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Radiocarbon-Based Assessment of Heterotrophic Soil Respiration in Two Mediterranean Forests

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Abstract

The amount of soil organic carbon (SOC) released into the atmosphere as carbon dioxide (CO_2), which is referred to as heterotrophic respiration (Rh), is technically difficult to measure despite its necessity to the understanding of how to protect and increase soil carbon stocks. Within this context, the aim of this study is to determine Rh in two Mediterranean forests dominated by pine and oak using radiocarbon measurements of the bulk SOC from different soil layers. The annual Rh was 3.22 Mg C ha^{-1} y^{-1} under pine and 3.13 Mg C ha^{-1} y^{-1} under oak, corresponding to 38 and 31% of the annual soil respiration, respectively. The accuracy of the Rh values was evaluated by determining the net primary production (NPP), as the sum of the Rh and the net ecosystem production measured by eddy covariance, then comparing it with the NPP obtained through independent biometric measurements. No significant differences were observed, which suggested the suitability of our methodology to infer Rh. Assuming the C inputs to soil to consist exclusively of the aboveground and belowground litter and the C output exclusively of the Rh, both soils were C sinks, which is consistent with a previous modeling study that was performed in the same stands. In conclusion, radiocarbon analysis of bulk SOC provided a reliable estimate of the average annual amount of soil carbon released to the atmosphere; hence, its application is convenient for calculating Rh because it utilizes only a single soil sampling and no time-consuming monitoring activities.

Key words: heterotrophic respiration; net ecosystem production; net primary production; soil carbon quality; soil fluxes; soil carbon sink.

Introduction

Soils of areas with a Mediterranean type of climate are relatively poor in organic matter, and global warming could further decrease their carbon (C) stocks (Davidson and Janssens 2006; Jones and others 2005). If the expected warming scenarios occur, the decrease in soil water availability rather than the increase in air temperature will particu-

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larly affect the soil carbon dioxide (CO_2) efflux (Rey and others 2002, 2005; Unger and others 2009) and the relative contribution of its two components: (i) autotrophic respiration by roots and the associated mycorrhizae and (ii) heterotrophic respiration (Rh) by microorganisms and soil fauna that decompose the aboveground litter and soil organic matter (SOM). The assessment and monitoring of the relative contribution of these two CO_2 sources becomes crucial for predicting how climate change is affecting the biogeochemical C cycle (Giardina and Ryan 2000). In Mediterranean environments, where climate change is expected to be more marked than elsewhere (IPCC 2013), the estimated contribution of Rh to annual soil CO_2 efflux is reported to range from 48 to 77% (Wang and others 2000; Rey and others 2002), mostly depending on the vegetation cover and the methods used to partition soil CO_2 efflux (for example, Subke and others 2006; Hanson and others 2000). Climate change in Mediterranean environments could induce marked modifications in soil respiration, particularly in its heterotrophic component (Inglima and others 2009).

In recent decades, new techniques have been developed to better investigate the nature and turnover time (TT) of soil organic carbon (SOC). In particular, tracing radiocarbon (^{14}C) throughout terrestrial ecosystems has emerged as a viable tool for discriminating the heterotrophic component of soil CO_2 efflux (Chiti and others 2011; Schuur and Trumbore 2006). In the 1950s and 1960s, nuclear tests released large amounts of ^{14}C into the atmosphere—the so-called ''bomb carbon''—that almost doubled the atmospheric ^{14}C concentration. Bomb carbon has been consequently transferred from the atmosphere to the vegetation via photosynthesis and, finally, to SOC. This process enabled the development of various models based on bomb carbon as a tracer to calculate SOC TT, as explained in Gaudinski and others (2000). Assuming that autotrophic respiration has much shorter TT and younger age than heterotrophic respiration, where soil decomposers can access old and protected SOM, the assessment of the SOC TT allows one to estimate the heterotrophic respiration in a single soil layer or horizon using the ratio between the stock and TT of SOC (for example, Gaudinski and others 2000; Harrison and others 2000). For this purpose, radiocarbon was initially measured in bulk soil samples (Harrison 1996), whereas in recent decades the attention shifted to specific soil fractions (Karhu and others 2010), as much as possible reflecting different SOC pools in terms of chemical composition and, above all, TT. The quality of SOC is the main controlling factor of SOC TT (Poirier and others 2003); hence, the type of vegetation plays a major role in driving SOC dynamics (Quideau and others 2001). Nevertheless, the stability of SOC also depends on the chemical interactions with the mineral phase (Mikutta and others 2006), the physical protection into aggregates (Wagai and others 2009), and the oxygen availability to decomposers (Munir and others 2014). The classic *C*/*N* ratio is a reliable indicator of the bioavailability of any plant residues to decomposers, whereas the chemical structure of C is the ultimate driving factor of decay. Solid-state ^{13}C nuclear magnetic resonance (NMR) is a powerful tool to obtain information on the chemical structure of SOC (Forte and others 2006; Simpson and others 2011) and thus to infer its ability to resist degradation.

The main aims of this study are (1) to quantify Rh in two Mediterranean forests in Italy by measuring the ^{14}C concentration in the bulk SOC from various soil depths and (2) to verify the reliability of the obtained Rh using data derived from eddy covariance measurements (for example, net ecosystem production—NEP), biometric measurements (for example, net primary production—NPP), and soil respiration measurements. An ancillary aim is to check, by solid-state ^{13}C NMR spectroscopy, if the presumed different quality of SOC at the two sites may actually explain a different Rh.

