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Separation of soil respiration; a site-specific comparison of partition methods

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Abstract. Without accurate data on soil heterotrophic respiration (Rh), assessments of soil carbon (C) sequestration rate and C balance are challenging to produce. Accordingly, it is essential to determine the contribution of the different sources of the total soil CO_2 efflux (Rs) in different ecosystems, but to date, there are still many uncertainties and unknown regarding the soil respiration partitioning procedures currently available. This study compared the suitability and accuracy of five different Rh/Rs partitioning methods in a subtropical forest: (1) regression between root mass and root derived CO_2; (2) root exclusion bags with intact soil blocks; (3) root exclusion bags with hand-sorted roots; (4) lab incubations with minimally disturbed soil microcosm cores; and (5) soil $\delta^{13}C$-CO_2 natural abundance. The relationship between Rh and soil moisture and temperature was also investigated. A qualitative evaluation table of the partition methods with five performance parameters was produced. The Rs was measured weekly from February 3rd to April 19th 2017 and found to average 6.1 ±0.3 Mg C ha^{-1} y^{-1}. During this period, the Rh measured with the in-situ mesh bags with intact soil blocks and hand-sorted roots were estimated to contribute 49 ±7% and 79 ±3% of Rs respectively. The Rh percentage estimated with the root mass regression, microcosm incubation and $\delta^{13}C$-CO_2 natural abundance were 54 ±41%, 8-17% and 61 ±39% respectively. Overall, no systematically superior or inferior Rh/Rs partition method was found. The paper discusses the strengths and weaknesses of each technique with the conclusion that combining two or more methods optimizes Rh assessment reliability.

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1 Introduction

During the 2016 Convention of Parties (COP21) of the United Nations Framework Convention on Climate Change (UNFCCC) in Paris, the goal of increasing global soil organic carbon (SOC) stocks by 0.4 percent per year was set, with the aim of mitigating global anthropogenic greenhouse gas emissions (Minasny et al., 2017). This ambitious target was set based on the concept that the SOC in the top soil layer is sensitive and responsive to management changes and may offer opportunities to mitigate the current increases in atmospheric CO_2 concentration (McConkey et al., 2007). Of the carbon (C) that enters into ecosystems via photosynthesis, a fraction is directly respired by the roots and above ground plant parts (autotrophic respiration) to produce energy (i.e. adenosine-5'-triphosphate), with the other fraction synthesized into organic molecules. Some of these C-containing compounds are harvested or consumed by herbivores and the remainder is added to the soil as plant residues (Janzen et al., 1998). Subsequently, a portion of these fresh organic compounds are respired by organisms (heterotrophic respiration) and the other portion is converted into SOC by the genesis of soil organic matter (SOM) (Janzen, 2006; Lal, 2005). If the amount of new organic residues added to the soil is greater than the C lost by SOC decomposition, SOC content increases (Ellert and Bettany, 1995).

Typically, many years (up to decades) are needed to assess SOC stock changes over time in order to evaluate which management practices are beneficial for SOC sequestration (Harmon et al., 2011; Wood et al., 2012). This timeframe is impractical for policy makers to evaluate the mitigation potential of different land management practices, in particular with the pressing need of the UNFCCC goal of increasing the global SOC stocks by 0.4 percent per year. An alternative approach that allows a more rapid evaluation of these long term impacts is to combine the SOC stock change procedure (e.g. VandenBygaart et al., 2008) with the soil C efflux balance approach (i.e. Hergoualc'h and Verchot, 2011), which although demanding and with some uncertainties can provide results on soil dynamics over an annual basis. The soil C efflux balance approach involves calculating the rate of C entry and exit in the soil. However, the total CO_2 efflux (Rs) from soil does not provide the necessary information to estimate whether the soil is a net source or net sink for atmospheric CO_2 (Kuzyakov and Larionova, 2005). Total soil efflux is a combination of root based respiration (autotrophic (Ra)) and heterotrophic respiration (Rh). Autotrophic respiration does not contribute to net C losses to the atmosphere as it is cycled within the ecosystem, whereas Rh represent net C losses. However, the boundary between Ra and Rh is not easy to distinguish (i.e. the rhizo-microbial respiration is linked to both) and realistic Rh assessments are difficult to produce (Braig and Tupek, 2010).

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Review of Rh-Rs segregation methods have been made (e.g. Kuzyakov, 2006) but no site specific study has been made analysing several different partition techniques simultaneously. The goal of our study was to compare five different partitioning methods to separate CO_2 efflux into its Rs and Rh component in a subtropical secondary forest in Hong Kong. The influence of soil moisture and temperature on CO_2 efflux was also analyzed.

2 Methodology

The research was conducted in a subtropical secondary forest of Hong Kong (Tai Po Kau Nature Reserve; 22° 27´N, 114° 11´E). The landscape is typical of the escarpment of the Tai Mo Shan mountain range, the system formed by volcanic activities in the Late Jurassic epoch (Langford et al., 1989). The rocks are mainly rhyodacite to rhyolite from the Tsuen Wan Volcanic Group (Davis et al., 1997). The study site was approximatively 600 m. above sea level and the slope surfaces were stable and vegetated. The forest was approximatively 50 years old and was covered with continuous canopy. More than 100 plant species were registered in the Nature Reserve. The following genera were surveyed in the study area: *Machilus sp, Meliosma sp, Garcinia sp, Engelhardia sp, Psychotria sp, Ilex sp, Eurya sp and Lithocarpus sp* (Tong, 2015). The mean annual temperature was 23.3°C and annual precipitation 2400 mm with a hot-humid season (April–September) and a cool-dry season (October–March) (Hong Kong Observatory). The study area was 0.5 ha and was located inside a long-term research site belonging to the Chinese University of Hong Kong. The canopy was closed in the area with an average solar radiation at 2 m high of 13.8 W/m² (non-published data).

2.1 Root exclusion bag methods

To partition the CO_2 efflux in-situ into Rs and Rh using mesh bags, two different approaches were followed: 1) the traditional dug soil with hand-sorted root removal and refilling method (HS) (Fenn et al., 2010; Hinko-Najera, 2015) and 2) a variant of it with intact soil blocks (IB). The HS method consisted of digging a pit for each bag with a size matching the bag dimensions (20 × 20 cm, depth: 25 cm) where the soil is excavated in layers (to maintain soil horizons) and visible roots are removed before repacking the bag inside the pit with the removed soil. The IB variant of this technique consisted in extracting a cube as intact as possible from the soil (20 × 20 cm, depth: 25 cm). Then, tightly placing the soil block into the micromesh bag and inserting it back into its original pit. For both methods, the same type of micromesh bags (38µm nylon mesh), closed at the bottom but open at the top were used. This mesh size was used to impede roots from entering inside the bags, but allowed mycorrhiza to penetrate (Moyano et al., 2007). Collars

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measuring 10 cm diameter were installed on the soil in the center of each bag to a depth of 8 cm, for heterotrophic emissions sampling.

