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Soil CO_2 efflux and fungal and bacterial biomass in a plantation and a secondary forest in wet tropics in Puerto Rico

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Abstract

We examined the effects of root and litter exclusion on the rate of soil CO_2 efflux and microbial biomass using trenching and tent separation techniques in a secondary forest (SF) and a pine (*Pinus caribaea* Morelet) plantation in the Luquillo Experimental Forest in Puerto Rico. Soil surface CO_2 efflux was measured using the alkali trap method at 12 randomly-distributed locations in each treatment (control, root exclusion, litter exclusion, and both root and litter exclusion) in the plantation and the SF, respectively. We measured soil CO_2 efflux every two months and collected soil samples at each sampling location in different seasons to determine microbial biomass from August 1996 to July 1997. We found that soil CO_2 efflux was significantly reduced in the litter and root exclusion plots (7-year litter and/or root exclusion) in both the secondary forest and the pine plantation compared with the control. The reduction of soil CO_2 efflux was 35.6% greater in the root exclusion plots than in the litter exclusion plots in the plantation, whereas a reversed pattern was found in the secondary forest. Microbial biomass was also reduced during the litter and root exclusion period. In the root exclusion plots, total fungal biomass averaged 31.4% and 65.2% lower than the control plots in the plantation and the secondary forest, respectively, while the total bacterial biomass was 24% and 8.3% lower than the control plots in the plantation and the secondary forest, respectively. In the litter exclusion treatment, total fungal biomass averaged 69.2% and 69.7% lower than the control plots in the plantation and the secondary forest, respectively, while the total bacterial biomass was 48% and 50.1% lower than the control plots in the plantation and the secondary forest, respectively. Soil CO_2 efflux was positively correlated with both fungal and bacterial biomass in both the plantation the secondary forest. The correlation between soil CO_2 efflux and active fungal biomass was significantly higher in the plantation than in the secondary forest. However, the correlation between the soil CO_2 efflux and both the active and total bacterial biomass was significantly higher in the secondary forest than in the plantation in the day season. In addition, we found soil CO_2 efflux was highly related to the strong interactions among root, fungal and bacterial biomass by multiple regression analysis ($R^2 > 0.61$, $P < 0.05$). Our results suggest that carbon input from aboveground litterfall and roots (root litter and exudates) is critical to the soil microbial community and ecosystem carbon cycling in the wet tropical forests.

Introduction

The total CO_2 efflux from forest soil is a combination of the contribution of autotrophic roots (including associated rhizospheric microbial population) and heterotrophic organisms. The knowledge of their relative contributions to total soil respiration will lead to a better understanding of carbon cycles in terrestrial ecosystems as well as between the ecosystems and the atmosphere (Edward, 1998; Luo et al., 1996). However, partitioning the total soil CO_2 efflux into autotrophic and heterotrophic respiration has been

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fraught with challenge because of there are no effective approaches for separating them during soil respiration measurements without disturbing the activities of roots and microbial organisms (Buchmann, 2000; Hanson et al., 2000; Hendrick and Pregitzer, 1993; Kurtz and Kimmins, 1987). Therefore, using indirect approaches to separate the two components, such as root removal (Hanson et al., 1993; Laudelout and Thierron, 1996), trenching (Edwards, 1998; Varner et al., 2003) and gap analysis (Brumme, 1995; Nakane et al., 1996), may help with gaining insight into the relative contribution of autotrophic and heterotrophic respiration to total soil CO_2 efflux.

In almost all ecosystems, microorganisms are responsible for most of the respiration and a large portion of the nutrient cycling. Microorganisms are generally considered the driving force behind soil decomposition processes (Smith and Paul, 1995). Knowledge of the roles of microbial biomass and their interactions with soil matrix in determining the rate of soil CO_2 efflux is critical for improving the scientific basis for forest management decisions and soil organic matter transformation modeling. It has been observed that seasonal change in microbial biomass could have important ramifications for nutrient cycling and ecosystem functioning (Lipson et al., 1999; Wardle, 1998; Zak et al., 1990).

