs by roots and prime SOM decomposition, thereby increasing N availability for tree uptake. Soil C sequestration would therefore decrease, whereas the extra N released during SOM decomposition can promote tree growth and ecosystem C sink allocation in aboveground biomass. Conversely, C is sequestered in soil when low soil C:N promotes microbial C use efficiency and new SOM formation and stabilization on clay particles.

Keywords: Net Root-derived Carbon, Ingrowth Cores, Soil C:N, Carbon Sequestration, Carbon Partitioning, Isotopes

Introduction

Forest ecosystems worldwide are currently acting as carbon (C) sinks (Pan et al. 2011). Several factors may, however, influence the magnitude and direction of the net C balance, including recovery from historical land use (e.g., abandoned agricultural land reverting to forested land), increases in atmospheric CO₂ concentration and nitrogen (N) deposition, and climate change (Schimel et al. 2001, Thomas et al. 2010). Nonetheless, while much research has been done to understand the controls on net ecosystem C balance (Valentini et al. 2000, Rustad et al. 2001, Reichstein et al. 2007a), we know little about the controls on C sink partitioning between plant biomass and soil organic matter (SOM) pools. Soils may store C for long periods of time (Lal 2005), accumulating on average three times the C in terrestrial vegetation (Post et al. 1982). On the other hand, more N is required per unit of C stored in soil as compared to plant biomass (Yang & Luo 2011). Hence, while an allocation to SOM may increase C sequestration in the long term, a preferential allocation to plant biomass is a more nutrient-efficient C sequestration process in the shorter term. Studying ecosystem C sink partitioning is challenging due to the difficulties associated with quantifying the different ecosystem fluxes. Especially complex is the assessment of rapid and small changes in SOM which are linked to the balance between microbial respiration and plant inputs, including both litter and root-derived C (Schwitz et al., 2011). Thus, belowground C allocation and subsequent C dynamics are still far from being accurately quantified and understood (Phillips et al. 2011, Vicca et al. 2012). Root C inputs have been shown to influence soil
C sequestration, but both the magnitude and direction of this root effect are variable (Karlen & Cambardella 1996, Parton et al. 1996, Cardon et al. 2001, Rasse et al. 2005, Dykstra & Cheng 2007). A robust definition of net ecosystem production (NEP) should be based on a full ecosystem mass balance (Randerson et al. 2002), which accounts for both plant and soil sinks. When it is flux-based, NEP is defined as the difference between ecosystem-level gross photosynthetic gain of C (gross primary production, GPP) and ecosystem respiratory losses (ER). Alternatively, NEP (g C m$^{-2}$ y$^{-1}$) can be expressed as (Campbell et al. 2004 - eqn. 1): \[
\text{NEP} = \Delta C_{\text{biomass}} + \Delta C_{\text{soil}}
\]

In deciduous forest ecosystems, $\Delta C_{\text{biomass}}$ is the annual change in plant biomass (wood, branches, coarse roots), and $\Delta C_{\text{soil}}$ is the annual net change in soil organic C (SOC) stock. In this equation, litterfall and fine root turnover are considered as soil C input and therefore contributing to the $\Delta C_{\text{soil}}$ (see eqn. 2).

Net ecosystem productivity can be directly determined using eddy covariance techniques starting from net ecosystem exchange (NEE = $-$NEP - Baldocchi 2003, Aubinet et al. 2012). Plant biomass changes are usually estimated via a combination of repeated inventories and allometric relationships (Clark et al. 2001). On the other hand, direct SOC determination methods are generally unable to quantify $\Delta C_{\text{soil}}$ in the short term (Schrumpf et al. 2011), and, at annual timescales, alternative methods are required to estimate soil C changes.

Considering that the dissolved organic C (DOC) is typically negligible, representing around 1% of forest NPP (Luyssaert et al. 2010), $\Delta C_{\text{soil}}$ can also be written as (eqn. 2):
\[
\Delta C_{\text{soil}} = \text{Input}_{\text{soil}} - \text{Output}_{\text{soil}} = \text{Input}_{\text{root}} - R_{\text{C, rhizosphere}} - Rh
\]

where $\Delta C_{\text{soil}}$ is the aboveground litterfall (i.e., leaves, branches, wood, etc.), $\text{Input}_{\text{root}}$ is the root-derived C input (i.e., exudates, root slashing and turnover), $R_{\text{C, rhizosphere}}$ is the rhizosphere respiration of root-derived C, and Rh is the heterotrophic respiration. Litter input is conventionally measured by litter traps, while wood input is measured using repeated sampling (Harmon & Sexton 1996), and rhizosphere and heterotrophic respiration can be estimated by a variety of methods (e.g., trenching, girdling, isotopes), as reviewed by Subke et al. (2006) and Kuzyakov (2006). The largest challenge is estimating gross root inputs. However, methods exist to estimate net annual root-derived C input ($Net-C_{\text{root}}$), which is the difference between $\text{Input}_{\text{root}}$ and $R_{\text{C, rhizosphere}}$ (eqn. 3):
\[
Net-C_{\text{root}} = \text{Input}_{\text{root}} - R_{\text{C, rhizosphere}}
\]

Different tracer methods have been used to date to estimate $Net-C_{\text{root}}$, such as pulse labeling, continuous labeling, and $^{13}$C natural abundance (Kuyzakov & Domanski 2000). The latter uses the difference in the stable C isotope composition of native SOM and new plant-derived organic matter to quantify $Net-C_{\text{root}}$. When natural isotope abundances do not allow the use of this approach, distinct C isotope signatures in the soil organic C (SOC) pool and plant-derived organic matter can be obtained in manipulation experiments, by growing C plants ($^{13}$C of approximately -27‰) in soil with organic matter derived from C$_2$ plants ($^{13}$C of approximately -12‰) or vice versa. This approach has been successfully applied in pot (Ineson et al. 1995, Vicca et al. 2010) and field studies (Hoosbeek et al. 2004, Cotrufo et al. 2011) and was used in this investigation.