Seven plots were randomly distributed inside the study area. In each plot, an IB bag was paired with a HS bag with space of 150 cm between them. The root exclusion bags were installed during the month of October 2016 and were let to stabilize for three months. At 1 m distance from each root exclusion bag, a collar was inserted into non-disturbed soil to measure Rs. To assess Rs and Rh without the influence of litterfall decomposition, the collars were cleared of leaves and flowers on a weekly basis.

From February 3rd to April 19th 2017 the collars were measured weekly with an IRGA (Environmental Gas Monitor, EGM-4, PP Systems, UK) attached to a soil respiration chamber (SRC-1, PP Systems, UK). Soil temperature and soil moisture were measured in the area located between the collar and the edge of the bag (to 10cm depth, HH2, Delta-T Devices, Cambridge- England). At the end of the study all the root exclusion bags were removed from the soil and inspected to ensure that no root had penetrated inside. The soil inside the measurement collars was then collected to assess bulk density (van Reeuwijk, 1992). Mathematical calculation and descriptive statistical analyses were done with Microsoft Excel XP®.

2.2 Root and carbon dioxide efflux regression method

The regression technique is based on the relationship between the CO_2 emitted by the root-rhizosphere and root biomass and the CO_2 efflux derived from SOM decomposition (i.e. Rh), corresponding to the intercept of the linear regression line (Kucera and Kirkham, 1971). This method was made following Farmer (2013) with 22 sampling spots. Each spot was a square of 20 x 20 cm randomly distributed in the study area. In each spot, Rs was determined per triplicate using a portable IRGA as described above. Concurrently with CO_2 efflux measurements, air and soil (10 cm depth) temperatures and soil volumetric moisture content were measured at each sampling spot. Immediately after the Rs measurement, the 20 x 20 cm squares were excavated to 25 cm depth. All the visible roots (diameter larger than 0.1 cm) from the excavated soil were collected. In the lab, the roots were washed and then oven dried at 60°C until a steady dry weight was attained, which was then recorded. A linear regression report between root quantity and CO_2 efflux was performed using the program R Foundation for Statistical Computing version 2.8.1 (R Development Core Team, 2008).

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2.3 Lab incubations

For the lab incubations, undisturbed soil cores of volume 98 cm^3 (inner diameter 5 cm, height 5 cm) were collected using a stainless-steel core soil sampler from the upper part of the soil profile (0–5 cm). In the study area, four groups of four soil cores were collected then pooled per group and brought to the lab. Subsequently all visible roots were removed but with special care to not destroy the small aggregates. The soil was then repacked to original bulk density in minimally disturbed soil microcosm cores of 45 cm^3 (inner diameter 3.5 cm, height 5 cm). The soil cores were separated in four groups of different volumetric moisture content (i.e. 15, 25, 35 and 45). These moisture levels corresponded to the natural annual fluctuation in the field (i.e. from dry to moist season) (Cui and Lai, 2016). After moisturizing the samples, each individual soil core was placed into a hermetically sealed 2.9 dm^3 plastic container. The experiment lasted four weeks and had four different incubation temperature levels (one per week; 14°C, 20°C, 26°C and 32°C) corresponding to the minimum, intermediate and maximum soil temperature values in the field based on preliminary studies (Cui and Lai, 2016). At the beginning of each week, the soil cores were pre-incubated in their incubation box to their corresponding weekly temperature (i.e week #1, 14°C … week #4, 32°C) for 3 days and then opened and vented for one minute. From all the boxes gas samples were collected (20 ml) with an air-tight syringe (t= 0, 24, 72 hour) after box closure. The CO_2 concentrations were analyzed within 48 hours with a gas chromatograph (GC system 7890A, Agilent Technologies). The GC system was equipped with a flame ionization detector and an electron capture detector to quantify and CO_2. Between each measurement session, the boxes opened to vent and the moisture of the soil cores was re-adjusted if needed.

Gaussian 3D regression fitted curve was derived as shown in equation 1. using SigmaPlot version 10.0 (Systat Software, San Jose, CA).

$$f(x,y) = a \times \exp\left[-0.5 \times \left(\frac{x-xo}{b}\right)^2 + \left(\frac{y-yo}{c}\right)^2\right] \quad (1)$$

where a, b and c are constant coefficients; x is the soil temperature (ºC); y is the soil moisture content (%); x_0 is the average temperature; y_0 is the average soil moisture.

2.4 $\delta^{13}C$ natural abundance method

Millard et al. (2010) have demonstrated that the natural abundance $\delta^{13}C$ (‰) of Rs falls between the $\delta^{13}C$ values of the Rh and Ra. The $\delta^{13}C$ of Rs/Rh respiration was determined following Lin et al. (1999) and Millard et al. (2010). The

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isotopic partitioning experiment assessed values of the $\delta^{13}C$ of the Rs, Ra and Rh. The sampling took place on March 15th 2017. A closed chamber (10 cm diameter, 10 cm high) was positioned on each emissions measurement collar (described in section 2.1). The chambers were flushed for 2 minutes with CO_2-free air to remove all the atmospheric air trapped within the headspace. Chambers were left to incubate for 40 minutes to ensure the concentration of the chamber sample reached above 400 ppm of CO_2 from which a duplicate sample of the gas in the chamber headspace were extracted into evacuated vials to give the $\delta^{13}C$ of the Rs. Subsequently, the soil under the chamber was dug and immediately brought to the lab (less than 30 minutes travel) where the soil and the roots were carefully separated. The roots were gently washed with water to remove adhered soil aggregates and slightly dried afterward with paper towels. Samples of 5 g of root and 10 g of root-free soil per chamber were incubated in CO_2 free air in 250 ml airtight glass bottles to give the $\delta^{13}C$ of the Ra and Rh respectively. The bottles were left to incubate for 90 minutes before duplicate extraction into evacuated vials. As recommended by Midwood et al., (2006), previous to gas sample extraction, the butyl rubber septa used to seal the vials were heated at 105°C for 12 h. The C isotope ratio of the CO_2 in all samples was analyzed using a Gas-bench II connected to a DeltaPlus Advantage isotope ratio mass spectrometer (both Thermo Finnigan, Bremen, Germany) at the James Hutton Institute Scotland UK. The $\delta^{13}C$ ratios, all expressed relative to Vienna-Pee-Dee Belemnite (VPDB), was calculated with respect to CO_2 reference gases injected with every sample and traceable to International Atomic Energy Agency reference material NBS 19 TS-Limestone. Measurement of the individual signatures of the natural abundance $\delta^{13}C$ of the Rs, Rh and Ra allowed partitioning between the different sources using the mass balance mixing model (Lin et al., 1999; Millard et al., 2010):

$$\%Rh = \frac{\delta Rs - \delta Rh}{\delta Ra - \delta Rh} \text{ X } 100 \quad (2)$$

where *%Rh* is the proportion of Rh from Rs, and *δRs*, *δRh* and *δRa* are the $\delta^{13}C$ isotopic signatures.