Although there are a number of studies on soil CO_2 efflux and microbial biomass comparisons among various forest types, only a few studies have characterized soil CO_2 efflux and microbial biomass under tropical plantations, secondary forests and agro-forestry systems (Amatya et al., 2002; Lugo, 1992; Tufekcioglu et al., 2001). Secondary forests account for 40% of the total area of tropical forests and this percentage is still increasing throughout the tropics (Brown and Lugo, 1990). Secondary forests provide many economically valuable products, such as fruits, medicinal plants, and construction materials. Sustainable use of secondary forests could reduce the human-induced pressures on primary forests and prevent the primary forests from over harvesting. The success of management of tropical forests in the future might well depend upon the adequacy of our ecological understanding of secondary forests (Cheng, 1993). Tree plantations, covering 11 million ha of the tropics (Lanly, 1982), are rapidly increasing in area in the tropics given the high demand for timber supply. In addition, short-rotation plantation, referred to as Kyoto forest, has been recommended in the Kyoto Protocol as an effective measure to reduce atmospheric CO_2 concentration. Reforestation through plantation on abandoned and degraded agricultural lands in the tropics has been proposed as an effective carbon management approach (Montagnini and Porras, 1998).

Using a 20-year old pine plantation and a secondary forest originating from the same abandoned farmland in the wet tropics in Puerto Rico, we examined soil CO_2 efflux and microbial biomass after 7-year litter exclusion, root exclusion, and both litter and root exclusion treatments in the plantation and the secondary forest. Specifically, the objectives of the present study are to examine (1) the effects of root and litter on soil CO_2 efflux after a seven-year continuous exclusion of roots and aboveground litter; (2) the effects of the root and litter exclusion on total microbial biomass and the biomass of different functional groups, such as active fungi, active bacteria, total fungi, and total bacteria; and (3) the relationships between soil CO_2 efflux and fungal and bacterial biomass and their interactions in the plantation and the secondary forest.

Methods

Study sites

The study was conducted on two sites that were within 100 m of each other. One site was in a *Pinus caribaea* (Morelet)-dominated plantation and the other was in a secondary forest. Both sites were located in the Guzman sector of the Luquillo Experimental Forest in northeastern Puerto Rico (18°18′ N, 65°50′ W). The plantation and the secondary forests originated from the same abandoned agricultural land with the same cropping system and management/disturbance history (Lugo, 1992). The sites were characterized by a wet tropical climate with mean annual precipitation of 3920 mm and mean annual air temperature of 22.3 °C (Lugo, 1992). The temperature was mild and stable with diurnal and seasonal temperature ranges of 3–4 °C (Figure 1). Precipitation showed a seasonal variation with greater between August and October than from January to March (Figure 1). Soils were classified as mixed isothermic tropohumult in both the plantation and the secondary forest. The sites were relatively flat with a slope of < 5 degrees and an elevation of about 400 m above sea level.

The tree plantation was established on the abandoned cropland in 1976 as part of a reforestation program of the United States Forest Service (Lugo,

