

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

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10 **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 CO₂ efflux (Rs) in different ecosystems, but to date, there are still many uncertainties and unknowns 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 CO₂; (2) lab incubations with minimally disturbed soil microcosm cores; (3) root exclusion bags with hand-sorted roots; (4) 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 δ¹³C-CO₂ 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 δ¹³C-CO₂ 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

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25 the strengths and weaknesses of each technique with the conclusion that combining two or more methods optimizes Rh assessment reliability.

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, 30 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 CO₂ 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 35 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).

40 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 45 change procedure (e.g. VandenBygaart et al., 2008) with the soil C efflux balance approach (i.e. Hergoualc'h and Verhot, 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 CO₂ efflux (R_s) from soil does not provide the necessary information to estimate whether the soil is a net source or net sink for atmospheric CO₂ (Kuz'yakov and Larionova, 2005). Total soil efflux is a combination of root 50 based respiration (autotrophic (R_a)) and heterotrophic respiration (R_h). Autotrophic respiration does not contribute to net C losses to the atmosphere as it is cycled within the ecosystem, whereas R_h 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).

55 | Reviews 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 CO₂ efflux into its Rs and Rh component in a subtropical secondary forest in Hong Kong. The influence of soil moisture and temperature on CO₂ efflux was also analyzed. The goal of our study was to compare partitioning methods to separate CO₂ efflux into its Rs and Rh component in a subtropical secondary forest in Hong Kong. Five methods were selected based on their suitability in the studied ecosystem. Three methods, were traditional techniques (i.e. regression between root mass and root derived CO₂; root exclusion bags with hand-sorted roots; and soil δ¹³C-CO₂ natural abundance) and two were innovative variation of existing methods (i.e. root exclusion bags with intact soil blocks; and lab incubations with minimally disturbed soil microcosm cores). The influence of soil moisture and temperature on CO₂ efflux was also analyzed.~~

2 Methodology

65 | 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 approximately 600 m. above sea level and the slope surfaces were stable and vegetated. The forest was approximately 50 years old and was covered with

70 | continuous canopy. More than 100 plant species were registered in the Nature Reserve. The following genera were surveyed found 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.

75 | The canopy was closed in the area with an average solar radiation at 2 m high of 13.8 W/m² (non-published data).

2.5.1 Soil general characterization

Four soil profiles were dug in the study area, characterizing the different landforms (i.e. back-slope and foot-slope) 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 saturating the soils, allowing them to freely drain for 24 h and determining gravimetric water content after oven-drying at 105 °C 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).

2.2 Root and carbon dioxide efflux regression method

The regression technique is based on the relationship between the CO₂ emitted by the root-rhizosphere and root biomass and the CO₂ 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 CO₂ 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 analysis between root quantity and CO₂ efflux was performed using the program R Foundation for Statistical Computing version 2.8.1 (R Development Core Team, 2008).

2.3 Lab incubations

For the lab incubations, undisturbed soil cores with a volume of 98 cm³ (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 micro-aggregates. The soil was then repacked to original bulk density in minimally disturbed soil microcosm cores of 45 cm³ (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 dm³ plastic container and left to stabilize in dark for two weeks at 25°C. After that, 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 CO₂ 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 CO₂ concentration. Between each measurement session, the boxes were 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).

$$f(x, y) = a \times \exp \left[-0.5 \times \left(\frac{x-x_0}{b} \right)^2 + \left(\frac{y-y_0}{c} \right)^2 \right] \quad (1)$$

where $f(x, y)$ is the CO₂ efflux function; a , b and c are constant coefficients; x is the soil temperature (°C); y is the soil moisture content (%); x_0 is the average temperature; y_0 is the average soil moisture.

2.14 Root exclusion bag methods

To partition the CO₂ 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 (38 μm 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 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.

135 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 R_s . To assess R_s and R_h without the influence of litterfall decomposition, the collars were cleared of leaves and flowers on a weekly basis.