2.5 Soil general characterization

Four soil profiles were dug in the study area, characterizing the different landforms present at the site. Morphological description was done according to Jahn (2006) and the soil was classified with the World Reference Base (IUSS-Working-Group-WRB, 2014). Soil pH was determined with a glass–calomel electrode pH meter (McLean, 1982). Rainfall and air temperature were recorded hourly with a HOBO Weather station (rain gauge, S-RGB-M002; air temperature/RH, sensor S-THB-M008, Onset Computer Corp., USA). Water holding capacity was assessed by

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saturating the soils, allowing them to freely drain for 24 h and determining gravimetric water content after oven-drying at 105 ºC following Arcand et al. (2016). Root biomass was measured by collecting soil cores (inner diameter 5 cm, height 5 cm) and determined using the approach of Tufekcioglu et al. (1999). The soil was dried, finely ground, and subsequently analyzed for total C and N content using a CNS Analyzer System (Perkin Elmer 2400 Series II CHNS/O Analyzer, USA).

2.6 Qualitative comparison of segregation methods

Isotopic partitioning methods are recognized as a more accurate approach to segregation of Rh/Rs than non-isotopic techniques (Paterson et al., 2009; Kuzyakov, 2006). Therefore, the soil $\delta^{13}C$ natural abundance method was used as reference point for segregation accuracy. Partition methods that had Rh%: <10, 10-20 and >20 lower or larger than the $\delta^{13}C$-CO_2 natural abundance were categorized as high, intermediate and low accuracy, respectively. The level of precision of the segregation methods was determined with the statistical variance associated with the Rh/Rs averages. High, intermediate and low precision were attributed to Rh% standard errors of <10, 10-20 and >20, respectively. The level of complexity was evaluated with the number of steps required to complete each method. For example, the hand-sorted root exclusion bags technique was judged as a four steps method (pit excavation, root removal, bag/pit refiling, and CO_2 efflux measurements). Methods with five steps or less were deemed simple and six steps or more deemed as complex. The time inversion needed to set up the experiment was assessed by counting the number of working hours (eight hours equal one day) required prior to the start of the measurements. The time inversion needed to produce seasonal trends was the number of months of measurements (in the field or in the lab) required to characterize the Rh at the different temperature and moisture levels of the year.

3 Results

3.1 Soil characteristics

According to their morphology and diagnostic properties, the soil was classified as Alic Umbrisol (Nechic) and Haplic Alisol (Nechic) (IUSS-Working-Group-WRB, 2014). The difference between the two soil groups was the thickness of humus-containing horizon (between 20 and 30 cm for the Umbrisol; while, 10 to 20 cm for the Alisol). The A horizon had high organic C content (3.2 ±0.2%) and high acidity (pH_{H2O} 4.2) (Table 1). The sub-superficial soil was represented by clayey yellow-colored profiles with an argic horizon. Soil texture was heavier in the argic horizon than in the topsoil and parent material. The structure in all the soil profiles was predominantly granular in the upper

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horizons, whereas the argic horizon was characterized by subangular blocky structure (Table 1). The argic horizon was deemed to be of high-activity clays and low cation base status based to previous results in the area (Tong, 2015), along with soil acidity, type parent material and level of mineralization of the rock in the soil pits.

3.2 Environmental parameters and root exclusion bag methods

During the root exclusion bags measurements period (Feb-Apr 2017), the average air temperature was 16ºC and the total rainfall 107 mm and the Rs averaged 6.1 Mg C ha^{-1} y^{-1} (Fig. 1). One of the requirements for the suitability of root exclusion bag methods to estimate Rh is that soil bulk density, soil temperature and moisture are statistically equal inside and outside of the bags. In this experiment, no significant differences were detected regarding the bulk density and soil temperature (p=0.87 and p=0.15, respectively) but the volumetric soil moisture in the HS bags was on average 17% lower than outside the root exclusion bags (p=0.04) (Table 2). As would be expected, all Rh IB and Rh HS efflux rates were lower than the Rs efflux at each measurement date. Throughout the experiment, the Rh IB was repetitively lower than the Rh HS except on March 31st (Fig. 1b).

3.3 Root regression and lab incubation

The 22 quadrats used for the root regression assessment yielded average Rs of 0.46 ±0.04 g CO_2 m^2 h^{-1}. The regression of the CO_2 efflux against root density produced a statistically significant slope correlation of 0.08 ±0.04 g CO_2 m^2 h^{-1} per mg root cm^{-3} (p=0.03), and set the intercept at 0.25 ±0.10 g CO_2 m^2 h^{-1} (p=0.02) which represented the basal efflux in absence of root i.e. the Rh (Fig. 2 and Table 3). The Rs measured when the root regression technique was performed (October 2016) was 11.1 ±1 Mg C ha^{-1} y^{-1} (Table 6), equivalent to 54% of the Rs.

During the incubation with minimally disturbed soil microcosms, the average CO_2 efflux at 14, 20, 26 and 32ºC was 0.0151 ±0.021, 0.0282 ±0.016, 0.0585 ±0.038 and 0.0938 ±0.058 g CO_2 m^2 h^{-1}, respectively (Fig. 3). The exponential relationship between CO_2 efflux, soil temperature and moisture is presented in Table 4.

3.4 Soil $\delta^{13}C$-CO_2 natural abundance

The $\delta^{13}C$-CO_2 natural abundance determination satisfactorily segregated the three respiration components (Table 5). The fact that the $\delta^{13}C$-CO_2 of the Rh HS, Rh IB and Rh lab were in a very close range indicated that in the field the efflux measured in the root exclusion bags were not contaminated with root respiration. Based on the $\delta^{13}C$-CO_2 of the Rs, the Rh lab and the Ra lab the percentage of heterotrophic respiration was 61 ±39% (Table 6). The notably large

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standard error of the percentage of heterotrophic respiration was due to the large variance in the $\delta^{13}C\text{-}CO_2$ of the three respiration components.

Comparing with the Rh from the $\delta^{13}C\text{-}CO_2$ method, the root regression, lab incubation, hand-sorted and intact block (IB) root exclusion techniques were 11% below, 72-87% below, 30% above and 20% below, respectively (Table 6).