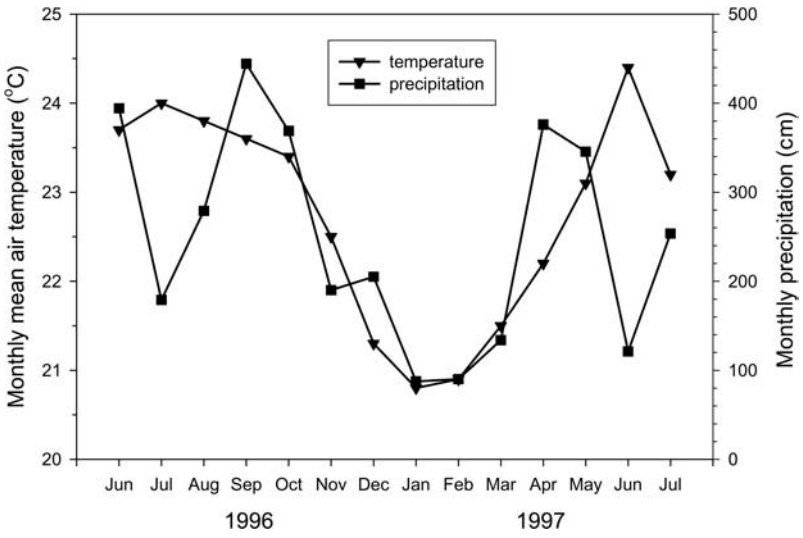

Figure 1. Monthly precipitation (mm) and air temperature (°C) in the Luquillo Experimental Forest in Puerto Rico from June 1996 to July 1997.

1992). The secondary forest had naturally developed on the same abandoned cropland since 1976, too. The plantation was dominated by *Pinus caribaea* (Morelet) with small trees and grass species underneath the canopy. When our study started in 1996, the average tree height was about 15 m and the average diameter at breast height (DBH) was 22 cm in the plantation. The secondary forest was characterized by a sparse overstory and a dense understory with abundant shrubs and grasses comparing to the plantation but there were still a lot of spaces among the vegetations. The dominant canopy species in the secondary forest include *Myrcia splendens* (Sw.), *Miconia prasina* (Sw.) and *Casearia arborea* (L C Richard). The major understory species include *Casearia sylvestris* (Sw.), *Miconia mirabilis* (Aubl.) and *Tabebuia heterophylla* (DC.).

Field measurement

This study was a part of a long-term experiment known as 'Soil Organic Matter Dynamic' (SOMD) in the Luquillo Experimental Forest. Three blocks were arranged in a square plot of 0.25 ha in the plantation and in the secondary forest initially established by the SOMD project in 1990. Three treatments (root exclusion, litter exclusion, and root+litter exclusion) and a control plot were established in each block. Within each treatment including the control, we randomly selected 4 sampling locations as independent replicates for soil CO_2 efflux and microbial biomass measurement. The litter exclusion treatment was implemented by building a tent 2 m above the ground surface using mesh screen with openings of 2 × 2 mm. The litterfall on roofs of the tents was cleared every seven days to allow sunlight to reach the soil surface covered by the tent. The root exclusion treatment was applied by trenching soil along the four sides of the plot to a depth of 1m and then a car tarp sheet was buried into the soil to keep roots from entering the plots. The litter-and-root exclusion (litter + root) treatment was conducted by building a tent and also burying a car tarp sheet as described above. The plots of root exclusion treatment were monitored frequently after the initial trenching to ensure no plants grow into it. The control plots were clearly marked and natural condition maintained.

Total C and N in soils were obtained using a Perkin Elmer CHN analyzer. Soil pH was measured in a 1:1 ratio of water to soil fresh weight with a combination electrode. Soil moisture contents were determined by oven-drying 10 g of fresh soil sampled 105 °C for 48 h. We did not measure soil temperature because the diurnal and seasonal temperature varied <3.5 °C at the weather station near our study plots. Additionally, the spatial variation of temperature among the plots is also expected to be minimal due to the small size and vicinity of the plots and relatively homogenous vegetation and topography in our study area.

Soil CO_2 efflux was measured bi-monthly in both the plantation the secondary forest from August 1996 to June 1997. We selected open space to place the chamber between the vegetation. Soil CO_2 efflux was measured using the alkali trap method (Carter, 1993).

