A new approach to trenching experiments for measuring root–rhizosphere respiration in a lowland tropical forest

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ABSTRACT

Soil respiration in tropical forests is a major source of atmospheric CO₂. The ability to partition soil respiration into its individual components is becoming increasingly important to predict the effects of disturbance on CO₂ flux from the soil as the responses of heterotrophic and autotrophic respiration to change are likely to differ. However, current field methods to partition respiration suffer from various methodological artefacts; root–rhizosphere respiration is particularly difficult to estimate. We used trenched subplots to estimate root–rhizosphere respiration in large-scale litter addition (L+), litter removal (L−) and control (CT) plots in a lowland tropical semi-evergreen forest in Panama. We took a new approach to trenching by making measurements immediately before-and-after trenching and comparing them to biweekly measurements made over one year. Root–rhizosphere respiration was estimated to be 38%, 17% and 27% in the CT, L+, and L− plots, respectively, from the measurements taken immediately before and one day after trenching in May–June 2007. Biweekly measurements over the following year provided no estimates of root–rhizosphere respiration for the first seven months due to decomposition of decaying roots. We were also unable to estimate root–rhizosphere respiration during the dry season due to differences in soil water content between trenched and untrenched soil. However, biweekly measurements taken during the early rainy season one year after trenching (May–June 2008) provided estimates of root–rhizosphere respiration of 39%, 24% and 36% in the CT, L+, and L− plots, respectively, which are very similar to those obtained during the first day after trenching. We suggest that measurements taken immediately before and one day after root excision are a viable method for a rapid estimation of root–rhizosphere respiration without the methodological artefacts usually associated with trenching experiments.

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1. Introduction

Litterfall and fine roots are two of the most dynamic components of carbon cycling in forest ecosystems as they have high turnover rates (Trumbore, 2000) and a large proportion of soil respiration is derived from live roots, root products, and the decomposition of litter (Kuzyakov, 2006). Globally, soil respiration releases approximately 80 Pg of carbon into the atmosphere per year, to which tropical and subtropical forests contribute more than any other biome (Raich et al., 2002); thus, the potentially critical role of tropical soils as future sinks or sources of atmospheric CO₂ has led to increased interest in soil carbon cycling in tropical forests.

Soil respiration is the sum of heterotrophic respiration (soil fauna, bacteria and fungi) during decomposition of organic matter, and autotrophic (root) respiration. The ability to partition soil respiration into these components is becoming increasingly important in the context of predicting the effects of environmental change because the sensitivity to disturbance of autotrophic and heterotrophic respiration is likely to differ (Subke et al., 2006). There is evidence that heterotrophic respiration and root respiration may respond differently to increased temperature (e.g. Boone et al., 1998; Epron et al., 2001), drought (e.g. Borken et al., 2006) and elevated atmospheric CO₂ (e.g. Edwards and Norby, 1998). Root respiration is strongly influenced by aboveground assimilation and growth (Horwath et al., 1994; Robinson and Sarmiento, 1995). The heterotrophic component of soil respiration is strongly influenced by substrate availability (Raich and Tufekcioglu, 2000; Vasconcelos et al., 2004), which is closely related to aboveground litterfall on a global scale (Raich and Nadelhoffer, 1989; Davidson et al., 2002), and is thus also ultimately driven by aboveground growth and production (Rey et al., 2002). Recently, a microcosm study showed that increased litter

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inputs under elevated CO\textsubscript{2} would greatly increase microbial respiration in the soil (Liu et al., 2008) and FACE experiments have shown large increases in root respiration, which may be related to increased root biomass or higher specific root respiration rates (Andrews et al., 1999; King et al., 2001; Pregitzer et al., 2008).

Partitioning soil respiration in the field is difficult because it usually involves a large amount of disturbance and changes in the abiotic environment. For example, litter exclusion can result in soil drying caused by greater evaporation from the unprotected soil surface and differences in soil water balance (Walsh and Voight, 1977; Sayer, 2006). Reviews and meta-analyses of respiration partitioning studies highlight the difficulties involved in the different methods employed and generally agree that isotopic labelling is the most accurate method with the fewest methodological artefacts (e.g. Hanson et al., 2000; Subke et al., 2006; Kuzyakov, 2006). However, isotope methods are costly and not readily applicable to most field studies (Kuzyakov, 2006) and thus litter and root exclusion techniques remain the most feasible way to partition soil respiration directly in the field. While some of the unwanted side effects of litter exclusion can be reduced by using artificial litter that does not contribute to CO\textsubscript{2} efflux (Ewel et al., 1987), measuring root respiration is much more problematic.