Materials and Methods

Study Sites and Soil Sampling

The study was conducted at two coastal forests of Central Italy, San Rossore and Castelporziano. Both sites overlook the Tyrrhenian Sea (Figure 1) and are characterized by a Mediterranean type of climate, which essentially consists of moist early spring and late autumn and a long, warm and dry summer. The stand at San Rossore (Chiesi and others 2005) is a 37-year-old plantation of maritime pine (*Pinus pinaster* Ait.) growing on recent (post-Roman) eolian sand deposits. The current pine vegetation replaced the natural forest of holm oak (*Quercus ilex* L.) originally present on most of the western coasts of Italy. The stand at Castelporziano (Garbulsky and others 2008) is a 59-year-old holm oak coppice under conversion to high forest. It is characterized by Holocene quartziferous sand of eolian origin, reddened by iron oxides that are the weathering products of the adjacent Pleistocene outcrops of pozzolana and pyroxene-rich lithoid tuff (Table 1). Additional information on the San Rossore and Castelporziano study sites is provided by Gellini and others (1983) and Manes and others (1997), respectively. Both sites have

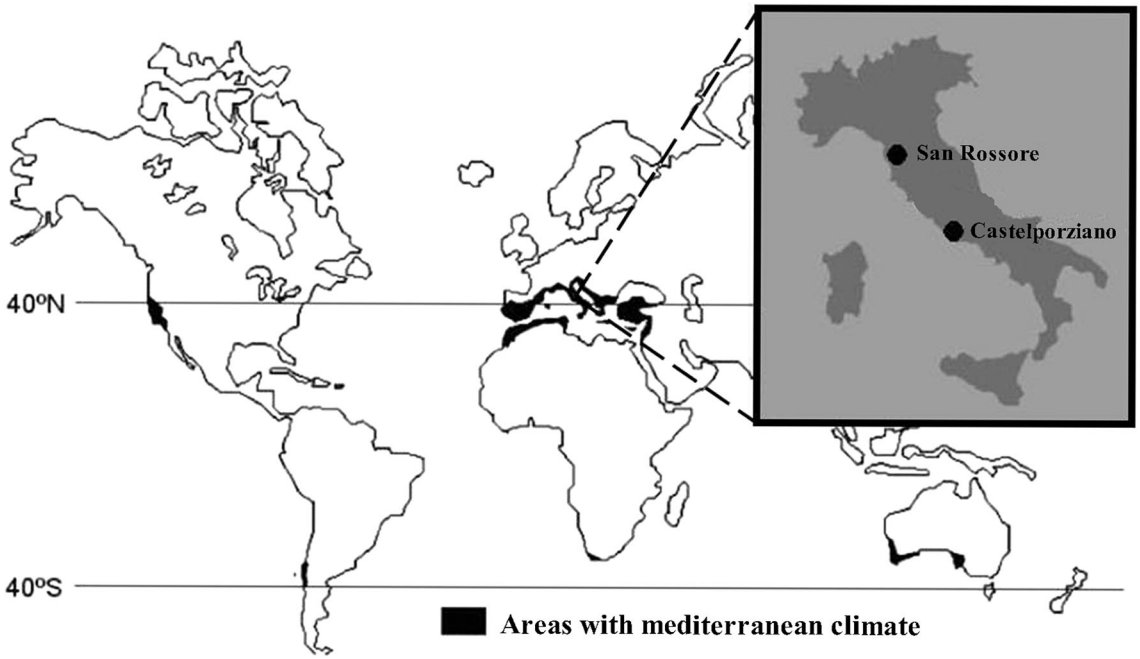

Figure 1. World distribution of areas experiencing a Mediterranean type of climate. *Inset* is the location of the two investigated forests on the western coast of Italy.

eddy covariance systems equipped with instruments for measuring CO_2 and water and energy fluxes, as well as meteorological variables; fluxes were calculated following the method described in Aubinet and others (2000). Both study sites are part of the FLUXNET international network (http://fluxnet.ornl.gov), and their eddy covariance-related measurements are available in the European Fluxes Database (www.europe-fluxdata.eu). According to the FLUXNET terminology, the sites will be hereafter called IT-SRo (San Rossore) and IT-Cpz (Castelporziano).

In spring 2004, three 1.5-m-width soil trenches were opened to 1 m depth within a 0.5 ha area at both sites. Soil samples were collected from 0 to 10, 10 to 20, 20 to 30, 30 to 50, 50 to 70, and 70 to 100 cm depth intervals at the opposite profiles of each trench, resulting in six replicates per sampling depth and per site. Samples were taken by a cylinder of known volume (diameter = 8 cm; height = 10 cm) to determine bulk density. The organic horizon was sampled at 10 random points within a 20-cm by 20-cm frame down to the mineral soil. Samples were oven-dried (60°C to constant weight) and in the case of the mineral soil sieved at 2 mm to remove rock fragments. They were then finely ground in a ball mill to reach the highest homogenization and measured for total C and N via dry combustion (Perkin-Elmer 2400 Series 2, Waltham, MA). Total C was measured on

Table 1. General Features of the Two Study Sites

Site	Forest vegetation	Latitude (°)	Longitude (°)	MAT[1] (°C)	MAP[2] (mm)	Elevation (m a.s.l.)	Parent material	Soil type[3]
San Rossore (IT-SRo)	*Pinus pinaster* Ait.	43.73N	10.29E	14	920	4	Post-Roman quartziferous eolian sand	Albic Arenosol
Castelporziano (IT-Cpz)	*Quercus ilex* L.	41.71N	12.38E	15	740	2	Holocene quartziferous eolian sand	Brunic Arenosol

[1]*Mean annual temperature.*
[2]*Mean annual precipitation.*
[3]*According to IUSS Working Group WRB (2014).*

all samples from the organic horizon (n = 10) and the mineral soil (n = 6). The possible occurrence of inorganic carbon was checked as well in all samples by a Dietrich-Fruhling volumetric calcimeter.

Radiocarbon Measurements

The ^{14}C concentration was measured in the organic horizon and the mineral soil from 0 to 10, 10 to 20, and 20 to 30 cm depth intervals on three composite samples for each layer created by combining equal aliquots of the two samples from the opposite profiles of each trench. Despite previous observations suggesting that the value of SOC from soil layers deeper than 30 cm is often marginally or not affected by bomb C (Phillips and others 2013), which accounts for a slow turnover and a minor Rh contribution to total soil CO_2 efflux, we also analyzed a single composite sample from the 30–50 cm soil layer at both sites. This allowed us to exclude the presence of bomb C below 30 cm in our soils. Inorganic C only occurred in the soil at the IT-SRo site, except in the 0–10 cm layer, where it had apparently decomposed because of acidity. As a consequence, soil samples from 10 to 20, 20 to 30, and 30 to 50 cm depths were treated with HCl to remove carbonates, according to the ''aqueous method'' by Verardo and others (1990). Graphite targets of all samples were prepared according to Steinhof and others (2004) and sent to the Keck Carbon Cycle AMS laboratory of Irvine, California, for ^{14}C measurement (Southon and others 2004). The results were expressed as percent Modern (pM) according to Stuiver and Polach (1977). Samples with a pM greater than 97.5 pM revealed the presence of ^{14}C produced by nuclear weapons testing in the 1950s and 1960s (''bomb C''). They were thus labeled as ''modern,'' as it is impossible to infer their age because of the fast and irregular increase in atmospheric ^{14}C during the nuclear weapons testing period (Meijer and others 1994). By contrast, pM values lower than 97.5 pM indicated that the sample incorporated minor amounts of bomb C.