$Net-C_{\text{root}}$ combined with aboveground inputs to the soil (litter and dead wood), also provides interesting information about soil C dynamics. For soils at steady-state ($\Delta C_{\text{soil}}$=0), the sum of $Net-C_{\text{root}}$ and aboveground inputs is the amount of C that replaces SOC decomposition, thus becoming a measure for SOC turnover. For soils which are net C sinks ($\Delta C_{\text{soil}}>0$), this sum exceeds SOC mineralization and a fraction of it enlarges the SOC pool, thus leading to soil C sequestration. In this context, for soils which are net C sinks, the ratio between $Net-C_{\text{root}}$ and $Net-C_{\text{root}}$ + aboveground inputs indicates the fate of C input: the higher the ratio, the larger the contribution of fresh C to soil C sequestration. The opposite is true for soils that are net C sources ($Net-C_{\text{root}}$>0).

Root C input rates vary considerably depending on tree species, mychorrhizal associations and environmental factors (Lynch & Whippes 1990), with values of up to 40% of net assimilated C being reported (Van Veen et al. 1991). According to the microbial efficiency-mineral stabilization (MEMS) framework (Cotrufo et al. 2013), the fraction of $Net-C_{\text{root}}$ inputs sequestered in the soil depends on the efficiency of decomposers to convert C into bio-products as compared to the amount of C lost as CO$_2$ (Six et al. 2006) and on soil matrix interactions (Sollins et al. 1996, Kleber et al. 2007). Soil organic matter mineralization is driven by both substrate stoichiometry and microbial demand for resources (Melillo et al. 1982, Hessen et al. 2004): when N is limiting, microbes use labile substrate to mineralize recalcitrant SOM (Moorhead & Simsabaugh 2006, Craine et al. 2007). Root exudates can thus prime SOM decomposition (Lohnis 1926, Bingeman et al. 1953, Fontaine et al. 2004). Clearly, root-derived soil C inputs can either stimulate soil C sequestration or, conversely, induce primary processes with consequent losses of stabilized SOM but likely enhancements in N availability, which in turn can stimulate plant growth. The key factors determining the direction (and magnitude) of this effect are, however, not yet clear. Understanding the fate of root-derived C, and its effects on N dynamics and ecosystem C sequestration, is relevant from an ecological perspective and is also an urgent challenge to address, particularly in the context of global changes such as atmospheric CO$_2$ increase and N deposition.

The aims of the present study were: (1) to obtain an estimate of $Net-C_{\text{root}}$ in six different forest ecosystems; (2) to partition NEP into aboveground tree biomass production and soil C sinks; and (3) to investigate the controls of this partitioning. Specifically, we tested the hypothesis that soil C:N stoichiometry controls ecosystem C uptake (GPP) and sink partitioning (ANPP vs. soil C) across forest ecosystems. To verify if our hypothesis could be generalized to other forests, we tested it on several world forest sites for which ANPP, GPP and soil C:N data were available in the literature.

Materials and methods

**Study sites**

Six forests were considered in the present study. Three sites were in central Italy, two sites in northern Italy; and one in Croatia. All sites were equipped with an eddy covariance tower for mass, momentum and energy ecosystem exchange measurements and can be classified as high fertility sites, according to key soil properties (Vicca et al. 2012 - see also Appendix 1). Site characteristics and flux data are reported in Tab. 1, while a brief description for each site is given below.

Quercus rossica (42° 24’ N, 11° 55’ E - Claus & George 2005, Tedeschi et al. 2006) is a Turkey oak (Quercus cerris L) coppice forest about at 235 m a.s.l. in central Italy. Mean annual temperature is 14 °C and mean annual rainfall is 755 mm. Soil is sandy clay Luvisol (which is typically nutrient rich), derived from sedimentary material of volcanic origin and marine deposits, and is moderately acid (pH=5.7), with a total depth > 100 cm (Rey et al. 2002). Cation exchange capacity (CEC) is high, ranging between 19 and 42 meq 100g$^{-1}$ in the different soil layers (Tedeschi et al. 2006). The forest has been managed as a “coppice with standards” over the last 200 years, with a rotation cycle varying between 15 and 20 years. Two stands were selected: a 6-year-old coppice (RO1) and a 15-year-old coppice (RO2).