There is to date no widely accepted field method to differentiate between autotrophic respiration from roots, respiration by associated mycorrhizae and the heterotrophic respiration of labile C compounds from roots (e.g. root exudates and sloughed-off root cells; Andrews et al., 1999; Kuzyakov, 2006), consequently these components are usually estimated together (sensu Wiant, 1967) and we henceforth use the term ‘root—rhizosphere respiration’ to include both autotrophic root respiration and heterotrophic respiration in the rhizosphere.

Tree-girdling has been used successfully to estimate root—rhizosphere respiration in a northern Scots pine forest (Högberg et al., 2001) and has the advantage of not changing the abiotic soil environment (Subke et al., 2006), however it has been less successful in studies of tropical trees because of large carbohydrate reserves in the existing root system of some species, which provide C for the maintenance of fine roots when the supply of C from aboveground assimilation is disrupted (Binkley et al., 2006; Nottingham et al. unpublished data). Thus, most attempts at estimating root—rhizosphere respiration in tropical and subtropical forests have used either an indirect mass balance approach (e.g. Trumbore et al., 1995; Silver et al., 2005) or trenching experiments (e.g. Li et al., 2005; Silver et al., 2005).

In trenching experiments a trench is cut around a block of soil to kill all roots and mycorrhizal hyphae within the area and a solid barrier is installed to exclude root regrowth; respiration rates in the root-free soil are then compared to respiration rates in control plots with roots present and root—rhizosphere respiration is estimated from the difference. Although this approach is fairly simple and can be carried out in most ecosystems, it is quite labour-intensive and entails several inherent problems in estimating root—rhizosphere respiration: i) decomposition of the cut roots can lead to high heterotrophic respiration (Hanson et al., 2000; Subke et al., 2006) which can last for several months to over a year, depending on soil type (Silver et al., 2005); ii) the presence or absence of roots in the soil leads to differences in soil water content which can greatly affect heterotrophic respiration rates (Subke et al., 2006); iii) seasonal differences in respiration rates, soil water content and soil temperature; and iv) roots have been shown to grow underneath installed barriers and recolonize root-free soil (Edwards and Norby, 1998; Tanner and Barberis, 2007). Estimates of root—rhizosphere respiration in tropical forests range widely from 27 to 76\% of total soil respiration (Subke et al., 2006) and it is uncertain how much of this variation results from differences in methodology.

We used a new approach to measure root—rhizosphere respiration in a lowland tropical forest by taking measurements immediately before trenching and for several days afterwards (before-and-after approach); we then continued to take measurements for the following 13 months (classic trenching experiment) to compare the results from the two methods. We hypothesized that isolating a block of soil by trenching would cause an immediate halt in root—rhizosphere respiration. Thus, the decrease in soil respiration in the days following root excision would provide a good estimate of root—rhizosphere respiration without being affected by the methodological problems usually associated with trenching experiments. Furthermore, the trenching experiment was carried out within a large-scale litter manipulation project to determine whether previously observed changes in fine root biomass in the litter manipulation treatments (Sayer et al., 2006) also affected the contribution of roots and the rhizosphere to total belowground respiration.

2. Materials and methods

2.1. Study site

The study was carried out within an ongoing large-scale, long-term litter manipulation experiment located on the Gigante Peninsula of the Barro Colorado Nature Monument in Panama, Central America. Nearby Barro Colorado Island (4.5 km from the study site) has a mean annual rainfall of 2600 mm with a strong dry season from January to April and an average temperature of 27° C (Leigh, 1999). The forest under study is old-growth moist lowland tropical forest. The soil is an oxisol with pH c. 5.0, low concentrations of extractable inorganic phosphorus (Bray’s and Mehlich III), but high base saturation and cation exchange capacity (Cavelier, 1992; Sayer et al., 2006).

Fifteen 45-m × 45-m plots were established in 2000. The plots were trenched to a depth of 0.5 m to minimize nutrient- and water-import via the root/mycorrhizal network and the sides of the trenches were double-lined with plastic and backfilled; a 7.5 m buffer was left around the inside of the trenches to eliminate trenching effects, resulting in a measurement plot size of 30-m × 30-m. Starting in January 2003, the litter (including small branches) in five plots was raked up once a month, resulting in low, but not entirely absent, litter standing crop (L− plots). The removed litter was immediately added to five further plots, where it was spread out as evenly as possible (L+ plots); five plots were left undisturbed as controls (CT plots). Treatments were applied in a stratified random design according to mass of pre-treatment litterfall (Sayer et al., 2007).