4 Discussion

4.1 Soil $\delta^{13}C$ natural abundance method

The three respiration components of this method (i.e. $\delta^{13}C\text{-}CO_2$ from Rs, Rh and Ra) had large standard errors (Table 5) that produced a high uncertainty value in the Rh/Rs assessment (61 ±39 %, Table 6). This method was accordingly deemed of low precision (Table 7). This, in turn, impeded to produce an Rh/Rs assessment in the individual collars. This large $\delta^{13}C\text{-}CO_2$ variance was likely caused by variability of $\delta^{13}C$ in soil and plants residues and also due to ^{13}C discrimination by plants that is affected by moisture content and nitrogen availability (Hogh-Jensen and Schjoerring, 1997). In addition, other studies reported the variability of $\delta^{13}C$ in soil or plants of at least 1–2‰, which in some cases can limit the capacity to produce soil respiration segregation assessments (Accoe et al., 2002; Cheng, 1996; Farquhar et al., 1989). Because soils are porous mediums, excluding any atmospheric CO_2 that has a different isotopic composition (i.e. $\delta^{13}C$ -7.5 to -8.5 ‰) to that of the Rs efflux is challenging and potential air contaminations have to be considering when analyzing the results (Millard et al., 2010). In our study, the Rh $\delta^{13}C$ was measured in the field (IB and HS; potentially air contaminated) and from airtight containers in lab incubations of root free soil (Rs lab; not potentially air contaminated). Both ways produced $\delta^{13}C$ in a close range and without statistical differences between them (Table 5). This indicates that the chamber system used in the field to collect the $\delta^{13}C$ efflux samples was adequately effective to prevent air contamination. Overall, the soil $\delta^{13}C$ natural abundance method was fast to setup but was relatively complex to perform with a field and lab component to be accomplished within a short period of time (Table 7).

4.2 Root exclusion bags methods

The HS and IB methods had %Rh of 79 ±3 and 49 ±7 %, respectively. These variances around the means (i.e. ±3 and ±7, respectively) were the lowest of all the field segregation methods tested (Table 6). Comparing the %Rh of the HS and IB with the $\delta^{13}C$ natural abundance technique, they resulted 18% above and 12% below, respectively. Thus the root exclusion bags methods were judged of intermediate accuracy and high precision. Also, the HS and IB methods were fast and simple to setup (Table 7).

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The micromesh size used in the root exclusion bags was 38µm which was reported to impede root penetration but to allow arbuscular mycorrhizal to spread inside the bags (Moyano et al., 2007; Rühr and Buchmann, 2010). In turn, Fenn et al. (2010) stated that in the mycorrhizal structures the arbuscules exist within roots, and therefore, the CO_2 efflux from these bags could contains some portions of the roots respiration. Contrary to this, the IB and HS air samples analyzed for $\delta^{13}C$ had an isotopic signature close and not statistically different from the gas samples collected in the lab airtight glass bottle of fresh soil without roots. This indicates that the root exclusion bags (both IB and HS) did not comprise traces of root respiration that had a significantly larger $\delta^{13}C$-CO_2 signature (Table 5). After the three months of soil stabilization period, both bag methods for partitioning total soil respiration and root-free soil respiration components successfully produced Rs>Rh in every sampling dates indicating that efflux rates within the bags had reached an apparent post disturbance equilibrium (Fig. 1). Also, in both IB and HS, soil temperature and bulk density were statistically equal to the surrounding soil (i.e. Rs) (Table 2). This indicates that the environmental conditions inside and outside of the bags were similar in respect to these two parameters. However, the soil moisture of the IB was statistically equal than the surrounding soil but for HS it was significantly lower. This was likely caused by the breakdown of the original soil structure at the moment of root removal that increased the drainage inside the HS bags. Moyano et al. (2007) also found that soil moisture can be affected by the hand-sorted root exclusion bag method. Overall, HS had a moisture level 20% lower and an Rh efflux 60% larger than IB (Table 3 and 6, respectively). Although not statistically significant, the HS and IB soil moisture parameter in the regression fit (i.e. y0, Table 4) showed that maximum Rh was when moisture content was relatively low (9.5 and 21.4%, respectively). Accordingly, this could partly explain the larger HS Rh efflux. In addition, the breakdown of numerous soil aggregates during the root removal likely allowed the soil microorganisms to thrive in previously encrusted SOM domains of the HS soil. It has been shown that the part of the SOM that is located in the interior of the soil aggregates is hardly accessible to microorganisms, and thus not easily mineralized unless the aggregates are shattered (Goebel et al., 2005).

4.3 Root and carbon dioxide efflux regression technique

As demonstrated by Gupta and Singh (1981) the intercept of the regression line between the independent variable (i.e. root biomass) and the dependent variable (i.e. Rs) corresponds to soil respiration in absence of root (i.e. Rh) (Fig. 2). In this study the regression had ten points (45%) outside the confidence interval but the intercept (0.25 ±0.10 g CO_2 m^2 h^{-1}) and slope (0.08 ±0.04 g CO_2 X mg root cm^3) were still statistically significant (P = 0.02 and 0.03, respectively) (Fig.

2, Table 4). These large coefficients of variance caused the largest standard error value in the Rh/Rs assessment (54 ±41 %, Table 6). The uncertainty in the regression fit was likely caused in large part by the older roots which are bulkier but respire less than fine and young roots (Behera et al., 1990). However, this method had the closest average Rh/Rs to the $\delta^{13}C$ natural abundance technique. Consequently the root regression technique was assessed as high accuracy and low precision (Table 7). Previous studies also highlighted large variation of CO_2 efflux and root biomass which causes relatively low coefficient of determinations (Behera et al., 1990; Farmer, 2013). In accordance to Kuzyakov (2005), this method was comparatively simple (Table 7).

4.4 Lab incubation method

Interpreting soil respiration processes in response to seasonal changes is generally challenging because soil temperature and moisture regularly covary (Carbone et al., 2011; Davidson et al., 1998). The lab incubation technique was the only method capable of dividing the effect of soil temperature and moisture on Rh and to produce a significant Gaussian regression fit (Table 4). However, the microcosm incubation produced Rh values notably lower than the other techniques (Table 6). This might be due to the fact that the soil column in the incubation microcosms were 5 cm high while the A horizon in the field (i.e. where the Rh assessments from the other techniques were made) was 10 cm thick (Table 2). Further studies should test the effect of microcosm height on Rh in relation to field measurements. The low Rh of the lab incubation method could also be attributed in part to the fact that this technique did not contain any rhizomicrobial respiration and its priming effect. That is, this method produced Rh from basal microbial respiration which is considered to be from stabilized SOM with slow turnover rates (Kuzyakov, 2006 Neff et al., 2002). In view of that, with additional field and lab methods development it would be possible to further segregate Rh assessments into percentage of rhizomicrobial respiration, decomposition of plant residues and basal decomposition of SOM. Overall, the lab incubation technique was slightly more complex than the non-isotopic field Rh assessment methods but allowed a prompt determination of Rh whilst simulating year round field environment (Table 7).

4.5 Comparison of methods and recommendations

The analysis of the five different Rh/Rs partitioning methods examined in this study shows that none of them was fully satisfactory. That is, each technique had strengths and weaknesses (Table 7).