Lecceto (LE - 43° 18’ N, 11° 16’ E) is a Holm oak (Quercus ilex L.) coppice with a rotation period of 18-20 years at about 300 m a.s.l. in central Italy. Holm oak represents
Table 1 - General characteristics for the six forest sites used in this study. \((\Delta C_{\text{wood}})\): change in aboveground wood biomass; \((\Delta C_{\text{root}})\): change in coarse root biomass; \((\text{NEP})\): net ecosystem production; \((\text{NEE})\): net ecosystem exchange; \((\text{GPP})\): gross primary production; \((R_{\text{eco}})\): ecosystem respiration; (RO1): Roccarsampampani site 1; (RO2): Roccarsampampani site 2; (LE): Lecceto; (JA): Jastrebarsko; (LM): La Mandria; (CO): = Collelongo. (a): N wet deposition in 1990 were derived for all sites using published gridded maps with 0.5° × 0.5° resolution derived from interpolated (kried) ground data (available at http://www.daac.ornl.gov). Total wet depositions (kg N ha\(^{-1}\) y\(^{-1}\)) were then computed as the sum of aqueous NO\(_3^+\) and NH\(_4^+\) fields, which were available. (b): For Collelongo, the reported number refers to direct measurements available for the period 2002-2009 (Piechulla et al. 2011).

| Group | Parameters | VEGETATION CHARACTERISTICS | RO1 | RO2 | LE | JA | LM | CO |
|-------|------------|----------------------------|-----|-----|----|----|----|----|
| Management | Turkey oak | coppice with standards | 6 (approx. 70 standards ha\(^{-1}\) of 20-40 years-old present) | 15 (approx. 70 standards ha\(^{-1}\) of 20-40 years-old present) | 15 | 35 | 80 | 110 |
| Mean stand age in 2006-2007 (years) | Turkey oak | coppice with standards | 20-40 years-old present | | 15 | 35 | 80 | 110 |
| Aboveground biomass (kg C m\(^{-2}\)) | Holm oak | coppice with standards | 1.9 | 4.5 | 5.0 | 6.3 | 7.8 | 13.7 |
| Wet N deposition\(^{\text{a}}\) (kg N ha\(^{-1}\) y\(^{-1}\)) | Pedunculate oak | high forest | 10.0 | 10.0 | 8.6 | 11.2 | 9.4 | 10.8 \(^{\text{b}}\) |
| Soil type | Pedunculate oak - Hornbeam | high forest converted to high forest since 1950 | 8.6 | 7.4 | 27.4 | 7.7 | 7.4 | 14.7 |
| Soil C stock 0-30 cm (kg C m\(^{-2}\)) | Beech | | 0.8 | 0.5 | 1.3 | 0.7 | 0.3 | 0.9 |
| Soil N stock 0-30 cm (kg N m\(^{-2}\)) | | | 10 | 14 | 22 | 11 | 24 | 16 |
| C:N | | | 52 | 52 | 40 | 18 | 6 | 6 |
| Sand (%) | | | 12 | 12 | 35 | 28 | 80 | 50 |
| Silt (%) | | | 35 | 35 | 25 | 54 | 14 | 44 |
| Clay (%) | | | 1577 | 1356 | 901 | 1633 | 754 | 1258 |
| Soil water repellency (mm s\(^{-1}\)) | | | 1060 | 810 | 368 | 1049 | 183 | 722 |
| Root:shoot ratio | | | 161 | 315 | 334 | 325 | 360 | 363 |
| GPP (g C m\(^{-2}\) yr\(^{-1}\)) | | | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.28 |
| Reco (g C m\(^{-2}\) yr\(^{-1}\)) | | | (Mokany et al. 2006) | (Mokany et al. 2006) | (Mokany et al. 2006) | (Mokany et al. 2006) | (Mokany et al. 2006) | (assessed at the site) |
| NEE (g C m\(^{-2}\) yr\(^{-1}\)) | | | 48 | 95 | 100 | 98 | 108 | 102 |
| NEC = NEC + NEC + NEC + NEC + NEC + NEC (g C m\(^{-2}\) yr\(^{-1}\)) | | | 209 | 410 | 435 | 423 | 468 | 464 |
| Litterfall (g C m\(^{-2}\) yr\(^{-1}\)) | | | 47 | 123 | 107 | 203 | 223 | 245 |
| NEE = NEC (g C m\(^{-2}\) yr\(^{-1}\)) | | | 517 | 545 | 533 | 584 | 571 | 535 |

81% of the total tree canopy; others species include Arbatus uso L., Juniperus communis L., Quercus pubescens L., Phillyrea latifolia L., Fraxinus ornus L. Mean annual temperature is 13.5 °C and annual average rainfall is 780 mm. Jastrebarsko (JA = 45° 37' N, 15° 41' E; Marjanovic et al. 2010, 2011) is a 35-year-old forest in Croatia dominated by pedunculate oak (Quercus robur L.) with 19% of black alder (Alnus glutinosa Haer.), 14% hornbeam (Carpinus betulus L.) and 9% of narrow-leaved ash (Fraxinus angustifolia L.). Mean annual temperature is 10.4 °C with mean monthly temperatures of -0.2 °C and 20.7 °C in January and July, respectively. Average annual precipitation is 900 mm year\(^{-1}\), of which around 500 mm falls during the active vegetation period (April-September). Soil is a Luvic Stagnosol with a upper mineral layer (0-20 cm) that linearly increases to neutral pH at depths > 100 cm. At the beginning of the growing season, the soil drains and water content soon drops below water holding capacity (46% v/v) allowing enough oxygen supply for root growth and substantially increasing nutrient availability in these soils, where nutrient availability can be constrained by high water levels. La Mandria (LM = 45° 09' N, 7° 34' E) is an 80-year-old pedunculate oak-hornbeam forest (Quercus robur L. and Carpinus betulus L.) in northern Italy. Mean annual temperature at the site is 11.6 °C and annual precipitation is 1030 mm. Soil is Typic Fragiudalf with adequate moisture content throughout the year, neutral pH and good CEC (ranging from 17 to 11 meq 100 g\(^{-1}\) at soil surface and Bb horizons, respectively). Collelongo (CO = 41° 52' N, 13° 38' E; Valentini et al. 1996, Scarzetta et al. 2004)
is an 110-years-old pure beech (Fagus sylvatica L.) forest in northern Italy that has been part of the network of Long Term Ecological Research sites (LTER) Italy since 2006. Mean annual temperature at the site is 7.1 °C and mean annual rainfall is 1188 mm. The soil is a Humic Alisol with volcanic ash also present. Both CEC and N content are high in the different soil layers, ranging from 14.8 to 23.3 meq 100 g⁻¹ and from 4 to 7.3 mg N g⁻¹, respectively (Persson et al. 2000). Wet N de- 