Heterotrophic Efflux from Soil

The ^{14}C approach used to determine the annual Rh relies on the ''box model,'' as reported by Harrison and others (2000) and Rodeghiero and others (2013). The box model assumes the same order of magnitude for the annual input and output of C into and out of different SOC compartments, called ''boxes.'' Boxes can be genetic horizons, depth intervals, and physical or chemical SOC fractions. In each box, the SOC pool is assumed to have reached the steady state, and dissolved organic carbon (DOC) losses are assumed to not occur. The ^{14}C concentration of the C input into the plant box reflects the atmospheric CO_2 concentration at the moment of photosynthesis. However, the model requires accounting for the residence time of C in the plant before it can be incorporated into the soil. The TT is adjusted for each box; thus, the calculated ^{14}C content of the box matches that measured in the year of sampling.

The heterotrophic C output from a box can be calculated via the C stock of the box and the average TT of such C stock according to equation (1):

$$\mathrm{Rh}(\mathrm{Mg\ C\ ha^{-1}\ y^{-1}}) = \frac{\mathrm{SOC\ stock\ (Mg\ C\ ha^{-1})}}{\mathrm{SOC\ TT\ (years)}}, \quad (1)$$

where total soil Rh is the sum of the Rh from the various boxes (depth intervals in this study).

To determine the TT of SOC from each box, we used a time-dependent steady-state (TDSS) model, as discussed in detail by Gaudinski and others (2000). The TDSS model assumes that the time lag between photosynthesis of CO_2 and its release to soil is 1 year or less and that radiocarbon does not fractionate during respiration. Following Hakkenberg and others (2008), we considered a time lag of 3 years for the pine stand and 2 years for the oak stand. Assuming steady-state conditions, the TT for a given pool of C is simply its mean residence time (MRT), and it thus estimates both the average time the carbon atoms stay in the pool and the average age of the pool (Trumbore 2000). To develop an atmospheric radiocarbon dataset, we referred to the ^{14}C time record of the Northern Hemisphere air published by Levin and Hesshaimer (2000) for the period 1900–1950, and by Hua and others (2013) for the period 1951–2004 (Northern Hemisphere, zone 2). Radiocarbon concentrations on the bomb-spike curve result in two possible TTs (Marín-Spiotta and others 2008), one on the rising side of the ^{14}C peak and the other on the decreasing side. In these cases, we identified the most likely solution based on the aboveground litterfall rate and the carbon stock of the considered soil layer (McFarlane and others 2013). For example, a ^{14}C pM value of 116% for the 0–10 cm soil layer under oak corresponded to two possible TTs, 14 and 40 years. The consideration that a TT of 14 years for the 0–10 soil layer required the same C input as the above-lying organic horizon, whereas a TT of 40 years required just one-third of the annual aboveground litterfall, led us to assume 40 years as the most likely solution.

It is important to note that TT integrates over time, and thus the ^{14}C value of the bulk soil changes relatively little on an annual basis.

Heterotrophic Soil Respiration Assessment

To assess the consistency of the Rh value obtained by applying the box model, we compared our results to estimates of NEP, as measured by the eddy covariance system in the same year of the soil sampling (that is, 2004). The eddy covariance data were post-processed (including quality filtering and gap filling) using standard approaches (Papale and others 2006; Reichstein and others 2005), to obtain the annual NEP values. The eddy covariance methodology provided the net exchange of CO_2 between the ecosystem and atmosphere, named net ecosystem exchange (NEE), which, when cumulated, is equivalent to NEP but with the opposite sign (see Aubinet and others 2012). We used equation (2) to determine the net primary productivity as the sum of the NEP and the C lost via heterotrophic respiration (Lovett and others 2006):

$$NPP = NEP + Rh. \quad (2)$$

This equation is based on the assumption that there are no C losses as DOC or via erosion or grazing. Such an assumption is reasonable, especially because the annual DOC export is minor in many Mediterranean systems (Bernal and others 2002; Pardini and others 2003), even one to two orders of magnitude lower than the DOC losses documented for humid forest areas (Hagedorn and others 2000; Meyer and Tate 1983). To check the reliability of our approach, the NPP resulting from equation (2) was compared with the NPP measured using biometric methods at the two study sites, in 2001 at IT-SRo (Chirici and others 2007) and in 2000 at IT-Cpz (Tirone and others 2003). The temporal discrepancy between the measurement of NPP at the two sites and the year we sampled and analyzed the soil samples should not be a problem because both stands are mature forests; hence, their annual NPP is confidently assumed to be constant across years. The reliability of this assumption was verified at the IT-SRo site, where a tree ring study revealed no significant increases in aboveground biomass during the considered period (Baabst and others 2014). No data are available for the IT-Cpz site to confirm the assumption. Nevertheless, the older age of the stand and the similar climate conditions lead us to hypothesize a similar behavior. For further corroboration of the obtained ^{14}C-based Rh values, we compared them with the respective annual soil CO_2 efflux measured at both sites in 2000, 2001, and 2002 using the ''dynamic chamber'' method by Tirone (2002).

Results

SOC Concentrations and Stocks

We found inorganic C only under pine at the IT-SRo site, except in the 0–10 cm layer where evidently carbonates had dissolved because of natural soil acidification. The organic C was calculated as the difference between total C and inorganic C. At IT-SRo, the organic horizon contained 8.6 Mg C ha^{-1} and the top meter of virtually stone-free mineral soil 81.1 Mg C ha^{-1}, one-third of which was in the top 20 cm. Both the SOC and the *C*/*N* ratio decreased with depth (Table 2).

Under oak at IT-Cpz, the organic horizon and the top meter of mineral soil contained 6.5 and 55.9 Mg C ha^{-1}, respectively (Table 2). In the mineral soil, where more than 60% of C was confined in the top 20 cm, the C concentration and *C*/*N* ratio decreased with depth, as in the other site.