Using $\delta^{13}C\text{-}CO_2$ is acknowledged as the preeminent way to segregate Rh/Rs (Cheng, 1996; Kuzyakov, 2006). However, we found several shortcomings to this technique. First, the conjunction of field and lab procedures makes it

difficult to complete this method in one day as needed. Second, the air flushing with CO_2 free gas in the field (to prevent ambient $\delta^{13}CO_2$ contamination) makes that technique more complex than the other methods to assess Rh%. Third, the ability to perform this technique in remote areas is limited because the $\delta^{13}C\text{-}CO_2$ needs to be quickly assessed with a calibrated and accurate spectrometer (Midwood et al., 2006). Fourth, in our study we found large variance in $\delta^{13}C\text{-}CO_2$ of the respiration components (i.e. Ra, Rh and Rs) that impeded the assessment of Rh% per individual collar. Accordingly, further studies should analyze the spatial relationships of $\delta^{13}C\text{-}CO_2$ with soil properties and root characteristics. As standalone, the $\delta^{13}C\text{-}CO_2$ technique was unable to produce assessment of soil CO_2 efflux; thus needed to be performed in conjunction with field Rs measurements. In this regards, the $\delta^{13}C\text{-}CO_2$ complemented well with root exclusion bags methods because it allowed to determine if the buried bags had teared and been invaded by roots and to standardized Rh% determination.

The root regression method had the advantage to be simple, to produce an average Rh% close to the $\delta^{13}C\text{-}CO_2$ natural abundance and the disadvantage to require a high number of replicates due to low coefficient of determination between CO_2 efflux and root biomass. Another disadvantage of the root regression technique is that in order to produce seasonal trends, the labor intensive procedures (i.e. pit digging, CO_2 measurements and root counting) need to be reinitiated several times during the years. This shortcoming can be particularly impractical in small plot experiments. Complementary studies should assess thresholds of root size to be included in the regression fit in order to optimize the correlation fit and use the $\delta^{13}C\text{-}CO_2$ natural abundance method to determine the effect of root size on the isotopic signature.

The root exclusion bags methods (i.e. HS and IB) had the advantage to be easy to monitor throughout the year. That is, because the % of Rh is unlikely to be constant in time it is important to assess it periodically. The bags methods can be considered as a miniaturization of the traditional soil trenching method. However, contrasting with large trenches (e.g. Comeau et al., 2016; Fisher and Gosz, 1986) the root exclusion bags had the advantage to be simpler to establish and to allow mycorrhiza development inside the mesh bags (Moyano et al., 2007). Conversely, due to the relatively small bag sizes, root webs on the outside edge could potentially contaminate Rh assessment. In this study, the $\delta^{13}C\text{-}CO_2$ determination made with the collars located in the center of the bags showed no isotopic signature of root respiration. Similarly with the trenching method, the root exclusion bag methods had the disadvantages to require several months of soil stabilization before starting CO_2 efflux measurements. Compared with the $\delta^{13}C\text{-}CO_2$ natural abundance method, the HS and IB overestimated and underestimated %Rh by 18 and 12%, respectively. The divergences were likely

caused by soil disturbances, alteration in root demise dynamic and lack of root exudates. Correspondingly, Carbone et al. (2016) found 11% differences in Rh% assessment between an isotopic partition method and the trenching technique. Comparing the HS and IB, the former created more soil disturbances but the latter would not be suitable for soil with high amount of sand, gravel or rock because the intact blocks would collapse.

The lab incubation with minimally disturbed microcosms was the only method that had absolutely no influence of root or mycorrhiza. Thus the results from this method exclusively represented the CO_2 efflux originating from the mineralization of the slow turnover SOC pool (i.e. basal soil respiration) (Pell et al., 2006). Assessment of basal soil respiration in relationship with the total Rh is of great importance in evaluating the dynamic of the stabilized SOC. In this study, the Rh% from the lab incubation was 8-17% while the $\delta^{13}C$-CO_2 natural abundance had an average of 61% Rh. Thus, if the soil incubation results were not affected by the height of the soil columns (as discussed above), basal respiration represented approximately one fifth of the Rh. Because stabilized SOC is a key indicator of soil quality and health (Creamer et al., 2014), further research should study the relationship between basal soil respiration and rhizosphere derived Rh.

Overall, results from field experiments exhibited a range of potential Rh between 2.5 and 6.0 Mg CO_2-C ha^{-1} y^{-1}. With the publication of the total annual life biomass growth (i.e. including root and above-grown biomass) at the study site (Tai Po Kau Nature Reserve) assessment of net ecosystem C balance will then be possible.

5 Conclusions

Methods for determining ecosystem C fluxes need to be improved and applied to allow a quantitative understanding of the biological processes underlying SOC balance. This study compared five methods to assess Rh and our results showed large variance of effluxes and Rh/Rs ratio between the different techniques analyzed. The data revealed that the hand-sorted root exclusion bags and the intact root exclusion bags methods produced similar Rh efflux values and these efflux were slightly lower than the one produced by the root regression method but notably larger than the lab incubation with soil cores We found that methods with higher accuracy (soil $\delta^{13}C$-CO_2 natural abundance and root regression) had lower precision (i.e. large variance) and methods with higher precision (root exclusions bags and lab incubation) had lower accuracy. Based upon these results, we suggest that when assessing rate of heterotrophic emissions and their contribution to total soil based emissions, two or more methods should be performed to produce more integral assessments.

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References

Accoe, F., Boeckx, P., Van Cleemput, O., Hofman, G., Zhang, Y., Rong hua, L., and Guanxiong, C.: Evolution of the d13C Signature Related to Total Carbon Contents and Carbon Decomposition Rate Constants in a Soil Profile Under Grassland, Rapid Commun. Mass Spectrom., 16, 2184–2189, doi:10.1002/rcm.767, 2002.

Arcand, M. M., Helgason, B.L., and Lemke, R. L.: Microbial crop residue decomposition dynamics in organic and conventionally managed soils, Applied Soil Ecology, 107, 347–359, 2016.

Behera, N., Joshi, S. K., and Pati, D. P.: Root contribution to total soil metabolism in a tropical forest soil from Orissa, India, Forest Ecology and Management, 36, 125–134, 1990.

Braig, E., and Tupek, B.: Separating soil respiration components with stable isotopes: natural abundance and labelling approaches, IFOREST., 3, 92-94, 2010.

Carbone, M. S., Richardson, A. D., Chen, M., Davidson, E. A., Hughes, H., Savage, K. E., and Hollinger, D. Y. Constrained partitioning of autotrophic and heterotrophic respiration reduces model uncertainties of forest ecosystem carbon fluxes but not stocks, J. Geophys. Res. Biogeosci., 121, 2476–2492, 2016.

Carbone, M. S., Still, C. J., Ambrose, A. R, Dawson, T. E., Williams, A. P., Boot, C. M., Schaeffer, S. M., and Schimel, J. P.: Seasonal and episodic moisture controls on plant and microbial contributions to soil respiration, Oecologia, 167, 265–278, 2011.

Cheng, W.: Measurement of rhizosphere respiration and organic matter decomposition using natural 13C, Plant Soil, 183, 263–268, 1996.

Comeau, L-P., Hergoualc'h, K., Hartill, J., Smith, J., and Verchot, L.V.: How do the heterotrophic and the total soil respiration of an oil palm plantation on peat respond to nitrogen fertilizer application?, Geoderma, volume 268, 41-51. doi:10.1016/j.geoderma.2016.01.016, 2016.