position rates in the period 2002-2009 averaged 10.8 kg N ha⁻¹ yr⁻¹ (Flechard et al. 2011)

Net root-derived C input to soil

Cotrufo et al. (2011) quantified using the in-growth core isotope technique, following Cotrufo et al. (2011). A soil depleted in 13C (δ13C = -17.22‰) was collected from the USDA-ARS Central Plains Experimental Range located in NE Colorado, USA (40° 49' W). The soil is classified as a Zigweid gr. Wet N de-

position for brevity we call henceforth this soil as “C-13 soil”. Soil was air-dried prior to being sealed and boxed for shipment to Italy. Upon ar-

ival, the C-13 soil was ground and sieved to 2 mm and well mixed to make a homogeneous soil pool, before using it for in-growth cores and chemical (C% and δ13C) analyses as de-

scribed below.

At each forest site, six cores, made of a 2 mm mesh net (thus allowing the penetration of fine roots) with a diameter of 4 cm and a height of 30 cm, were placed randomly within the eddy covariance tower footprint in Oc-

tober 2006 (2008 for Jastrebarsko) and filled with the C-13 soil to a bulk density similar to the average bulk density for the site. At the top of each core the net was closed to avoid above-ground litter input. Cores were sam-

pled a year later, and the soil from each core was separated into 0-15 cm and 15-30 cm depth layers, except for Jastrebarsko, where the entire 0-30 cm core was considered.

All soil samples were sieved to 2 mm, and root samples carefully removed and washed with deionized water. Root samples were placed by site and depth, and each samples analyzed in triplicates. Both soil and root samples were oven-dried at 70 °C, pulvéri-

zed and analyzed for %C and δ13C by an ele-

mental analyzer (Flash EA 1112 NC, CE In-

strument, Wigan, UK) connected to an Isoto-

one Ratio Mass Spectrometer (IRMS, Delta Plus, Thermo-Finnigan, Bremen, Germany). Prior to C analyses, soil samples were treated with HCl to eliminate carbonates (Harris et al. 2001). The measured δ13C values were used to calculate the proportion of new C (fnew, i.e., the Net-Croot), by using a mass ba-

lance equation (Del Gado et al. 2003, Co-

truf et al. 2011 - eqn. 4):  

\[
f_{\text{new}} = \frac{\delta_{\text{new}} - \delta_{\text{old}}}{\delta_{\text{vg}} - \delta_{\text{old}}} 
\]

where δnew is δ13C of the organic matter of the C-13 soil collected from each core after one year of field incubation, δold is the δ13C of the organic matter of the C-13 soil measured before incubation, and δvg is the δ13C of the roots averaged by site and depth. The average δold value across all our sites was -28.11±0.29‰, while variation (standard deviation) within a site was between 0.15 and 0.577‰ at RO1 and RO2, respectively. Knowing the fvalues for the new C, the soil organic C concentra-


tions (%C), soil depth (D, m), and soil bulk density (σ, kg m⁻³), Net-Croot amounts (g m⁻²) were computed for all soil samples as fol-

ows (eqn. 5):  

\[C = f \cdot \%C \cdot \sigma \cdot D\]

Estimates of Net-Croot using this method (Cotrufo et al. 2011) rely on the assumptions that: (1) root inputs are the same inside and 

outside the in-growth bags and are independent of the C-13 soil properties; and (2) that there is no isotopic fractionation during the decomposi-
tion of the native SOM or forma-
tion of the new SOM from the root tissues. New studies applying this method should test these assumptions, since some fractiona-
tion could occur (Hobbie et al. 2004).

Ecosystem fluxes and primary production

Eddy covariance flux data from all five Ita-
lían sites were analyzed for the years 2006-

2007 (Tab. 1). Data of net ecosystem ex-

change (NEE), gross primary production (GPP) and ecosystem respiration (Rveg) at monthly time steps were downloaded from the central Fluxnet database (http://gaia.agra-

ria.unitus.it/database/). Specifically, we used the NEE gap-filled data using the Artificial Neural Network method (NEE ANN from level 4 dataset - Papale et al. 2006). Rveg was computed according to the short-term tempera-
ture response of night-time fluxes (Reich-

stein et al. 2005) and GPP values were de-

rived as sum of the absolute values of NEE files and Rveg. At sites where data for 

the years 2006 or 2007 were incomplete even after gap-filling because of missing weather data, data for 2008 were also inclu-
ded in the analysis for the calculation of an-

nual means. As for the Jastrebarsko site, 2009 eddy flux data were derived from Ma-

janovic et al. (2010).