SOC Turnover Time and Rh Fluxes

In the pine forest at IT-SRo, the organic horizon contained bomb C resulting in two possible average TTs, 3 or 85 years (Table 3). Considering that the needles of *P. pinaster* are reported to require 2–8 years to decompose in Mediterranean environments (Kurz and others 2000), the 3-year TT was the most plausible of the two values. The presence of bomb C was also evident in the 0–10 cm layer of mineral soil and, to a lesser extent, in the 10–20 cm layer. In the former depth interval, the average TT of 75 years was considered more plausible than 8 years on the basis of the C inputs required in that specific horizon. This choice is supported by other studies that suggest the longer of the two possible TTs as the most likely solution for mineral soil (Marín-Spiotta and others 2008; McFarlane and others 2013). Nonetheless, we used both TTs to calculate the heterotrophic efflux from the 0–10 cm layer to account for the error generated by the wrong choice of TT in those cases where helpful collateral information was missing. Below 10 cm, there was just one possible TT and bomb C was even undetectable in the 30-50 cm layer, where the TT of SOC amounted to more than two millennia (Table 3).

The main contributors to soil heterotrophic efflux were the organic horizon (2.86 Mg C ha^{-1} y^{-1}) and the top 10 cm of mineral soil which, depending on the selected TT, released 2.86 or 0.30 Mg C ha^{-1}

Table 2. Carbon Concentration (± standard deviation), *C*/*N* Ratio, and C Stocks at Different Depth Intervals Under Pine at IT-SRo and Oak at IT-Cpz

Depth (cm)	Pine			Oak		
	SOC (g C kg^{-1})	*C*/*N*	C stock (Mg C ha^{-1})	SOC (g C kg^{-1})	*C*/*N*	C stock (Mg C ha^{-1})
Organic horizon	416.2 ± 13.3	27.7	8.6 ± 3.3	327.4 ± 11.5	27.3	4.7 ± 1.5
0–10	16.6 ± 4.3	16.8	22.9 ± 4.1	31.7 ± 3.1	13.3	22.5 ± 5.1
10–20	4.8 ± 3.7	15.2	7.1 ± 2.2	10.5 ± 3.2	11.6	13.8 ± 2.1
20–30	6.8 ± 2.0	15.6	9.1 ± 3.2	5.3 ± 1.2	11.5	6.8 ± 1.3
30–50	4.5 ± 2.1	12.7	13.1 ± 4.2	2.4 ± 0.9	10.3	4.0 ± 1.2
50–70	4.3 ± 1.5	8.6	12.6 ± 4.1	2.1 ± 0.5	8.3	3.6 ± 1.3
70–100	3.8 ± 2.1	9.5	16.3 ± 4.2	2.1 ± 0.5	8.5	5.2 ± 2.2
Total			89.7 ± 9.7			62.4 ± 6.5

Numbers on the right side are standard deviations.

Table 3. Radiocarbon Activity (pM C ± σ) and Turnover Time (TT) of SOC Under Pine at IT-SRo and Oak at IT-Cpz

Depth (cm)	Profile	Pine		Oak	
		pM (%)	TT (years)	pM (%)	TT (years)
Organic horizon	1	115.6 ± 0.3	3 or 85	117.9 ± 0.4	2 or 94
Organic horizon	2	113.1 ± 0.2	2 or 82	118.1 ± 0.3	3 or 83
Organic horizon	3	117.6 ± 0.5	3 or 88	115.4 ± 0.3	2 or 96
Mean		115.3 ± 0.3	3 ± 1 or 85 ± 12	117.1 ± 0.3	2 ± 1 or 91 ± 7
0–10	1	113.0 ± 0.2	10 or 61	117.6 ± 0.5	15 or 33
0–10	2	107.1 ± 0.2	9 or 93	118.8 ± 0.2	17 or 29
0–10	3	109.4 ± 0.2	5 or 72	113.5 ± 0.2	9 or 59
Mean		109.8 ± 0.2	8 ± 2 or 75 ± 17	116.3 ± 0.3	14 ± 4 or 40 ± 16
10–20	1	99.8 ± 0.2	356	108.4 ± 0.3	4 or 119
10–20	2	105.0 ± 0.3	165	107.6 ± 0.2	3 or 131
10–20	3	105.5 ± 0.3	151	102.0 ± 0.2	276
Mean		103.4 ± 0.3	224 ± 114	106.0 ± 0.2	94 ± 157 or 175 ± 87
20–30	1	97.8 ± 0.2	332	103.5 ± 0.2	248
20–30	2	98.3 ± 0.2	377	105.3 ± 0.2	275
20–30	3	98.1 ± 0.3	413	100.7 ± 0.3	297
Mean		98.0 ± 0.3	374 ± 41	103.2 ± 0.2	273 ± 24
30–50	1	78.9 ± 0.2	2312	99.5 ± 0.2	362

σ is the measurement uncertainty. Soils were sampled in the summer of 2004 and analyzed in the same year.

y^{-1}. The Rh efflux from deeper layers was minor: 0.03 Mg C ha^{-1} y^{-1} in both the 10–20 and 20–30 cm layers and even less in the 30–50 cm layer. The total annual soil Rh, obtained as the sum of the Rh of each single layer, was thus 5.78 or 3.22 Mg C ha^{-1} y^{-1}, depending on the TT of SOC selected for the 0–10 cm layer (Table 4).

In the oak forest at IT-Cpz, the influence of bomb C was significant down to 50 cm. The average TT of SOC increased progressively with depth but to a lesser extent than at the other site (Table 3). Of the two possible TTs of the 0–10 and 10–20 cm layers, 40 and 175 years were assumed to be the correct ones based on the C inputs required by those layers, respectively (Table 3).