140 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 10 cm 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
145 assess bulk density (van Reeuwijk, 1992). Mathematical calculation and descriptive statistical analyses were done with Microsoft Excel XP[®].

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

2.45 $\delta^{13}\text{C}$ natural abundance method

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

isotopic partitioning experiment assessed values of the $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 before~~ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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):

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

where %Rh is the proportion of Rh from Rs, and δRs , δRh and δRa are the $\delta^{13}\text{C}$ 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-RCB-M002; air temperature/RH, sensor S-THB-M008, Onset Computer Corp., USA). Water holding capacity was assessed by~~

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215 **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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ -CO₂ 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 refilling, and CO₂ efflux measurements). Methods with five steps or less were deemed simple and six steps or more deemed as

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225 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

230 **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 ($3.2 \pm 0.2\%$) and high acidity (pH_{H₂O} 4.2) (Table 1). The sub-~~superficial~~ surface soil was

235 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

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 ~~upon~~ previous results in the area (Tong, 2015), along with soil acidity, type parent material and level of mineralization of the bedrock in the soil pits.

3.3.2 Root regression and

The 22 quadrats used for the root regression assessment yielded average Rs of $0.46 \pm 0.04 \text{ g CO}_2 \text{ m}^2 \text{ h}^{-1}$. The regression of the CO_2 efflux against root density produced a statistically significant slope correlation of $0.08 \pm 0.04 \text{ g CO}_2 \text{ m}^2 \text{ h}^{-1}$ per mg root cm^{-3} ($p=0.03$), and set the intercept at $0.25 \pm 0.10 \text{ g CO}_2 \text{ m}^2 \text{ h}^{-1}$ ($p=0.02$) which represented the basal efflux in absence of root i.e. the Rh (Fig. 2 and Table 3). The Rh measured when the root regression technique was performed (October 2016) was $6.0 \pm 2.4 \text{ Mg C ha}^{-1} \text{ y}^{-1}$, equivalent to 54% of the Rs (Table 6).

3.3.3 Lab incubation

The 22 quadrats used for the root regression assessment yielded average Rs of $0.46 \pm 0.04 \text{ g CO}_2 \text{ m}^2 \text{ h}^{-1}$. The regression of the CO_2 efflux against root density produced a statistically significant slope correlation of $0.08 \pm 0.04 \text{ g CO}_2 \text{ m}^2 \text{ h}^{-1}$ per mg root cm^{-3} ($p=0.03$), and set the intercept at $0.25 \pm 0.10 \text{ g CO}_2 \text{ m}^2 \text{ h}^{-1}$ ($p=0.02$) 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 $11.1 \pm 1 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ (Table 6), equivalent to 54% of the Rs.

During the incubation with minimally disturbed soil microcosms, the average (moisture levels combined) CO_2 efflux at 14, 20, 26 and 32°C was 0.015136 ± 0.50021 , 0.679282 ± 0.38016 , 0.0585140 ± 0.91038 and $2.2400938 \pm 1.39058 \text{ Mg CO}_2\text{-C ha}^{-1} \text{ y}^{-1}$, respectively (Fig. 3). The exponential relationship between CO_2 efflux, soil temperature and moisture is presented in Table 4.

3.2.4 Root exclusion bag methods

During the root exclusion bags measurements period (Feb-Apr 2017), the average air temperature was 16°C and the total rainfall 107 mm, ~~and the~~ During that period the Rs averaged $6.1 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ (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 $0.46 \pm 0.04 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$. The regression of the CO₂ efflux against root density produced a statistically significant slope correlation of $0.08 \pm 0.04 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ per mg root cm³ ($p=0.03$), and set the intercept at $0.25 \pm 0.10 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ ($p=0.02$) 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 $11.1 \pm 1 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ (Table 6), equivalent to 54% of the Rs.~~

~~During the incubation with minimally disturbed soil microcosms, the average CO₂ efflux at 14, 20, 26 and 32°C was 0.0151 ± 0.021 , 0.0282 ± 0.016 , 0.0585 ± 0.038 and $0.0928 \pm 0.058 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, respectively (Fig. 3). The exponential relationship between CO₂ efflux, soil temperature and moisture is presented in Table 4.~~