Mean annual temperature (MAT), mean annual precipitation (MAP), and soil C stocks (0-30 cm), as well as changes in wood biomass (stem and branches - ΔCwood), were derived from ancillary data files available at

the central database, updated to 2006-2007 when necessary, or using specific yield ta-

bles available at the site (e.g., Jastrebarsko). All data were checked, if necessary updated and completed by site Principal Investiga-
tors, who are co-authors of the present study. Changes in root biomass (ΔCroot) were derived from ΔCwood using root-to-shoot ratios reported by Mokany et al. (2006) or using site-specific relationships as in the case of Collelongo and do not include fine root pro-
ductivity.

ANPP was calculated as the sum between ΔCbiomass and NPPbiom (foliar net primary production). The latter corresponds to litter-
fall in the case of broadleaved forests, and was directly measured at the site (i.e., Rocca, Jastrebarsko, Collelongo) or assessed from NPPbiom using biomass expansion factors de-

rived at nearby sites with similar species composition and structure (i.e., La Mandria). In the case of Lecceto, where the dominant species is evergreen (Holm oak), we assumed that the system was at steady state and thus litterfall = NPPbiom. Then the ANPP: GPP ratio was calculated.

World forest sites data

In order to test if the relationship between ANPP:GPP and soil C:N, observed across our study sites, was generalizable across fo-

rest ecosystems, we searched published data-

sets (Litton et al. 2007, Luyssaert et al. 2007, Vicca et al. 2012) for forest sites that pro-
vided the data suitable to our analyses. Twenty-three additional sites were found in-

cluding ANPP and GPP data, as well as soil C:N (determined for a depth up to 45 cm) were found (Tab. 2). Fertility classification followed Vicca et al. (2012). More details are given in Appendix 1.

Data analysis

To test if the site the annual change in net soil C (ΔCroot - g C m⁻² y⁻¹) was calculated starting from eddy covariance NEE data and measu-

red changes in aboveground wood biomass (ΔCwood) and coarse roots (ΔCroot) by re-ar-

ranging eqn. 1 (eqn. 6):  

\[ΔC_{\text{root}} = \text{NEE} - ΔC_{\text{wood}} - ΔC_{\text{roots}}\]

\[\text{NEP} = ΔC_{\text{biomass}}\]

Statistical analyses were performed using the package SISVAR PLOT® 11.0 (Systat® Software, San José, CA, USA). Data were tested for normal distributions, using the Shapiro-Wilk’s test, and homogeneity of var-

iance, and log transformed when necessary. To assess differences in Net-Croot among si-
tes, a one way analysis of variance (one-way ANOVA) was used. Significant treatment (site) effects (P<0.05) were further explored via a treatment (site) comparison using the Least-Squares means test with Tukey’s ad-
justment for multiple comparisons. For sites
where data for 0-15 and 15-30 cm depths were available, a two-way ANOVA with site and depth as fixed factors was also performed.

A correlation analysis between all available variables was performed using the Spearman’s rank method through a correlation matrix in Stata10® (StataCorp®, College Station, TX, USA). For variables that were correlated with p<0.10, linear models were fitted to measured data.

Results

Net root-derived C input to soil

Total Net-C<sub>root</sub> in the top 30 cm soil layer ranged between 420 g C m<sup>-2</sup> year<sup>-1</sup> at Collelongo and 818 g C m<sup>-2</sup> year<sup>-1</sup> at Jastrebarsko (Fig. 1). Mean annual Net-C<sub>root</sub> across sites was 606 ± 164 g C m<sup>-2</sup> year<sup>-1</sup> (mean ± standard deviation). A significant difference in total Net-C<sub>root</sub> (0-30 cm) was detected among sites (ANOVA, p = 0.007). In particular, post-hoc Tukey’s tests showed a significant difference between Jastrebarsko and Collelongo (p = 0.013) and Lecceto and Collelongo (p = 0.041 - Fig. 1). For sites where data for 0-15 and 15-30 cm depths were available (i.e., all sites except Jastrebarsko), the two-way ANOVA applied showed significant differences among sites (p = 0.004), between depths (p = 0.024) and for site × depth interaction (p = 0.035). No differences among sites were detected at 0-15 cm depth (Tukey’s test: p > 0.05), while Net-C<sub>root</sub> at 15-30 cm in Lecceto was significantly different from Rocca1 (p = 0.002), Collelongo (p = 0.003) and Rocca2 (p = 0.021). Climate (i.e., MAT, MAP, soil water content) did not explain significant variability in Net-C<sub>root</sub> among the different sites (see Tab. S1 in Appendix 1). Moreover, total Net-C<sub>root</sub> was not significantly correlated to soil C:N.