The Rh contributions of the different soil layers followed the same depth trend found in the pine stand. The organic horizon was the main contributor (2.35 Mg C ha^{-1} y^{-1}), and the 0–10 cm soil layer the second one, with 1.61 or 0.56 Mg C ha^{-1} y^{-1} depending on the selected TT (Table 3). The contribution of the other depth intervals to total Rh was minor, the fluxes being 0.15 or 0.18 Mg C ha^{-1} y^{-1} at 10–20 cm, 0.02 Mg C ha^{-1} y^{-1} at 20–30 cm, and 0.01 Mg C ha^{-1} y^{-1} at 30–50 cm. Hence, the total annual soil Rh ranged from a maximum of

4.14 Mg C ha^{-1} y^{-1} to a minimum of 3.13 Mg C ha^{-1} y^{-1} (Table 4).

Rh Assessment

The values of NPP resulting from equation (2), using the NEP values for 2004 and the Rh obtained with the longer TT for the mineral soil, were 8.18 Mg C ha^{-1} y^{-1} under pine and 9.17 Mg C ha^{-1} y^{-1} under oak. Taking into account the errors (Table 4), these values are consistent with the NPP actually measured at the sites: 7.96 and 8.95 Mg C ha^{-1} y^{-1}, respectively. On the other hand, the values of NPP calculated by equation (2) using the shorter TT were also reasonable: 10.74 Mg C ha^{-1} y^{-1} under pine and 10.18 Mg C ha^{-1} y^{-1} under oak.

The heterotrophic soil respiration accounted for 38% of the total soil respiration in the pine forest at IT-SRo and 31% in the oak forest at IT-Cpz (Table 4).

Discussion

Soil Heterotrophic Efflux Assessment and Methodological Limitations

The application of radiocarbon measurements on bulk soil to assess heterotrophic soil respiration resulted in realistic and promising results. In fact, at both investigated sites, the NPP calculated using the ^{14}C-based Rh from this study and the NEP from eddy covariance was very similar to the NPP from biometric measurements (Table 4). We confidently assumed the value of Rh obtained using the longer TTs to be the correct one at both sites but, should this assumption be wrong, the overestimation would not be considerable, amounting to 16% at IT-SRo and 13% at IT-Cpz.

The ^{14}C-based Rh in the pine forest at IT-SRo matches the Rh value reported by Luyssaert and others (2007) for conifer forests in the Mediterranean area, that is, 5.3 ± 1.0 Mg C ha^{-1}. However, the relative contribution of Rh to total soil respiration at IT-SRo (38%) is in the lower range of values recorded for conifers in the same area (for example, Hanson and others 2000; Subke and others 2006). A substantial lack of Rh data for soils of broadleaf evergreen forests in the Mediterranean area, which in Italy are composed mainly of *Quercus ilex*, does not allow us to make an adequate comparison with our data from the oak forests from the IT-Cpz site. Nevertheless, the Rh contribution to soil respiration found at the IT-Cpz site is in the lower range of the values reported for broadleaf forests in the same climatic area (Subke and others 2006) and, more importantly, it is similar to the ^{14}C-based Rh of approximately 3.0 Mg C ha^{-1} y^{-1} determined in a *Quercus ilex* forest in Italy by Rodeghiero and others (2013). Measuring ^{14}C in the bulk soil from different depth intervals allowed us identifying the soil layer that interacts most with the atmosphere, which was different at the two sites. In the oak forest, the bomb C was incorporated into the soil down to 50 cm compared with only 30 cm in the pine forest. At both sites, radiocarbon revealed a major contribution of recently synthesized organic matter in the top 20 cm of mineral soil, where most SOC is stored and most Rh occurs. Similar to the results of this study, Albanito and others (2012) found at IT-SRo that the Rh from the mineral soil was almost exclusively from the top 20–30 cm.

The ^{14}C-based approach applied on bulk soil cannot be indiscriminately used for all soil types. In particular, it may provide misleading results in soils that are far from the steady state or experience major DOC losses. In fact, DOC mainly comprises

Table 4. Rh Values Derived from ^{14}C Measurements, NEP Data from Eddy Covariance System, Calculated NPP (NPP_Cal), Measured NPP (NPP_Bio), Annual Soil Respiration (SR_Cmb), and the Ratio Between Heterotrophic Respiration and Annual Soil Respiration (Rh/SR) Under Pine at IT-SRo and Oak at IT-Cpz

Site	Mg C ha^{-1} y^{-1}					Rh/SR (%)
	Rh_^{14}C	NEP_EC	NPP_Cal	NPP_Bio	SR_Cmb	
IT-SRo (pine)	3.22 ± 0.58	4.96 (4.76–5.07)	8.18 (7.40–8.76)	7.96 ± 0.65[1]	8.43 ± 0.52[3]	38 ± 7
IT-Cpz (oak)	3.13 ± 0.47	6.04 (5.98–6.05)	9.17 (8.64–9.65)	8.95 ± 0.84[2]	10.12 ± 0.84[2]	31 ± 5

Rh_^{14}C = Heterotrophic respiration based on ^{14}C; NEP_EC = NEP measured by eddy covariance; NPP_Cal = NPP calculated as NEP + Rh; NPP_Bio = NPP determined by biometric methods; SR_Cmb = soil respiration measured with dynamic chambers. Ranges in NEP and NPP_Cal are calculated on the basis of the main source of uncertainty (u filtering) and values in parenthesis are the 16th and 84th percentiles (equivalent to one standard deviation in case of normal distribution).*
[1]*From Chirici and others (2007).*
[2]*From Tirone and others (2003).*
[3]*From Tirone (2002).*

''young'' C, as for example, simple sugars or low-molecular weight organic acids (van Hees and others 2005; Sanderman and others 2008; Sanderman and Amundson 2009), and its loss implies a higher average TT of the remaining SOC, leading to underestimation of heterotrophic soil respiration. As observed by Sanderman and Amundson (2009), in those cases where DOC is composed of aged material the resulting lower average TT leads to an overestimation of Rh, because the young material is retained in the soil. SOC losses via dissolution are minor compared to those via respiration, as observed by Sanderman and Amundson (2009) in a Mediterranean forest. The sandy nature of the two studied soils prevents the formation of aggregates, which could offer physical protection to SOM against decomposition. Consequently, most organic matter occurs as particulate organic matter (POM), as highlighted by the studies by Forte and others (2006) and Piazzi (2005), which is a further guarantee that DOC production is limited. Additional causes of possible Rh underestimation include human-mediated inputs to soil of exogenous organic matter (Chiti and others 2009) and organic matter translocation through the soil profile, such as in podzols (Rumpel and others 2004). Actually, pedogenic processes may substantially affect the TT of SOM, as demonstrated by Trumbore (1993) and Bol and others (1999) from the investigation of contrasting soil types. Finally, bias in the radiocarbon values of SOC could arise from the acid pretreatment of the samples, which might selectively remove some SOC (Brodie and others 2011; Schlacher and Connolly 2014), and from the use of a one-pool C model instead of a multi-pool C model (Gaudinski and others 2000) due to the heterogeneity of SOM.