3.54 Soil δ¹³C-CO₂ natural abundance

The δ¹³C-CO₂ natural abundance determination satisfactorily segregated the three respiration components (Table 5). The fact that the δ¹³C-CO₂ 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 δ¹³C-CO₂ of the Rs, the Rh lab and the Ra lab the percentage of heterotrophic respiration was $61 \pm 39\%$ (Table 6). ~~The notably large standard error of the percentage of heterotrophic respiration was due to the large variance in the δ¹³C-CO₂ of the three respiration components.~~

~~Using the δ¹³C-CO₂ method as base-line, the percent increase/decrease of Comparing with the Rh from the δ¹³C-CO₂ method, the root regression, lab incubation, hand-sorted and intact block (IB) root exclusion techniques were -11% below, $-72-87\%$ below, $\pm 30\%$ above and -20% below, respectively (Table 6).~~

4 Discussion

4.1 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 and slope were still statistically significant. This 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 $\delta^{13}\text{C}$ 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).

4.2 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 3). However, the microcosm incubation produced Rh values notably lower than the other techniques (Table 5). This might mainly be due to three different causes. First, 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). Second, to prevent potential shifts in the microbial community during the incubations (i.e. adapting to lower resource availability), previous to the beginning of the experiment the microcosms were left stabilize for two weeks. Accordingly, the fresh and label organic residues that would in the other segregation methods contribute to the soil respiration had already decomposed before the beginning of the incubations. Third, 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 6). Further studies should test the effect of microcosm height on Rh in relation to field measurements and determine microbial C use efficiency by isothermal microcalorimetry during the incubations.

4.3 Root exclusion bags methods

320 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 5). Comparing the %Rh of the HS and IB with the $\delta^{13}\text{C}$ 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 6).

325 The micromesh size used in the root exclusion bags was $38\mu\text{m}$ 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 contain some portions of the roots respiration. Contrary to this, the IB and HS air samples analyzed for $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ - CO_2 signature (Table 4). After the three months of soil stabilization period, both bag methods for partitioning total soil respiration and root-free soil respiration

330 components successfully produced $R_s > R_h$ in every sampling dates indicating that efflux rates within the bags had reached an apparent post disturbance state (Fig. 1). Also, in both IB and HS, soil temperature and bulk density were statistically equal to the surrounding soil (i.e. R_s) (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 to the surrounding soil but for HS it was significantly lower. This was likely caused by the

335 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 5). The larger HS Rh efflux compared with IB Rh could be due in part to the lower soil moisture in the former. This likelihood is supported by the fact that in the regression fit the maximum Rh was when moisture content was relatively low (i.e. y_0 , Table 3).

340 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).

345 | 4.14 Soil $\delta^{13}\text{C}$ natural abundance method

The three respiration components of this method (i.e. $\delta^{13}\text{C}$ - CO_2 from Rs, Rh and Ra) had large standard errors (Table 54) that produced a high uncertainty value in the Rh/Rs assessment ($61 \pm 39\%$, Table 65). This method was accordingly deemed of low precision (Table 76). This, in turn, impeded to produce an Rh/Rs assessment in the individual collars. This large $\delta^{13}\text{C}$ - CO_2 variance was likely caused by variability of $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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. $\delta^{13}\text{C}$ -7.5 to -8.5 ‰) to that of the Rs efflux is challenging and potential air contaminations have to be considered when analyzing the results (Millard et al., 2010). In our study, the Rh $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ in a close range and without statistical differences between them (Table 54). This indicates that the chamber system used in the field to collect the $\delta^{13}\text{C}$ efflux samples was adequately effective to prevent air contamination. Overall, the soil $\delta^{13}\text{C}$ 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 76).