2.2. Respiration measurements

Four soil respiration collars were installed in each of the 15 litter manipulation plots in 2005 by sinking PVC tubes (20 cm inner diameter and 12 cm height) into the ground to 2 cm depth (Sayer et al., 2007). In February 2007, one 2-m × 2-m subplot was marked out at a randomly chosen location without stems along the edge of each litter manipulation plot and a fifth respiration collar was installed as described above in the centre of each subplot; In May 2007, respiration was measured over all the collars using a Li-8100 soil CO\textsubscript{2} flux system (LI-COR, Lincoln, USA) and then the subplots were trenched to 0.5 m depth, the inner side of the trenches were lined with four layers of construction plastic and the trenches were refilled. Trenching took place from 15 to 30 May 2007; three 2-m × 2-m subplots (one in each litter manipulation treatment) were simultaneously trenched, usually within two days. Trampling and disturbance inside the subplots was avoided as far as possible during trenching and casual observation showed that trenching to 0.5 m depth cut all fine roots and c. 90\% of larger roots. Respiration was measured over the collars 1, 3, 5, 7, and 14 days after trenching and then every 14 days (henceforth: biweekly) for one year until the end of June 2008. All respiration measurements were made
during one day each month between 8.00 h and 14.00 h but no measurements were taken during or immediately following heavy rainfall. Newly established seedlings and creepers were removed from the trenched subplots every two weeks. In May 2008, the trenches around the subplots were recut to a depth of 60 cm around the outer side using a machete to sever roots growing through or underneath the plastic barriers.

2.3. Soil temperature and soil water content

Soil temperature was recorded during respiration measurements within 0.5 m of the collars using a soil temperature probe inserted to a depth of 100 mm. Volumetric soil water content was measured from 0 to 60 mm depth using a thetaprobe (Delta-T Devices, Cambridge, UK), which was calibrated to the soil type in the plots following the procedure described by Delta-T. Due to technical problems, volumetric soil water measurements were not made from 26 December–30 January 2007, 26 March–8 April 2008 and 15 May–25 June 2008.

2.4. Data analysis

The relative contribution of roots to total belowground respiration was estimated using two different approaches:

1) The immediate effect of trenching on belowground respiration was assessed by comparing the respiration values taken before trenching (D0) with the values measured at 1, 3, 5 and 7 days after trenching (D1, D3, D5 and D7) by performing a paired t-test for each comparison (D0 vs. D1; D0 vs. D3; D0 vs. D5 and D0 vs. D7) and then applying the Bonferroni correction. As heavy rainfall strongly affected soil respiration in the first trenched subplots, only nine subplots (three from each of CT, L+ and L−) were included in the analysis. The relative contribution of roots to soil respiration in each treatment was estimated from the observed decreases in soil respiration during the first week after trenching.

2) The differences in respiration between trenched and untrenched soil for the rest of the study period (June 2007–June 2008) were investigated using repeated measures ANOVAs. As the original litter manipulation plots were not blocked, for analysis we considered ‘trenched’ or ‘untrenched’ as treatments and litter manipulation (CT, L+ and L−) as blocks. We divided the biweekly measurements into two periods for analysis: i) June–December 2007, when decomposition of the severed roots was thought to dominate soil respiration and ii) January–June 2008 when decomposing roots in the trenched subplots were thought to contribute little to soil respiration. This division was based on the finding that 50% of mass loss during root decomposition on nearby Barro Colorado Island occurred within the first seven months (Cusack et al., 2009). The relative contribution of roots and the rhizosphere to soil respiration in each treatment was then estimated as:

\[ R_{\text{ROOT-RHIZO}} = (R_{\text{SOIL-UNTRENCHED}}) - (R_{\text{SOIL-TRENCHED}}) \]

Differences between trenched and untrenched sites in soil water content and soil temperature were analysed with repeated measures ANOVAs for the whole study period as described above. The relationships between soil respiration and soil water content or soil temperature were investigated by regression curve estimation in SPSS 16 for Mac (SPSS Inc., Chicago, USA); all other analyses were carried out in Genstat 8 (VSN International Ltd., UK).

3. Results

3.1. The contribution of the root—rhizosphere to belowground respiration

Trenching immediately decreased soil respiration: on the first day after trenching soil respiration in the trenched subplots was on average 38% lower in the CT plots, 17% lower in the L+ plots and 27% lower in the L− plots compared to pre-trenching (D0) respiration rates (Bonferroni, \( P = 0.02 \); Fig. 1). Soil respiration remained significantly lower than the pre-trenching values for five days after trenching (Bonferroni, \( P \leq 0.02 \); Fig. 1); during this time soil respiration was on average 37% lower in the CT plots, 18% lower in the L+ plots and 36% lower in the L− plots compared to pre-treatment respiration rates. In the untrenched soil, respiration rates did not differ from D0 values during the first week after trenching and the measurements taken on the first day after trenching were the same as, or higher than, pre-trenching values (Fig. 1).