Soil Heterotrophic Efflux and Contribution at Ecosystem Level

Data on the aboveground and belowground C inputs and C outputs from soil via heterotrophic respiration allowed us to assess whether a soil is a C sink or source. At IT-Cpz, where the total C input to the soil from the aboveground and belowground biomass was 5.1 ± 1.8 Mg C ha^{-1} y^{-1} (Tirone and others 2003) and the ^{14}C-based Rh we determined was 3.1 Mg C ha^{-1} y^{-1}, we concluded that the soil was actually a C sink, accumulating 2.0 ± 1.6 Mg C ha^{-1} y^{-1}. Unfortunately, at IT-SRo only the C input from aboveground biomass—6.2 ± 1.5 Mg C ha^{-1} y^{-1} (Carsten Gruening, personal communication)—was available. Nevertheless, even neglecting the belowground C inputs and considering an Rh of 3.22 Mg C ha^{-1} y^{-1}, the soil is a C sink, accumulating 3.0 Mg C ha^{-1} y^{-1}. The sink can be much higher depending on the amount of belowground C inputs. On the other hand, the C sink behavior of both soils is consistent with the results of a modeling study previously performed in the same stands by Chiti and others (2010).

Concerning the whole forest ecosystem, the role of both forests as carbon sinks was evident on the basis of their NEP measured by the eddy covariance technique. Via Rh determination, we discriminated the contribution of soil from that of vegetation in total C sequestration. Considering the NEP measured by eddy covariance in 2004 (4.28 and 6.04 Mg C ha^{-1} y^{-1} in pine and oak, respectively) and the annually sequestered SOC, soil accounted for 70 and 31% of the total C sink at the ecosystem level in the pine and oak forests, respectively. The importance of determining the heterotrophic flux of C from the soil becomes even clearer when looking at the magnitude of the C fluxes at the ecosystem level. In the year of our analyses (2004), ecosystem respiration (Reco) measured by eddy covariance through partitioning was 12.1 Mg C ha^{-1} y^{-1} in the pine stand at IT-SRo and 13.6 Mg C ha^{-1} y^{-1} in the oak forest at IT-Cpz. Hence, according to our findings, 27 and 23% of Reco was due to soil Rh, which is in line with the results of Unger and others (2009) in oak-savanna-like woodlands with *Quercus ilex* and *Quercus suber* in Portugal (Mediterranean climate).

The similarity in soil Rh contribution to total ecosystem respiration at the two study sites is supported by ^{13}C NMR investigation on SOM, which did not reveal any major qualitative differences that can be related to the different vegetation types (see Supplementary Material).

Finally, the C stock down to 1 m in the soil of the pine forest at IT-SRo was consistent with the average reported by Chiti and others (2012) for conifer forests in the Mediterranean area, approximately 75 Mg C ha^{-1}. The same is true for the oak stand at IT-Cpz, where the SOC stock in the upper meter was within the range of Mediterranean evergreen broadleaf forests, which clusters at approximately 60 Mg C ha^{-1} (Chiti and others 2012; Tedeschi and others 2006). Such a matching supports our assumption that no major DOC losses were affecting the studied soils, which was necessary for relying on the box model.

Conclusions

Measuring ^{14}C in bulk soil from various depth intervals allowed us to reliably determine the heterotrophic component of soil respiration in both forests. This method also enabled us to identify the soil compartment that is significantly contributing to heterotrophic soil respiration and to disentangle the sink or source behavior of the studied soils. The method is time effective because it requires a single soil sampling and no monitoring activity. In combination with eddy covariance and biometric data, it may largely improve our understanding of the role of soils in the global C cycle and how they respond to climate change. In addition, information on the vertical distribution of Rh processes can yield important recommendations for the monitoring of climatic site variables, such as soil temperature and soil water content, that are important when modeling soil fluxes.

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REFERENCES

Albanito F, McAllister JL, Cescatti A, Smith P, Robinson D. 2012. Dual-chamber measurements of $\delta^{13}C$ of soil-respired CO_2 partitioned using a field-based three end-member model. Soil Biol Biochem 47:106–15.

Aubinet M, Vesala T, Papale D. 2012. Eddy covariance—a practical guide to measurement and data analysis. Berlin: Springer. ISBN 978-94-007-2351-1

Aubinet M, Grelle A, Ibrom A, Rannik U, Moncrieff J, Foken T et al. 2000. Estimates of the annual net carbon and water exchange of European forests: the EUROFLUX methodology. Adv Ecol Res 30:113–75.

Baabst F, Bouriaud O, Papale D, Gielen B, Janssens IA, Nikinmaa E, Ibrom A, Wu J, Bernhofer C, Köstner B, Grünwald T, Seufert G, Ciais P, Frank D. 2014. Above-ground woody carbon sequestration measured from tree rings is coherent with net ecosystem productivity at five eddy-covariance sites. New Phytol 201:1289–303.

Bernal S, Butturini A, Sabater F. 2002. Variability of DOC and nitrate responses to storms in a small Mediterranean forested catchment. Hydrol Earth Syst Sci 6:1031–41.

Bol RA, Harkness DD, Huang Y, Howard DM. 1999. The influence of soil processes on carbon isotope distribution and turnover in the British uplands. Eur J Soil Sci 50:41–51.

Brodie CR, Casford JSL, Lloyd JM, Leng MJ, Heaton THE, Kendrick CP, Yongqiang Z. 2011. Evidence for bias in C/N, δ13C and δ15N values of bulk organic matter, and on environmental interpretation, from a lake sedimentary sequence by pre-analysis acid treatment methods. Quat Sci Rev 30:3076–87.

Chiesi M, Maselli F, Bindi M, Fibbi L, Cherubini P, Arlotta E, Tirone G, Matteucci G, Seufert G. 2005. Modelling carbon budget of Mediterranean forests using ground and remote sensing measurements. Agric For Meteorol 135:22–34.