~~4.2 Root exclusion bags methods~~

~~The HS and IB methods had % Rh of 79 ± 3 and $49 \pm 7\%$, 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 $\delta^{13}\text{C}$ 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).~~

~~The micromesh size used in the root exclusion bags was 38 μm 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 contain some portions of the roots respiration. Contrary to this, the IB and HS air samples~~

analyzed for $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ - 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 $R_s > R_h$ 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. R_s) (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 R_h 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. y_0 , Table 4) showed that maximum R_h was when moisture content was relatively low (9.5 and 21.4%, respectively). Accordingly, this could partly explain the larger HS R_h 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. R_s) corresponds to soil respiration in absence of root (i.e. R_h) (Fig. 2). In this study the regression had ten points (45%) outside the confidence interval but the intercept ($0.25 \pm 0.10 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) and slope ($0.08 \pm 0.04 \text{ g CO}_2 \text{ X mg root cm}^{-3}$) 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 R_h/R_s assessment ($54 \pm 41\%$, 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 R_h/R_s to the $\delta^{13}\text{C}$ natural abundance technique. Consequently the root regression technique was assessed as high

400 accuracy and low precision (Table 7). Previous studies also highlighted large variation of CO₂ 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

405 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
410 (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
415 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
420 satisfactory. That is, each technique had strengths and weaknesses (Table 7). Using $\delta^{13}\text{C-CO}_2$ is acknowledged as the preeminent way to segregate Rh/Rs (Cheng, 1996; Kuzyakov, 2006); and accordingly accuracy was defined by the difference with this method. We recognize this was a precarious approach because the $\delta^{13}\text{C-CO}_2$ method had a large variation. Fortunately, the root regression, which is also recognized in the literature as a reliable method (Kuzyakov, 2006), gave a similar % Rh estimate. However, we found several other shortcomings with the $\delta^{13}\text{C-CO}_2$ method. First,
425 the conjunction of field and lab procedures makes it difficult to complete this method in one day as needed. Second, the air flushing with CO₂ free gas in the field (to prevent ambient $\delta^{13}\text{C-CO}_2$ 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 $\delta^{13}\text{C-CO}_2$ needs to be quickly assessed with a calibrated and accurate spectrometer (Midwood et al., 2006).

430 Fourth, ~~in our study we found the~~ large variation~~ance~~ in $\delta^{13}\text{C-CO}_2$ 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 $\delta^{13}\text{C-CO}_2$ with soil properties and root characteristics. As standalone, the $\delta^{13}\text{C-CO}_2$ technique was unable to produce assessment of soil CO_2 efflux; thus needed to be performed in conjunction with field Rs measurements. In this regard~~s~~, the $\delta^{13}\text{C-CO}_2$ 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 standardize~~d~~ Rh% determination.

435 The root regression method had the advantage to be simple, to produce an average Rh% close to the $\delta^{13}\text{C-CO}_2$ 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.

440 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 $\delta^{13}\text{C-CO}_2$ 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

445 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 $\delta^{13}\text{C-CO}_2$ determination made with the collars located in the center of the bags showed no isotopic signature of root respiration.

450 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 $\delta^{13}\text{C-CO}_2$ 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.

455 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 CO₂ efflux originating from the mineralization of the slow turnover SOC pool (i.e. basal soil respiration) (Pell et al., 2006). Assessment of basal soil
460 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 $\delta^{13}\text{C-CO}_2$ 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
465 rhizosphere derived Rh. Also, future studies on soil CO₂ efflux segregation should include other partition techniques like the trenching method and the radiocarbon-based assessment (Chiti et al., 2015). Overall, results from field experiments exhibited a range of potential Rh between 2.5 and 6.0 Mg CO₂-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.

470 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 variation ~~of~~ in 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
475 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 $\delta^{13}\text{C-CO}_2$ 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
480 more integral assessments.

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608 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 ₂ O)	pH (KCl)	WHC (g H ₂ O 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	-	-

609 ^a SL, sandy loam; CL, clay loam.

610 ^b gr, granular; sbk, subangular blocky.