Ecosystem C sink partitioning

All six sites were net C sinks with similar NEP values (average NEP was 547 ± 25 g C m<sup>-2</sup> year<sup>-1</sup>) but with large differences in annual GPP (Tab. 1). They actively sequestered C both aboveground and in the soil: A<sub>C</sub> was between 10 and 48% of annual GPP (RO1 and LM, respectively). A<sub>C</sub> was positive for all sites representing between 6 and 20% of annual GPP (CO and RO1, respectively). In accordance with our hypothesis, GPP and A<sub>C</sub> were correlated to soil C:N (p = 0.0048 and p = 0.07, respectively - Tab. S1) and soil C:N across gradients of forest stands and environmental conditions. Nutrient availability was assessed according to Vicca et al. (2012), when possible. (n.a.): not available. (1): Ryan et al. (2004), Binkley et al. (2004); (2) Ghosh et al. (1986), Gholz & Fisher (1982), Gholz et al. (1985); (3) Gower et al. (1997), Ryan et al. (1997), Online BOREAS dataset. Site: “NSA-OJP-90JP1”; (4): Malhi et al. (1999), Marland et al. (2004); (5) Malhi et al. (1999), Liu et al. (2004); (6): Chambers et al. (2004), Li et al. (2004); (7) Harris et al. (1975), Marland et al. (2004); (8): Kinerson et al. (1977); (9): Woodwell & Botkin (1970); ANPP = NPP/1.3; Marland et al. (2004); (10): Kutsch et al. (2001); (11) Dilly et al. (2002); (12): Sun et al. (2004); (13): Malhi et al. (2009); (14) Kellihier et al. (2004); (15) Kellihier et al. (2004); (16): Malhi et al. (2009); (17): Present study, ANPP = A<sub>C</sub> + litterfall.

### Tab. 2 - Studies used to validate the relationship between ANPP-GPP and soil C:N across gradients of forest stands and environmental conditions

| Forest type and location | Management or treatment | Nutrient availability | ANPP | GPP | ANPP : GPP | Depth (cm) | Soil C:N |
|--------------------------|-------------------------|-----------------------|------|-----|-----------|-----------|----------|
| Eucalyptus saligna, Pepekeo, HI<sup>1</sup> | 2 yr, 1x1 m | high | 1427 | 5057 | 0.28 | 0-45 | 16 |
| 2 yr, 1x1 m | high | 480 | 2369 | 0.20 | 0-45 | 15 |
| 2 yr, 3x3 m | high | 1456 | 4413 | 0.33 | 0-45 | 16 |
| 6 yr, 3x3 m | high | 828 | 2930 | 0.28 | 0-45 | 15 |
| Pinus radiata, Pepekeo, FL<sup>2</sup> | 20 yr C | medium | 599 | 2415 | 0.25 | 0-30 | 19 |
| Pinus elliotii, Pepekeo, FL<sup>2</sup> | 7-9 yr | low | 199 | 1407 | 0.14 | 0-15 | 12 |
| Picea mariana, N-BOREAS<sup>3</sup> | 150 yr | low | 132 | 563 | 0.23 | n.a. | 13 |
| Pinus banksiana, N-BOREAS<sup>4</sup> | 63 yr | low | 115 | 677 | 0.17 | n.a. | 15 |
| Oak-Hickory, Oak Ridge, TN<sup>5</sup> | 55 yr | low | 510 | 1329 | 0.38 | 0-20 | 16 |
| Tropical forest, Manaus Brazil<sup>6</sup> | Old growth | low | 870 | 2620 | 0.33 | 0-10 | 14 |
| Tropical forest, Manaus Brazil<sup>6</sup> | Old growth terra firme | low | 650 | 2860 | 0.23 | 0-10 | 12 |
| Liriiodendron, Oak Ridge, TN<sup>7</sup> | 50 yr | n.a. | 352 | 2162 | 0.16 | 0-20 | 14 |
| Pinus taeda, Oak Ridge, TN<sup>9</sup> | 16 yr | medium | 1490 | 4124 | 0.36 | 0-20 | 15 |
| Pinus-Quercus, Oak Ridge, TN<sup>9</sup> | 43 yr | medium | 462 | 1280 | 0.36 | 0-20 | 14 |
| Bornhoved Alder<sup>10</sup> | Temperate Humid-Broadleaved | low | 589 | 2420 | 0.24 | 0-30 | 18 |
| Bornhoved Beech<sup>10</sup> | Temperate Humid-Broadleaved | medium | 601 | 1324 | 0.45 | 0-5 | 15 |
| Cascade Head (1)<sup>11</sup> | Temperate Humid-Needle-leaved | high | 569 | 1400 | 0.41 | 0-30 | 21 |
| Cascade Head (1A)<sup>11</sup> | Temperate Humid-Broadleaved | high | 640 | 1558 | 0.41 | 0-30 | 20 |
| Caxium<sup>12</sup> | Tropical Humid-Broadleaved | low | 869 | 3630 | 0.24 | 0-30 | 13 |
| Jacaranda/K.34<sup>13</sup> | Tropical Humid-Broadleaved | low | 796 | 3040 | 0.26 | 0-30 | 17 |
| Metolius<sup>14</sup> | Temperate Semi-arid-Needle-leaved | medium | 183 | 1143 | 0.16 | 0-30 | 20 |
| Metolius young<sup>15</sup> | Temperate Semi-arid-Needle-leaved | medium | 104 | 724 | 0.14 | 0-30 | 21 |
| Tapajos 67<sup>16</sup> | Tropical Humid-Broadleaved | low | 1400 | 3141 | 0.45 | 0-30 | 15 |
| Roeca 1<sup>17</sup> | Mediterranean Turkey oak | high | 208 | 1577 | 0.13 | 0-30 | 10 |
| Roeca 2<sup>17</sup> | Mediterranean Turkey oak | high | 438 | 1356 | 0.32 | 0-30 | 14 |
| Lecceto<sup>17</sup> | Mediterranean Horn oak | high | 441 | 901 | 0.49 | 0-30 | 22 |
| Jastrebarsko<sup>17</sup> | Mediterranean Pedunculate oak | high | 528 | 1633 | 0.32 | 0-30 | 11 |
| La Mandria<sup>17</sup> | Mediterranean Pedunculate oak - Hornbeam | high | 583 | 754 | 0.77 | 0-30 | 24 |
| Collelongo<sup>17</sup> | Mediterranean mountain beech | high | 608 | 1258 | 0.48 | 0-30 | 16 |
in Appendix 1) and decreased linearly as soil C:N increased (Fig. 2). Moreover, GPP was linearly and inversely related to soil clay content (p = 0.05) but, similarly to \( \Delta C_{\text{soil}} \), it was not correlated with either MAT, MAP or stand age. \( \Delta C_{\text{soil}} \) was weakly related with soil C:N (p = 0.07), while \( \Delta C_{\text{soil}} \)-to-GPP ratio (Fig. 3.a) and ANPP-to-GPP ratio significantly increased with soil C:N (p < 0.0001 and p = 0.005, respectively - Tab. S1 in Appendix 1). In contrast to GPP, Spearman’s correlation analysis revealed a significant relationship between \( \Delta C_{\text{soil}} \) and both MAT and MAP (p = 0.05 and p = 0.008, respectively - Tab. S1 in Appendix 1). Similarly, ANPP was significantly correlated with MAT and MAP (p = 0.001 and p = 0.0003, respectively). Finally, the fate of root C input appeared to depend on soil C:N stoichiometry, with proportionally more Net-C\(_{\text{root}}\) being allocated to C sequestration with decreasing soil C:N (\( \Delta C_{\text{soil}} \) vs. Net-C\(_{\text{root}}\) p = 0.07) and soil C:N was able to explain 40% of the variation in the ratio of \( \Delta C_{\text{soil}} \) to Net-C\(_{\text{root}}\) + litterfall (Fig. 3.b).