Chirici G, Barbati A, Maselli F. 2007. Modelling of Italian net primary productivity by the integration of remotely sensed and GIS data. For Ecol Manag 246:285–95.

Chiti T, Díaz-Pines E, Rubio A. 2012. Soil organic carbon stocks of conifers, broadleaf and evergreen forests of Spain. Biol Fertil Soils 48:817–26.

Chiti T, Neubert REM, Jansenss IA, Yuste JC, Sirignano C, Certini G. 2011. Radiocarbon based assessment of soil organic carbon contribution to soil respiration in a pine stand of the Campine region, Belgium. Plant Soil 344:273–82.

Chiti T, Papale D, Smith P, Dalmonech D, Matteucci G, Yeluripaty J, Rodeghiero M, Valentini R. 2010. Predicting changes in soil organic carbon in Mediterranean and Alpine forests during the Kyoto Protocol commitment periods using the Century model. Soil Use Manag 26:475–84.

Chiti T, Neubert REM, Janssens IA, Certini G, Curiel Yuste J, Sirignano C. 2009. Radiocarbon dating reveals different past management of two adjacent forest soils in the Campine region, Belgium. Geoderma 149:137–42.

Davidson EA, Janssens IA. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–73.

Forte C, Piazzi A, Pizzanelli S, Certini G. 2006. CP MAS ^{13}C spectral editing and relative quantitation of a bulk soil sample. Solid State Nucl Magn Reson 30:81–8.

Garbulsky MF, Peñuelas J, Papale D, Filella I. 2008. Remote estimation of carbon dioxide uptake by a Mediterranean forest. Glob Change Biol 14:2860–7.

Gaudinski JB, Trumbore SE, Davidson EA, Zheng SH. 2000. Soil carbon cycling in a temperate forest: radiocarbon based estimates of residence times, sequestration rates and partitioning of fluxes. Biogeochemistry 51:33–69.

Gellini R, Pantani F, Grossoni P, Bussotti F, Barbolani E, Rinallo C. 1983. Survey of the deterioration of the coastal vegetation in the park of San Rossore in central Italy. Eur J For Pathol 13:296–304.

Giardina CP, Ryan MG. 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. Nature 404:858–61.

Hagedorn F, Schleppi P, Peter W, Hannes F. 2000. Export of dissolved organic carbon and nitrogen from Gleysol dominated catchments-the significance of water flow paths. Biogeochemistry 50:137–61.

Hakkenberg R, Churkina G, Rodeghiero M, Börner A, Steinhof A, Cescatti A. 2008. Temperature sensitivity of turnover times of soil organic matter in forests. Ecol Appl 18:119–31.

Hanson PJ, Edwards NT, Garten CT, Andrews JA. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48:115–46.

Harrison AF, Harkness DD, Rowland AP, Garnett JS, Bacon PJ. 2000. Annual carbon and nitrogen fluxes in soil along the European forest transect, determined using ^{14}C-Bomb. In: Schulze ED, Ed. Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies. Berlin: Springer. p 237–55.

Harrison KG. 1996. Using bulk soil radiocarbon measurements to estimate soil carbon turnover times: implications for atmospheric CO_2 levels. Radiocarbon 38:181–90.

Hua Q, Barbetti M, Rakowski A. 2013. Atmospheric radiocarbon for the period 1950-2010. Radiocarbon 55:2059–72.

Inglima I, Alberti G, Bertolini T, Vaccari FP, Gioli B, Miglietta F, Cotrufo MF, Peressotti A. 2009. Precipitation pulses enhance respiration of Mediterranean ecosystems: the balance between organic and inorganic components of increased soil CO_2 efflux. Glob Change Biol 15:1289–301.

IPCC. 2013. Climate change 2013: the physical science basis. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, Eds. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press. p 953–1015.

IUSS Working Group WRB. 2014. World Reference Base for Soil Resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports, Vol. 106Rome: FAO.

Jones C, McConnell C, Coleman K, Cox P, Falloon P, Jenkinson D, Powlson D. 2005. Global climate change and soil carbon stocks; predictions from two contrasting models for the turnover of organic carbon in soil. Glob Change Biol 11:154–66.

Karhu K, Fritze H, Hämäläinen K, Vanhala P, Jungner H, Oinonen M, Sonninen E, Tuomi M, Spetz P, Kitunen V, Liski J. 2010. Temperature sensitivity of soil carbon fractions in boreal forest soil. Ecology 91:370–6.

Kurz C, Coùteaux MM, Thiéry JM. 2000. Residence time and decomposition rate of *Pinus pinaster* needles in a forest floor from direct field measurements under a Mediterranean climate. Biogeochemistry 32:1197–206.

Levin I, Hesshaimer V. 2000. Radiocarbon: a unique tracer of global carbon cycle dynamics. Radiocarbon 42:69–80.

Lovett GM, Cole JJ, Pace ML. 2006. Is net ecosystem production equal to ecosystem carbon accumulation? Ecosystems 9:1–4.

Luyssaert S, Inglima I, Jung M et al. 2007. CO_2 balance of boreal, temperate, and tropical forests derived from a global database. Glob Change Biol 13:2509–37.

Manes F, Grignetti A, Tinelli A, Lenz R, Ciccioli P. 1997. General features of the Castelporziano test site. Atmos Environ 31:1352–2310.

Marín-Spiotta E, Swanston CW, Torn MS, Silver WL, Burton SD. 2008. Chemical and mineral control of soil carbon turnover in abandoned tropical pastures. Geoderma 143:49–62.

McFarlane KJ, Torn MS, Hanson PJ, Porras RC, Swanston CW, Callaham MA Jr, Guilderson TP. 2013. Comparison of soil organic matter dynamics at five temperate deciduous forests with physical fractionation and radiocarbon measurements. Biogeochemistry 112:457–76.

Meijer HAJ, van der Plicht J, Gislefloss JS, Nydal R. 1994. Comparing long-term atmospheric ^{14}C and ^{3}H records near Groningen, the Netherlands with Fruholmen, Norway and Izaña, Canary Islands ^{14}C stations. Radiocarbon 37:39–50.

Meyer JL, Tate CM. 1983. The effects of watershed disturbance on dissolved organic carbon dynamics of a stream. Ecology 64:33–44.