Trenching had no consistent effect on soil respiration from June to December 2007 (Fig. 2) and there was no significant time \( \times \) treatment interaction. From the beginning of January 2008 to the end of June 2008 trenching reduced soil respiration rates by 22% in the CT plots, 18% in the L+ plots and 30% in the L− plots (\( P = 0.011, df = 1.26, F = 7.58; \) Fig. 2). There was no block effect but there was a significant time \( \times \) treatment interaction (\( P = 0.003, df = 10, 274, F = 3.74 \)) indicating an effect of seasonality; soil respiration during the driest time of the year in March and April 2008 was similar in trenched and untrenched soil and differences between trenched and untrenched soil were only observed from January–February and May–June 2008 (Fig. 2). From the beginning of May 2008 to the end of June 2008, one year after the trenches were dug, trenching had reduced soil respiration rates by 39% in the CT plots, 24% in the L+ plots and 36% in the L− plots (Fig. 2), which was remarkably similar to the reductions measured a year earlier in the five days after the trenches were cut.

3.2. Soil water content and soil temperature

Soil water content was significantly increased by trenching (0–6 cm depth; \( P < 0.001, df = 1.25, F = 28.1 \)) especially during the driest time of the year in March and April 2008 when soil water content in the trenched soil was 65% higher in the CT plots, 84% higher in the L+ plots and 32% higher in the L− plots compared to measurements over untrenched soil (Fig. 3). Soil respiration tended to decrease at both low (<0.3 m3 m−3) and high (>0.5 m3 m−3) soil water contents and thus a quadratic regression model best described the relationship between soil water content and soil respiration. Soil water content (0–6 cm depth) was a reasonably good predictor of soil respiration only when roots and litter were present (i.e. in the untrenched soil in the CT and L+ plots), explaining 25% and 29% of the variance in soil respiration in the CT and L+ plots, respectively (\( P < 0.01 \)).

Soil temperature (0–10 cm depth) ranged from 23.6 °C to 27.4 °C over the study year but was not affected by trenching or litter manipulation. Soil temperature explained 26%, 42% and 24% of the variation in soil respiration in untrenched soil in the CT, L+ and L− plots, respectively (\( P < 0.001 \)) and the relationship between soil temperature and soil respiration was best described with a linear regression model. In the trenched soil, the relationship between soil temperature and soil respiration was only significant when litter was absent (L− plots: \( R^2 = 0.3, P < 0.001 \)).

4. Discussion

4.1. Root–rhizosphere respiration

The estimates of root–rhizosphere respiration obtained from measurements taken one day before and one day after trenching
were very similar to the values obtained at the same time of year (May–June) after one year’s biweekly measurements. In our study, the immediate decrease in soil respiration on the first day after trenching (D1) provided the most reliable estimate of root–rhizosphere respiration, as respiration rates in the untrenched soil did not decrease from D0 to D1 in any treatment but varied thereafter (Fig. 1); we can thus be confident that the decrease measured in the trenched soil on D1 was due to root excision. Furthermore, in a mesocosm study of soil dominated by root respiration, soil respiration declined to 50% of the starting value within four hours of root excision and to 5% within c. 24 h (Nottingham et al., unpublished data).

The estimated root–rhizosphere respiration in the control and L+ plots remained relatively constant during the first five days after trenching but it varied considerably in the L− plots. It is therefore likely that at least part of the observed variation in the L− plots during this time (D3 and D5; Fig. 1) was a result of the absence of litter on the forest floor. The estimated contribution of root–rhizosphere respiration to total belowground respiration in the control plots of 25% (January–February 2008)–39% (May–June 2008) also closely corresponds to the 24–35% estimated for clay soils in lowland tropical rainforest in the Amazon (Silver et al., 2005).

We had expected the lower relative contribution of root–rhizosphere respiration to total belowground respiration in the L+ plots (17%) compared to the control plots (38%) as fine root biomass was shown to be c. 30% lower in the L− plots (Sayer et al., 2006, 2007) and root–rhizosphere respiration is thought to be directly related to fine root biomass (Rout and Gupta, 1989). The lower relative contribution of root–rhizosphere respiration is apparent in both the before-and-after calculations and the classic trenching calculations, which allows us to conclude that our new approach can be used successfully to compare root–rhizosphere respiration in soils with different root densities.