Creamer, R. E., Schulte, R. P. O., Stone, D., Galb, A., Krogh, P. H., Lo Papa, G., Murray, P. J., Peres, G., Foersterg, B., Rutgers, M., Sousa, J. P., and Winding, A.: Measuring basal soil respiration across Europe: do incubation temperature and incubation period matter?, Ecol. Indic., 36, 409-418, 2014.

Cui, J., and Lai, D. Y. F.: Soil-atmosphere N2O and CH4 exchanges were suppressed by litter layer in a subtropical secondary forest, AGU Fall Meeting, San Francisco, USA. 12-16 December 2016.

Davidson, E. A., Belk, E., Boone, R. D.: Soil water content and temperature asindependent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biol., 4, 217–227, 1998.

Davis, D. W., Sewell, R. J., and Campbell, S. D. J.: U-Pb dating of Mesozoic igneous rocks from Hong Kong, Journal of the Geological Society, 154, 1067-1076, 1997.

Ellert, B. H., and Bettany, J. R.: Calculation of organic matter and nutrients stored in soils under contrasting management regimes, Can. J. Soil Sc., 75, 529-538, 1995.

Farmer, J.: Measuring and modeling soil carbon and carbon dioxide emissions from Indonesian peatlands under land-use change, Ph D, University of Aberdeen, UK, 2013.

Farquhar, G. D., Ehleringer, J. R., and Hubick, K. T.: Carbon isotope discrimination and photosynthesis, Annual Reviews in Plant Physiology, 40, 503–537, 1989.

Fenn, K. M., Malhi, Y., and Morecroft, M. D.: Soil CO2efflux in a temperate deciduousforest: environmental drivers and component contributions, Soil Biol. Biochem., 42, 1685–1693, 2010.

Fisher, F. M., and Gosz, J. R.: Effects of trenching on soil processes and properties in a New Mexico mixed-conifer forest, Biol. Fertil. Soils, 2, 35–42, 1986.

Goebel, M.-O., Bachmann, J., Woche, S. K., and Fischer, W. R.: Soil wettability, aggregate stability, and the decomposition of soil organic matter, Geoderma, 128, 80-93, 2005.

Gupta, S. R., and Singh, J. S.: Soil respiration in a tropical grassland, Soil Biol. Biochem., 13, 261–268, 1981.

Harmon, M. E., Bond-Lamberty, B., Tang, J. W., and Vargas, R.,: Heterotrophic respiration in disturbed forests: A review with examples from North America, J. Geophys. Res. Biogeosci,. 116:G00K04, 2011.

Hergoualc'h, K., and Verchot, L. V.: Stocks and fluxes of carbon associated with land use change in Southeast Asian tropical peatlands: a review, Global Biogeochemical Cycles 25:GB2001. DOI: 10.1029/2009gb003718, 2011.

Hinko-Najera, N., Fest, B., Livesley, S. J., and Arndt, S. K.: Reduced throughfall decreases autotrophic respiration, but not heterotrophic respiration in a dry temperate broadleaved evergreen forest, Agric. For. Meteorol., 200, 66–77, 2015.

Hogh-Jensen, H., and Schjoerring, J. K.: Effects of drought and inorganic N form on nitrogen fixation and carbon isotope discrimination in Trifolium repens, Plant Physiology and Biochemistry 35, 55–62, 1997.

Jahn, R., Blume, H.-P., Asio, V. B., Spaargaren, O., and Schad, P.: Guidelines for soil description, FAO, Rome, 97, 2006.

Janzen, H.H.: The soil carbon dilemma: shall we hoard it or use it?, Soil Biol. Biochem., 38, 419-424, 2006.

Janzen, H. H., Campbell, C. A., Izaurralde, R. C., Ellert, B. H., Juma, N., McGill, W. B., and Zentner, R. P.: Management effects on soil C storage on the Canadian prairies. In K. Paustian, et al. (ed.) Conference: Special issue: tillage and crop management impacts on soil carbon storage, Soil & Tillage Research 47, 181-195, 1998.

Kucera, C.L., and Kirkham, D. R.: Soil respiration studies in tallgrass prairie in Missouri, Ecology 52, 912–915, 1971.

Kuzyakov, Y.: Review: factors affecting rhizosphere priming effects, Journal of Plant Nutrition and Soil Science 165, 382-396, 2002.

Kuzyakov, Y.: Sources of CO2 efflux from soil and review of partitioning methods, Soil Biol. Biochem., 38, 425–448, 2006.

Kuzyakov, Y., and Larionova, A. A.: Review of methods and results of separation of root and rhizomicrobial respiration, Journal of Plant Nutrition and Soil Science 168, 503-520, 2005.

Lal, R.: World crop residues production and implications of its use as a biofuel, Environ. Int., 31, 575-584, 2005.

Langford, R. L. Lai, R. W., Arthurton, R. S., and Shaw, R.: Geology of the Western New Territories, Hong Kong Geological Memoir No. 3, Geotechnical Control Office, Hong Kong, 140 p., 1989.

Lin, G., Ehleringer, J., Rygiewicz, P., Johnson, M., and Tingey, D.: Elevated CO2 and Temperature Impacts on Different Components of Soil CO2 Efflux in Douglas-fir Terracosms, Global Change Biol., 5, 157–168, 1999.

McConkey, B. G., Angers, D., Bentham, M., Boehm, M., Brierley, T., Cerkowniak, D., Liang, B. C., Collas, P., de Gooijer, H., Desjardins, R., Gameda, S., Grant, B., Huffman, T., Hutchinson, J., Hill, L., Krug, P., Martin, T., Patterson, G., Rochette, P., and Worth, D.: Canadian Agricultural Greenhouse Gas Monitoring Accounting and Reporting System: Methodology and greenhouse gas estimates for agricultural land in the LULUCF sector for NIR 2006. Agriculture and Agri-Food Canada, 2007.

McLean, E. O.: Soil pH and lime requirement. Pp. 199-224 in A. L. Page, R. H. Miller, and D. R. Keeney, eds. Methods of soil analysis. Part 2. Chemical and microbiological properties, Agronomy 9, 2nd ed. ASA, SSSA. Publ., Madison, WI, 1982.

Midwood, A., and Millard, P.: Challenges in Measuring the δ13C of the Soil Surface CO_2 Efflux, Rapid Communications in Mass Spectrometry 25 (1) (January 15): 232–42. doi:10.1002/rcm.4857, 2011.

Midwood, A., Gebbing, T., Wendler, R., Sommerkorn, M., Hunt, J., and Millard, P.: Collection and Storage of CO_2 for 13 C Analysis : an Application to Separate Soil CO_2 Efflux into Root- and Soil-derived Components, Rapid Communications in Mass Spectrometry : RCM 20: 3379–3384. doi:10.1002/rcm, 2006.

Millard, P., Midwood, A., Hunt, J., Barbour, M., and Whitehead, D.: Quantifying the Contribution of Soil Organic Matter Turnover to Forest Soil Respiration, Using Natural Abundance δ13C, Soil Biol. Biochem., 42, 935–943. doi:10.1016/j.soilbio.2010.02.010, 2010.