611 WHC, water holding capacity

612 C, carbon; N, nitrogen

613

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

Method	Soil temperature (°C)	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

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

617

618 Table 3: Linear regression report between root density^a and CO₂ 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

619 Overall r² of the linear regression: 0.21.

620 ^a root density in unit of milligram, small (radius between 0.1-0.5 cm) dried roots (60°C) per cm³ of soil.

621 ^b SE, standard error.

622

623 Table 34: Parameter values of the Gaussian 3D regression fitted curve (equation 1)

Efflux	Parameter a (Mg ha ⁻¹ v ⁻¹ g m ² h ⁻¹)	Parameter xo	Parameter yo	Parameter b	Parameter c
Rh incubation	5.00-21 ***	49.2 ***	34.7 ***	15.7 ***	19.2 ***
Rs field	10.30-43 **	24.9 **	18.3 NS	9.6 NS	15.8 NS
Rh IB	5.70-24 NS	21.93 NS	21.4 NS	4.8 NS	13.4 NS
Rh HS	8.10-34 NS	21.7 NS	9.5 NS	5.1 NS	14.2 NS

624 Rh incubation, heterotrophic respiration from the soil cores incubation

625 Rs field, total soil respiration from outside of the root exclusion bags

626 Rh IB, heterotrophic respiration from the intact root exclusion bags

627 Rh HS, heterotrophic respiration from the hand-sorted root exclusion bags

628 ** and *** significant at p < 0.01 and p < 0.05, respectively; NS non-significant.

629 Parameters from equation 1. Parameter “a” correspond to the height of the maximum high of the curve (g CO₂ m⁻² h⁻¹); “xo” is
630 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

631 Gaussian root mean squared widths of the soil temperature and soil moisture, respectively.

632

633 | Table 45: $\delta^{13}\text{C-CO}_2$ results

Method	$\delta^{13}\text{C-CO}_2$ (‰)
Rs ^a	-18.21 (0.53) $\alpha\beta$
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

634 ^a Rs, gas samples collected from the field total soil respiration collars.

635 ^b Rh HS, gas samples collected from the field hand-sorted root exclusion bags collars.

636 ^c Rh IB gas samples collected from the field intact blocks root exclusion bags collars.

637 ^d Rh lab, gas samples collected from lab incubations of soil with freshly removed roots.

638 ^e Ra lab, gas samples collected from lab incubations of the roots extracted in Rh lab.

639 Values are means and standard error, n = 14 for Rs and Ra and n = 7 for HS, IB and Rh lab.

640 Values followed by a different Greek letter (α , β) are significantly different from each other at $\alpha=0.05$.

641

642 | Table 56: 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 $\delta^{13}\text{C-CO}_2$ natural abundance	-	-	61 (39)

643 Values are means and standard error, n = 22 for the root regression, n = 47 for soil incubation,

644 n = 28 for both root exclusion bags techniques.

645 ^a Rh, heterotrophic respiration.

646 ^b Rs, total soil efflux taken alongside the Rh efflux.

647 ^c Efflux range at temperature between 14°C and 26°C.

648 ^d Calculated as Rh from incubation at 14°C and 26°C divided by average field Rs at 14°C and 26°C respectively.

649

650 | Table 67: 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 $\delta^{13}\text{C-CO}_2$ natural abundance	High	Low	Complex	1-2 days	6 months to 1 year