**World forest sites**

The positive relationship between ANPP-to-GPP ratio and soil C:N found across our six study sites was confirmed also when iForest (early view): e1-e12
it is difficult to determine whether soil texture rates (shown to significantly influence rhizodeposition). Moreover, soil texture has also been associated with the in-growth core isotope technique. The six sites considered in the present study are labeled as reported in Tab. 1. Eucalyptus saligna plantations (Tab. 2) have been averaged by stand age and vertical bars indicate standard deviation. The reported R² is the adjusted R².

Discussion and conclusions

To our knowledge, this study is the first to quantify Net-C Newtown in a range of forest ecosystems. The measurement of Net-C Newtown in situ is difficult, thus measured values are lacking and modeled estimates cannot be validated. However, the in-growth core isotope technique has already been shown to allow detection of changes in Net-C Newtown in CO₂ and climate manipulation experiments (Hoosbeek et al. 2004, Cotrufo et al. 2011), even though it does suffer from several caveats related to the use of an exogenous soil and high spatial variability. Steingrobe et al. (2000) reviewed the in-growth core method for measuring gross root growth: a first shortcoming associated with this method is achieving the soil conditions inside the bag similar to the bulk soil. Moreover, soil texture has also been shown to significantly influence rhizodeposition rates (Scandellari et al. 2010), although it is difficult to determine whether soil texture influences rhizodeposition rates in our study.

Our estimates of Net-C Newtown using the in-growth core isotope technique were on average 606 g C m⁻² y⁻¹, which is higher than values reported by Cotrufo et al. (2011) for an Arbutus unedo L. coppice in dry Mediterranean conditions, but lower than values reported by Hoosbeek et al. (2004) for an irrigated and fertilized poplar plantation in central Italy. A possible overestimation of Net-C Newtown can be also related to the fact that a certain amount of fine root fragments could have passed through the 2 mm sieve. Such an amount is a function of root integrity as affected by plant age and sample processing. Being aware of this possible overestimation and of the above-mentioned limitations associated with the in-growth core isotope technique, in this study we used Net-C Newtown estimates solely as an indicator of differences in the effect of root-derived C on SOC sequestration through the calculation of the ratio ΔC Newtown (Net root-derived C + litterfall C).

Many factors have been suggested to affect soil C sequestration, including the characteristics of input material, soil texture and mineralogy, climatic factors, and soil nutrient status (Galantini et al. 1992, Andrén & Kätterer 1997, Janssens et al. 2010). We found that the proportion of root C input resulting in C sequestration at these high fertility sites was related to soil C:N ratio, and soil C sequestration was greater at low C:N (Fig. 2b) therefore confirming our hypothesis. Recently, Manzoni et al. (2012) suggested a C-to-nutrient stoichiometric control on microbial C use efficiency (CUE), which would increase with increasing nutrient availability. The importance of CUE as a determinant of the fate of plant inputs to soils has also been recognized by other recent studies (Schimel & Schaeffer 2012, Cotrufo et al. 2013) and some models have suggested that low nutrient availability, particularly N, might limit soil C storage through mechanisms that are still not completely understood (Rastetter et al. 1997, Hungate et al. 2003). Recently, Kirkby et al. (2013) hypothesized that the sequestration of C-rich crop residue material into SOM could be improved only by adding supplementary nutrients, as the more stable SOM fraction has more N, P and S per unit of C than the plant material input due to microbial reprocessing. Thus, the increase in soil C sequestration at lower soil C:N values observed in this study may be explained by a higher microbial CUE of root C inputs.