Mikutta R, Kleber M, Torn M, Jahn R. 2006. Stabilization of soil organic matter: association with minerals or chemical recalcitrance? Biogeochemistry 77:25–56.

Munir TM, Xu B, Perkins M. 2014. Responses of carbon dioxide flux and plant biomass to water table drawdown in a treed peatland in Northern Alberta: a climate change perspective. Biogeosciences 11:807–20.

Papale D, Reichstein M, Aubinet M, Canfora E, Bernhofer C, Longdoz B, Kutsch W, Rambal S, Valentini R, Vesala T, Yakir D. 2006. Towards a standardized processing of Net Ecosystem Exchange measured with eddy covariance technique: algorithms and uncertainty estimation. Biogeosciences 3:571–83.

Pardini G, Gispert M, Dunjo G. 2003. Runoff erosion and nutrient depletion in five Mediterranean soils of NE Spain under different land use. Sci Total Environ 309:213–24.

Phillips CL, McFarlane KJ, Risk D, Desai AR. 2013. Biological and physical influences on soil $^{14}CO_2$ seasonal dynamics in a temperate hardwood forest. Biogeosciences 10:7999–8012.

Piazzi A. 2005. Studio della sostanza organica in suoli mediterranei mediante tecniche di Risonanza Magnetica Nucleare allo stato solido. Master thesis at the University of Pisa, Italy. https://etd.adm.unipi.it/theses/available/etd-06292005-120844/

Poirier N, Derenne S, Balesdent J, Mariotti A, Massiot D, Largeau C. 2003. Isolation and analysis of the non-hydrolysable fraction of a forest soil and an arable soil (Lacadé, southwest France). Eur J Soil Sci 54:243–55.

Quideau SA, Chadwick OA, Trumbore SE, Johnson-Maynard JL, Graham RC, Anderson MA. 2001. Vegetation control on soil organic matter dynamics. Org Geochem 32:247–52.

Reichstein M, Falge E, Baldocchi D et al. 2005. On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. Glob Change Biol 11:1424–39.

Rey A, Pegoraro E, Tedeschi V, De Parri I, Jarvis PG, Valentini R. 2002. Annual variation in soil respiration and its components in a coppie oak forest in Central Italy. Glob Change Biol 8:851–66.

Rey A, Petsikos C, Grace J, Jarvis PG. 2005. The effect of soil temperature and soil moisture on carbon mineralisation rates in a Mediterranean forest soil. Eur J Soil Sci 56:589–99.

Rodeghiero M, Churkina G, Martinez C, Scholten T, Gianelle D, Cescatti A. 2013. Components of forest soil CO_2 efflux estimated from Δ^{14}C values of soil organic matter. Plant Soil 364:55–68.

Rumpel C, Eusterhues K, Kögel-Knabner I. 2004. Location and chemical composition of stabilized organic carbon in topsoil and subsoil horizons of two acid forest soils. Soil Biol Biochem 36:177–90.

Sanderman J, Baldock J, Amundson R. 2008. Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. Biogeochemistry 89:181–98.

Sanderman J, Amundson R. 2009. A comparative study of dissolved organic carbon transport and stabilization in California forest and grassland soils. Biogeochemistry 92:41–59.

Schlacher TA, Connolly RM. 2014. Effects of acid treatment on carbon and nitrogen stable isotope ratios in ecological samples: a review and synthesis. Methods Ecol Evol 5:541–50.

Schuur EAG, Trumbore SE. 2006. Partitioning sources of soil respiration in boreal black spruce forest using radiocarbon. Glob Change Biol 12:165–76.

Simpson AJ, McNally DJ, Simpson MJ. 2011. NMR spectroscopy in environmental research: from molecular interactions to global processes. Prog Nucl Magn Reson Spectrosc 58:97–175.

Southon J, Santos GM, Druffel-Rodriguez K, Druffel E, Trumbore S, Xu X, Griffin S, Ali S, Mazon M. 2004. The Keck Carbon Cycle AMS Laboratory, University of California, Irvine: two installations, initial operation, and a background surprise. Radiocarbon 46:33–9.

Steinhof A, Adamiek G, Gleixner G, Van Klinken GJ, Wagner T. 2004. The new C-14 analyses laboratory in Jena, Germany. Radiocarbon 46:51–8.

Stuiver M, Polach H. 1977. Reporting of ^{14}C data. Radiocarbon 19:355–63.

Subke JA, Inglima I, Cotrufo F. 2006. Trends and methodological impacts in soil CO_2 efflux partitioning: a metaanalytical review. Glob Change Biol 12:921–43.

Tedeschi V, Rey A, Manca G, Valentini R, Jarvis PG, Borghetti M. 2006. Soil respiration in a Mediterranean oak forest at different development stages after coppicing. Glob Change Biol 12:110–21.

Tirone G, Dore S, Matteucci G, Greco S, Valentini R. 2003. Evergreen mediterranean forests: carbon and water fluxes, balances, ecological and ecophysiological determinants. In: Valentini R, Ed. Fluxes of carbon, water and energy of European forest. Ecological Studies 163. Berlin: Springer. p 125–50.

Tirone G. 2002. Stima del bilancio del carbonio di due ecosistemi forestali Mediterranei. PhD dissertation. Università della Tuscia, Viterbo, Italy.

Trumbore SE. 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecol Appl 10:99–411.

Trumbore SE. 1993. Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements. Glob Biogeochem Cycles 7:275–90.

Unger S, Máguas C, Pereira JS, Aires LM, David TS, Werner C. 2009. Partitioning carbon fluxes in a Mediterranean oak forest to disentangle changes in ecosystem sink strength during drought. Agric For Meteorol 149:949–61.

van Hees PAW, Jones DL, Finlay R, Godbold DL, Lundström US. 2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biol Biochem 37:1–13.

Verardo DJ, Froelich PN, McIntyre A. 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 Analyzer. Deep Sea Res 37:157–65.

Wagai R, Mayer LM, Kitayama K. 2009. Nature of the ''occluded'' low-density fraction in soil organic matter studies: a critical review. Soil Sci Plant Nutr 55:13–25.

Wang Y, Amundson R, Niu XF. 2000. Seasonal and altitudinal variation in decomposition of soil organic matter inferred from radiocarbon measurements of soil CO_2 flux. Glob Biogeochem Cycle 14:199–211.