Minasny, B., Malone, B. P., McBratney, A. B., Angers, D. A., Arrouays, D., Chambers, A., ... and Field, D. J.: Soil carbon 4 per mille, Geoderma 292, 59-86, 2017.

Moyano, F. E., Kutsch, W. L., and Schultze, E.-D.: Response of mycorrhizal, rhizosphere and soil basal respiration to temperature and photosynthesis in a barley field. Soil Biol. Biochem., 39, 843-853, 2007.

Neff, J. C., Townsend, A. R., Gleixner, G., Lehman, S. J., Turnbull, J., and Bowman, W. D.: Variable effects of nitrogen additions on the stability and turnover of soil carbon, Nature 6910, 915–917, 2002.

Nelson, D. W. and Sommers L. E.: Total carbon, organic carbon, and organic matter, pp. 539-579 in A. L. Page, R. H. Miller, and D. R. Keeney, eds. Methods of soil analysis. Part 2. Chemical and microbiological properties. Agronomy 9, 2nd ed.ASA, SSSA. Publ., Madison, WI, 1982.

Paterson, E., Midwood, A. J., and Millard, P.: Through the Eye of the Needle: a Review of Isotope Approaches to Quantify Microbial Processes Mediating Soil Carbon Balance, New Phytologist 184, 19–33. doi:10.1111/j.1469-8137.2009.03001.x, 2009.

Pell, M., Stenström, J., and Granhall, U.: Soil Respiration. In: Bloem, J., Hopkins,D.W., Benedetti, A. (Eds.), Microbial Methods for Assessing Soil Quality, CAB International, Wallingford, Oxfordshire, U.K, 2006.

R Development Core Team, R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Version 2.8.1. (http://www.R-project.org) Accessed May 1st 2014.

Rühr, N. K., and Buchmann, N.: Soil respiration fluxes in a temperate mixed forest: seasonality and temperature sensitivities differ among microbial and root-rhizosphere respiration, Tree Physiol., 30, 165–176, 2010.

Steward, B. A., and Davies, B. R.: The influence of different litter bag designs on the breakdown of leaf, Hydrobiologia 183, 173-177, 1989.

Tan, K. H: Soil sampling, preparation, and analysis. Marcel Dekker, Inc., New York, NY. 264 pp., 1996.

Tong, C. H.: Effect of litter layer on soil GHG fluxes in subtropical forests, Bachelor Thesis. Chinese University of Hong Kong. 119 p., 2015.

Tufekcioglu, A., Raich, J. W., Isenhart, T. M., Schultz, R. C.: Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA, Agroforestry Systems 44, 163–174, 1999.

Van Reeuwijk, L. P.: Procedures for soil analysis, 3rd Ed. International Soil Reference and Information Center (ISRIC), Wageningen, the Netherlands. 34p., 1992.

VandenBygaart, A. J., McConkey, B. G., Angers, D. A., Smith, W., de Gooijer, H., Bentham, M., and Martin, T.: Soil carbon change factors for the Canadian agriculture national greenhouse gas inventory, Can. J. Soil Sci., 88, 671-680, 2008.

Wood, T. E., Cavaleri, M. A., and Reed, S. C.: Tropical forest carbon balance in a warmer world: a critical review spanning microbial- to ecosystem-scale processes, Biol. Rev., 87, 912-927, 2012.

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Table 1: Morphological description of the soil profiles at the study site

Horizon depth (cm)	Color (dry)	Color (moist)	Field texture[a]	Structure[b]	Rock fragments volume %	Roots mg root cm^{-3}	pH (H_2O)	pH (KCl)	WHC (g H_2O g $soil^{-1}$)	Soil organic C; N (%)
A 0~10	10 YR 6/2	10 YR 3/2	SL	gr	0	9.3 ±3.4	4.2	3.3	0.50 ±0.01	3.2 ±0.2; 0.24 ±0.02
AB 10~25	10 YR 6/3	10 YR 4/2	SL	gr	0	1.6 ±0.6	4.5	3.9	-	-
Bt 25~60	10 YR 8/8	7.5 YR 6/8	CL	sbk	10-20	0.5 ±0.2	4.7	4.0	-	-
C 60~100	10 YR 8/8	10 YR 7/8	CL	sbk	>60	0	4.7	4.0	-	-

[a] SL, sandy loam; CL, clay loam.
[b] gr, granular; sbk, subangular blocky.
WHC, water holding capacity
C, carbon; N, nitrogen

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478 Table 2: Comparison of environmental parameters inside and outside the root exclusion bags

Method	Soil temperature (ºC)	Soil moisture (vol. %)	Bulk density (g cm^{-3})
Inside hand-sorted root exclusion bags (HS)	22.4 (0.2) α	20.5 (1.2) β	1.16 (0.04) α
Inside intact root exclusion bags (IB)	22.6 (0.3) α	25.5 (1.4) α	1.13 (0.05) α
Outside root exclusion bags (Rs)	22.4 (0.2) α	24.8 (0.8) α	1.14 (0.03) α

479 Values are means and standard error. Values in the same column followed by a different Greek letter (α, β) are significantly
480 different from each other at α=0.05.

481

482 Table 3: Linear regression report between root density[a] and CO_2 efflux[b]

Parameter	Value	SE[b]	t value	P value
Intercept (g CO_2 m^2 h^{-1})	0.25	0.10	2.50	0.02
Slope (CO_2 X mg root cm^3)	0.08	0.04	2.31	0.03

483 Overall r^2 of the linear regression: 0.21.
484 [a] root density in unit of milligram, small (radius between 0.1-0.5 cm) dried roots (60°C) per cm^3 of soil.
485 [b] SE, standard error.

486

487 Table 4: Parameter values of the Gaussian 3D regression fitted curve (equation 1)

Efflux	Parameter a (g m^2 h^{-1})	Parameter xo	Parameter yo	Parameter b	Parameter c
Rh incubation	0.21 ***	49.2 ***	34.7 ***	15.7 ***	19.2 ***
Rs field	0.43 **	24.9 **	18.3 NS	9.6 NS	15.8 NS
Rh IB	0.24 NS	21.93 NS	21.4 NS	4.8 NS	13.4 NS
Rh HS	0.34 NS	21.7 NS	9.5 NS	5.1 NS	14.2 NS

488 Rh incubation, heterotrophic respiration from the soil cores incubation
489 Rs field, total soil respiration from outside of the root exclusion bags
490 Rh IB, heterotrophic respiration from the intact root exclusion bags
491 Rh HS, heterotrophic respiration from the hand-sorted root exclusion bags
492 ** and *** significant at $p < 0.01$ and $p < 0.05$, respectively; NS non-significant.
493 Parameters from equation 1. Parameter "a" correspond to the height of the maximum high of the curve (g CO_2 m^{-2} h^{-1}); "xo" is
494 the peak soil temperature point (°C) in the curve, "yo" is the peak soil moisture (%) point in the curve, and "b" and "c" are the
495 Gaussian root mean squared widths of the soil temperature and soil moisture, respectively.
496