Soil C Newtown exerted a strong control on GPP across our six forests and GPP increased with decreasing soil C:N (Fig. 2a). This relationship is based on six forest sites and we cannot exclude the possibility that other factors influenced this relation. At the ecosystem scale, variation in global plant productivity across ecosystems has often been related to environmental factors (Field et al. 1995, Reichstein et al. 2007b), but also to...
nutrient availability (Vicca et al. 2012). In this context, Zha et al. (2013) reported a strong positive relationship between GPP or NPP and total soil N. Across our sites, $\Delta C$ and ANPP increased slightly, but not at sites with increasing soil C:N, and showed significant correlations with MAT, MAP and stand age, thus confirming previous studies (Curtis et al. 2002, Hsu et al. 2012, Robinson et al. 2012, He et al. 2012). How such different behavior between $\Delta C$ and ANPP with respect to soil C:N could be explained? We suggest that this result is due to the lower demand for N by woody tissues (which comprise the largest fraction of the tree and are characterized by very high C:N) as compared to green leaves (which control GPP, and have much lower C:N than woody tissues). As a result of these variations in both GPP and ANPP, the ratio between ANPP and GPP varied substantially among our six forest sites.

Following the distinct patterns of ANPP and GPP versus soil C:N, the ANPP-to-GPP ratio significantly increased with increasing soil C:N (Fig. 2a). At first sight, this seems to contradict the current understanding that partitioning of photosynthates into above-ground biomass increases with increasing nutrient availability across a wide range of forests (Vicca et al. 2012). However, all six forest sites had high nutrient availability, but, at those sites, where soil C:N presumably exceeded tree demand for wood growth (i.e., sites with low C:N), root C inputs were probably responsible for the higher net soil C sequestration. We speculate that the link between soil C:N stoichiometry and microbial activity controls C sequestration belowground, as well as for the increase in ANPP-to-GPP ratio with increasing soil C:N across the high fertility forests in our dataset. At site C:N below 15, CUE is expected to be high, and more of the fresh C input is used for microbial products, resulting in the net formation of new SOM. Conversely, when C:N is high, microbes have a low C use efficiency and therefore they respire more of the fresh C inputs and prime SOM decomposition (Fontaine et al. 2004), which increases N availability and supports a higher allocation of fixed C (GPP) to ANPP. Our observations of increasing ANPP-to-GPP ratio, and the tendency for a decrease in soil C sequestration with increasing soil C:N (Fig. 2b), support this hypothesis.

In order to further test this hypothesis, we analyzed a larger dataset. Also in this case, ANPP-to-GPP ratios were quite variable (average ANPP-GPP = 0.28 with SD = 0.10; Tab. 2) and our analysis confirmed the relationship between ANPP-to-GPP ratio and soil C:N at sites with high fertility (Fig. 4). At sites where overall nutrient availability was low, this relationship did not hold. Variation in partitioning of GPP to ANPP at these sites is probably driven by the need for plants to invest in the nutrient acquiring system (i.e., roots and root symbionts - Vicca et al. 2012). When nutrient availability is limited, belowground input by plants may be the dominant control of microbial activity and SOM mineralization (Hamilton & Frank 2001, Wardle et al. 2004, De Deyn et al. 2008, De Graaff et al. 2010), thereby influencing mineral nutrient availability for plant uptake. Our speculation is also consistent with other recent findings. At the Duke Free Air CO₂ Enrichment (FACE) experiment, the increase in the belowground C flux stimulated microbial activity, accelerated SOM decomposition, and stimulated tree uptake of N bound to this SOM, sustaining ANPP (Drake et al. 2011, 2013, Phillips et al. 2012). Yin et al. (2013) found that an increase in the release of root exudates into the soil was an important physiological mechanism to sustain growth responses of plants to experimental warming.

At our study sites, soil C:N stoichiometry appeared to be weakly controlled by the soil clay content (r = 0.15 - Tab. S1 in Appendix 1), decreasing with increasing %clay in soil. This is consistent with our knowledge of soil primary organo-mineral particles, which describes clay-associated SOM as the fraction with the highest microbial contribution and lowest C:N ratio (Christensen 1992, Grandy & Neff 2008).

In conclusion, our results suggest that a specific site property, such as soil texture, could drive soil C:N stoichiometry in which it would control ecosystem C uptake and partitioning within forests of high nutrient availability. While GPP strongly and linearly increased with increasing soil N, above-ground tree biomass demand for N appeared to saturate, possibly because of the higher C:N of wood vs. green leaves, and, at high nutrient availability, NPP became limited by other environmental factors. When this occurs, more C is sequestered by soil (Fig. 5), where the high N availability promotes CUE efficiency and new SOM formation.

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Supplementary Material

Appendix 1

Box S1 - Nutrient classification.
Tab. S1 - Spearman correlation matrix for the six Mediterranean forests considered in the study.

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