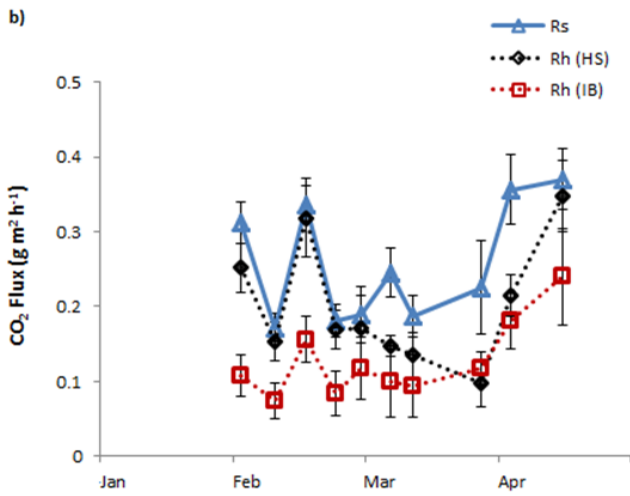
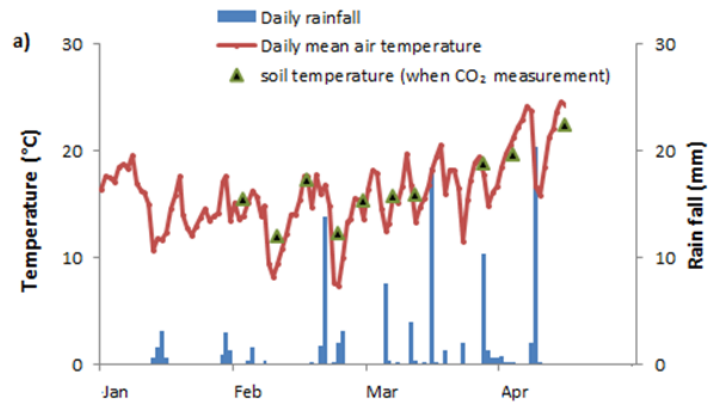
651 ^a Partition methods that had Rh%: <10, 10-20 and >20 lower or larger than the $\delta^{13}\text{C-CO}_2$ natural abundance were categorized as high, intermediate and low accuracy, respectively.

652 ^b High, intermediate and low precision were attributed to Rh% standard errors of <10, 10-20 and >20, respectively.

653 ^c Methods with five steps or less were deemed simple and six steps or more deemed as more complex.

654 ^d The time needed to setup experiment was assessed with the number of working hours required prior to be able to start the measurements.

655 ^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
 656 year.
 657



660 **Figure 1: a) Soil and air temperature and daily rainfall over the study period; b) Total soil CO₂ efflux (Rs), heterotrophic CO₂ efflux (Rh) from hand-sorted root exclusion bags (HS), and Rh from intact block root exclusion bags (IB).**

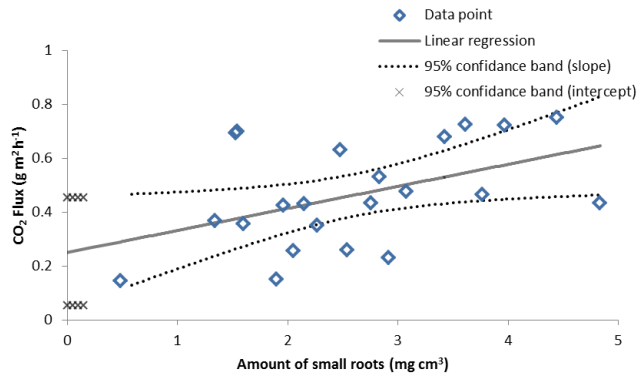


Figure 2: Linear regression between root quantity and CO₂e flux.

665

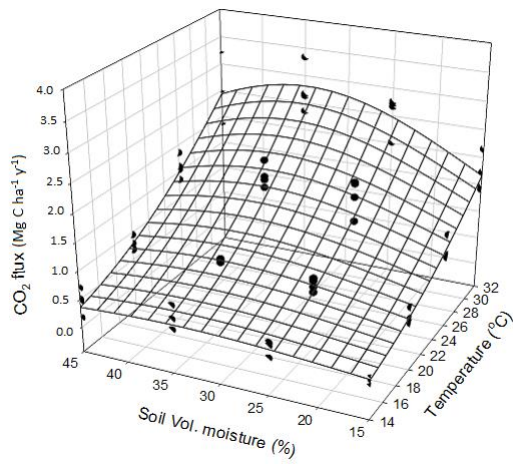


Figure 3: Results from the lab incubation; regression between incubation temperature, moisture and CO₂ efflux.