Thus, by taking measurements before and intensively in the days after trenching we were able to estimate root–rhizosphere respiration within a week of cutting the roots and thus eliminate the major methodological issues that can affect results in trenching experiments: 1) decomposition of excised roots, 2) differences in soil water content and temperature between trenched and untrenched plots, 3) effects of seasonality and 4) root regrowth into trenched plots.

One week after trenching the differences in soil respiration between trenched and untrenched soil disappeared and there was no effect of trenching on soil respiration rates for the next seven months, which we had expected as a result of increased heterotrophic respiration from the decomposition of the cut roots in the trenched subplots (e.g. Hanson et al., 2000). Similarly, Silver et al. (2005) showed high rates of root decomposition during nine months after trenching in similar soils in Amazonian rainforest.

Soil water content did not differ between trenched and untrenched soil during the first week after trenching, probably because it takes several days for the absence of live roots to affect soil water content. Thus, our new approach reduced the likelihood of differences in soil respiration between treatments resulting from changes in soil water whereas our biweekly measurements from June 2007 to June 2008 were affected by the consistently higher soil water content in the trenched subplots. It is very likely that rainfall seasonality combined with the differences in soil water content between trenched and untrenched soil masked decreases in soil respiration due to trenching: we were unable to estimate root–rhizosphere respiration during the driest months of the year (March and April 2008) as the soil water content in the trenched soil was 32–84% higher than in the untrenched soil due to the lack of uptake by roots. These large differences in soil water content most likely lead to increased heterotrophic respiration rates in the trenched plots during the dry season (Subke et al., 2006), resulting in similar respiration rates in trenched and untrenched soil in the CT and L+ plots (Fig. 2). Interestingly, the greatest differences in soil water content between trenched and untrenched soil during the dry season (84% higher in trenched subplots) were observed in the L+ plots where the thick

![Fig. 1. Soil respiration in trenched subplots (dashed lines) and untrenched soil (solid lines) in litter manipulation plots in lowland tropical forest in Panama, Central America, in May 2007; squares are controls, triangles are litter addition, circles are litter removal. Top panels show measured soil CO2 efflux during the first 14 days after trenching in a) controls, b) litter addition and c) litter removal treatments; error bars are standard errors of means for N = 3. Bottom panels show soil CO2 efflux during the first 14 days relative to pre-trenching values in d) controls, e) litter addition and f) litter removal treatments.](image-url)
litter cover protected the soil from evaporation and drying (Matthews et al., 2008), and the smallest difference (32% higher in trenched subplots) was observed in the L/C0 plots (Fig. 3), where the lack of litter probably lead to greater evaporation from the soil surface (Walsh and Voight, 1977; Sayer, 2006).

4.2. Treatment effects on the relationships between soil respiration, soil temperature and soil water content

Soil respiration is generally strongly influenced by soil temperature (Singh and Gupta, 1977; Lloyd and Taylor, 1994); in our study, despite a very small temperature range (<4 °C), soil temperature at 0–10 cm depth explained 24–42% of the variation in soil respiration when roots were present, while in the trenched subplots, soil respiration was only related to soil temperature in the litter removal treatments. Increased sensitivity of soil respiration in the presence of roots has previously been shown for temperate forests (Boone et al., 1998) and it appears that a similar relationship may exist in tropical forests even though they have a much smaller range in soil temperatures.

Soil water content was somewhat less important in explaining variation in soil respiration but respiration was generally suppressed at high (>0.5 m³ m⁻³) and low soil water content (<0.3 m³ m⁻³) when roots were present. The lack of relationship between soil water content and soil respiration in the trenched subplots was likely a consequence of smaller variation in soil water content in the absence of water uptake by roots.

4.3. Conclusions

We propose that measurements taken one day before and one day after trenching can give a rapid accurate estimate of root—rhizosphere respiration. The method eliminates many of the methodological artefacts associated with ‘classic’ trenching experiments, which are carried out for a year or longer, and is much less labour-intensive than using long-term trenched plots. Our new procedure has several potential uses in future studies of root—rhizosphere respiration: 1) it can be used to compare root—rhizosphere respiration in different soils with different root decay rates 2) it can be done repeatedly to
gain insights into seasonal changes in root–rhizosphere respiration, 3) it can be done in a less destructive manner than trenching by simply cutting around blocks of soil instead of digging out a trench, and 4) it can be combined with measurements of root biomass from soil cores to quantify the relationship of root–rhizosphere respiration and root biomass. We recommend that measurements be taken over several days after root excision to ensure that the maximum decrease due to root excision occurs on the first day. We also recommend that measurements be taken over collars in untrrenched soil at the same time to control for any changes in soil respiration due to environmental factors (e.g. rainfall or temperature).

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