Table 5: $\delta^{13}C\text{-}CO_2$ results

Method	$\delta^{13}C\text{-}CO_2$ (‰)
Rs[a]	-18.21 (0.53) αβ
Rh HS[b]	-16.65 (0.44) β
Rh IB[c]	-16.52 (1.07) β
Rh lab[d]	-16.75 (0.54) β
Ra lab[e]	-20.44 (0.65) α

[a] Rs, gas samples collected from the field total soil respiration collars.
[b] Rh HS, gas samples collected from the field hand-sorted root exclusion bags collars.
[c] Rh IB gas samples collected from the field intact blocks root exclusion bags collars.
[d] Rh lab, gas samples collected from lab incubations of soil with freshly removed roots.
[e] Ra lab, gas samples collected from lab incubations of the roots extracted in Rh lab.
Values are means and standard error, n = 14 for Rs and Ra and n = 7 for HS, IB and Rh lab.
Values followed by a different Greek letter (α, β) are significantly different from each other at α=0.05.

Table 6: Comparison of heterotrophic respiration assessment methods

Method	Rh efflux[a]	Rs efflux[b]	Rh / Rs
	------Mg CO_2–C ha^{-1} y^{-1}-------		--- % ---
Root regression	6.0 (2.4)	11.1 (1.0)	54 (41)
Soil cores incubation	0.4-1.9[c]	-	8-17[d]
Hand-sorted root exclusion bags (HS)	4.8 (0.3)	6.1 (0.3)	79 (3)
Intact root exclusion bags (IB)	3.0 (0.3)	6.1 (0.3)	49 (7)
Soil $\delta^{13}C\text{-}CO_2$ natural abundance	-	-	61 (39)

Values are means and standard error, n = 22 for the root regression, n = 47 for soil incubation,
n = 28 for both root exclusion bags techniques.
[a] Rh, heterotrophic respiration.
[b] Rs, total soil efflux taken alongside the Rh efflux.
[c] Efflux range at temperature between 14°C and 26°C.
[d] Calculated as Rh from incubation at 14°C and 26°C divided by average field Rs at 14°C and 26°C respectively.

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514 Table 7: Comparison of partition methods

Segregation method	Accuracy[a]	Precision[b]	Complexity of procedures[c]	Time needed to setup experiment[d]	Time needed to produce seasonal trends
Root regression	High	Low	Simple	2-3 days	6 months to 1 year
Soil cores incubation	Low	High	Complex	5-7 days	<1 to 2 months
Hand-sorted root exclusion bags (HS)	Intermediate	High	Simple	2-3 days	6 months to 1 year
Intact root exclusion bags (IB)	Intermediate	High	Simple	2-3 days	6 months to 1 year
Soil $\delta^{13}C$-CO_2 natural abundance	High	Low	Complex	1-2 days	6 months to 1 year

515 [a] Partition methods that had Rh%: <10, 10-20 and >20 lower or larger than the $\delta^{13}C$-CO_2 natural abundance were categorized as high, intermediate and low accuracy, respectively.
516 [b] High, intermediate and low precision were attributed to Rh% standard errors of <10, 10-20 and >20, respectively.
517 [c] Methods with five steps or less were deemed simple and six steps or more deemed as more complex.
518 [d] The time needed to setup experiment was assessed with the number of working hours required prior to be able to start the measurements.
519 [e] The time needed to produce seasonal trends was the number of months of measurements required to characterize the Rh at the different temperature and moisture levels of the
520 year.
521

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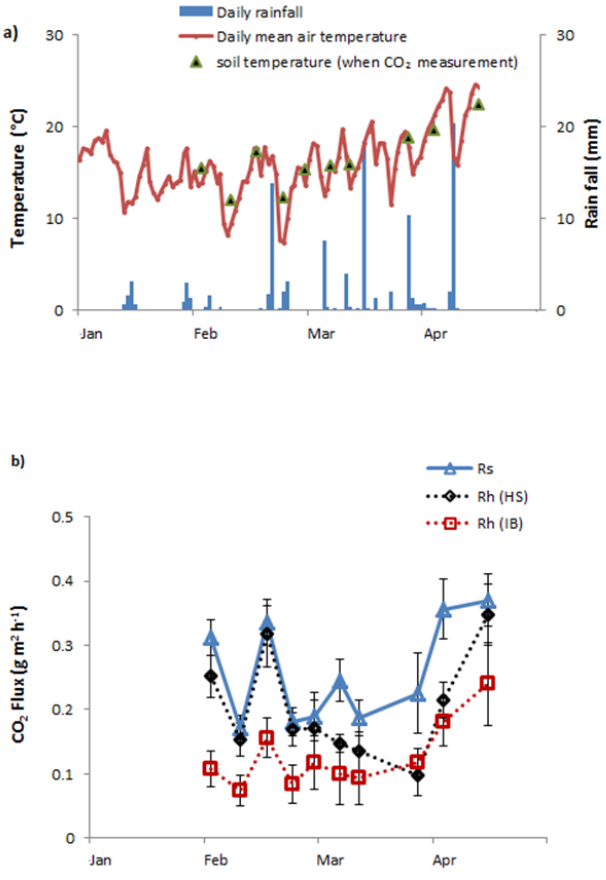

523
524 **Figure 1: a) Soil and air temperature and daily rainfall over the study period; b) Total soil CO_2 efflux (Rs), heterotrophic**
525 **CO_2 efflux (Rh) from hand-sorted root exclusion bags (HS), and Rh from intact block root exclusion bags (IB).**
526

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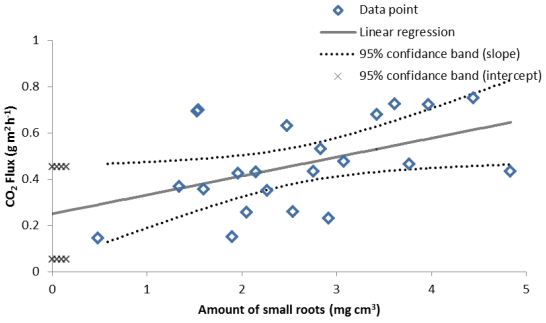
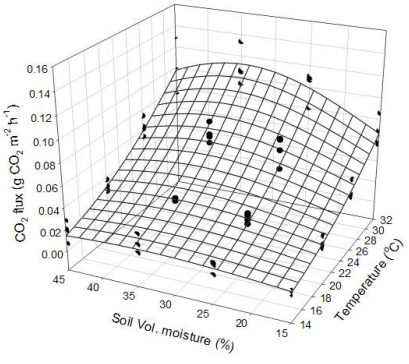


527

528 **Figure 2: Linear regression between root quantity and CO_2 efflux.**



529

530 **Figure 3: Results from the lab incubation; regression between incubation temperature, moisture and CO_2 efflux.**