A plastic chamber with an opening of 102.5 cm^2 and a height of 20 cm and a plastic cup with a diameter of 5 cm and a height of 7.5 cm were used for soil CO_2 efflux measurements at each location. The chamber and the cup were made of polyethylene and the CO_2 absorption/emission by the chamber wall was negligible. At each measurement location a trap unit was prepared by pipetting 15 mL 1.0 M NaOH solution into a plastic cup and placing it on the soil surface. A chamber was immediately placed over the alkali cup and its edge was pressed into the soil for 2 cm to ensure the chamber was well-sealed. Another cup was filled with the same solution and tightly sealed and placed outside of the chamber as the control to consider the CO_2 absorption during the solution transport. After 24 h, the cup was removed, enclosed with a lid, and taken to the laboratory for analysis. In the laboratory, alkali solutions were titrated with 1 M HCl solution to the phenolphthalein end point to determine the amount of NaOH left after excess $BaCl_2$ was added to the NaOH solution to precipitate the carbonate as insoluble $BaCO_3$. Soil CO_2 efflux was measured about every two months from August 1996 to June 1997.

Total microbial biomass was measured using a fumigation-incubation procedure (Jenkinson and Powlson, 1975) in August 1996 and March 1997, which represented a wet and a dry season, respectively. A soil sample from seven cores (3.75 cm in diameter) from each plot was collected to a depth of 10 cm and weighed for soil bulk density determination. Approximately 15 to 20 g of each soil sample was oven-dried at 105 °C for three days for determination of soil water content. Two sets of soil samples were prepared: one as a control and the other for fumigation treatment. Each sample containing 30 g of soil was placed into a 100 mL beaker. For the fumigation treatment, beakers were placed in a clean glass vacuum desiccator that was lined with moistened filter paper. A beaker containing 40 mL of alcohol-free chloroform was placed into the desiccator, which was then evacuated for 2–3 min. During the evacuation period, the chloroform was boiled vigorously until the vapor in the desiccator was near saturation. Samples were left in the desiccator for 18–24 h. The fumigated samples were vacuumed three times to extract the remaining chloroform in the soil. Each sample was inoculated with 1.0 g of well mixed inoculum and then placed into a 2-liter wide-mouth glass jar. A beaker containing 20 mL of 1 M NaOH was placed into each jar. The jars were closed using lids with rubber septa and incubated for 10 days at 25 °C. The quantity of NaOH that remained in each beaker was titrated with 1 M HCL with phenolphthalein as an indicator. The same soil sample was incubated with another 20 mL of 1 M NaOH for another 10 days and the remaining NaOH was titrated with HCl at the end of the incubation. Biomass of active and total fungi was estimated using the agar film techniques (Lodge and Ingham, 1991). Biomass of active and total bacteria was obtained using fluorescein isothiocyanate techniques (Zou and Bashkin, 1998). Microbial biomass was sampled twice in wet and dry seasons with four replicates in each sampling subplot.

Root biomass was determined by sampling four soil columns of $0.25 \times 0.25 \times 0.25$ m in each of the plantation and the secondary forest sites. The measurement of root biomass was conducted in wet and dry seasons with four replicates in each sampling plot during the study period. Roots were divided into two groups with root diameter >5 mm and <5 mm and oven-dried at 75 °C for one week to determine root biomass.

Data analysis

Significant differences among means were determined by Scheffe's multiple range test at $P = 0.05$. Differences among treatments were analyzed with one-way ANOVA for each component (Montgomery, 2001). Simple and multiple linear regression analyses were used to examine the relationships between soil CO_2 efflux and root, fungal and bacterial biomass and their interactions.

Results

Soil CO_2 efflux

The rate of soil CO_2 efflux, on average, was higher in the secondary forest (2.65 ± 0.18 g C m^{-2} day-1) than in the plantation (2.33 ± 0.15 g C m-2 day^{-1}). The rate of CO_2 evolution was fairly constant from August 1996 to February 1997 in both the plantation and the secondary forest (Figure 2). Soil CO_2 efflux at both sites reached the lowest level in April 1997, immediately following the dry period in March in Puerto Rico. The soil CO_2 efflux decreased markedly in both plantation and secondary forest with aboveground litter and/or root exclusion for 7 years (Figure 3). Litter exclusion reduced 60.7% of soil CO_2 efflux after the 7-year litter exclusion, averaged over the two forests. Litter exclusion had a larger ($P \leq 0.05$) effect on soil

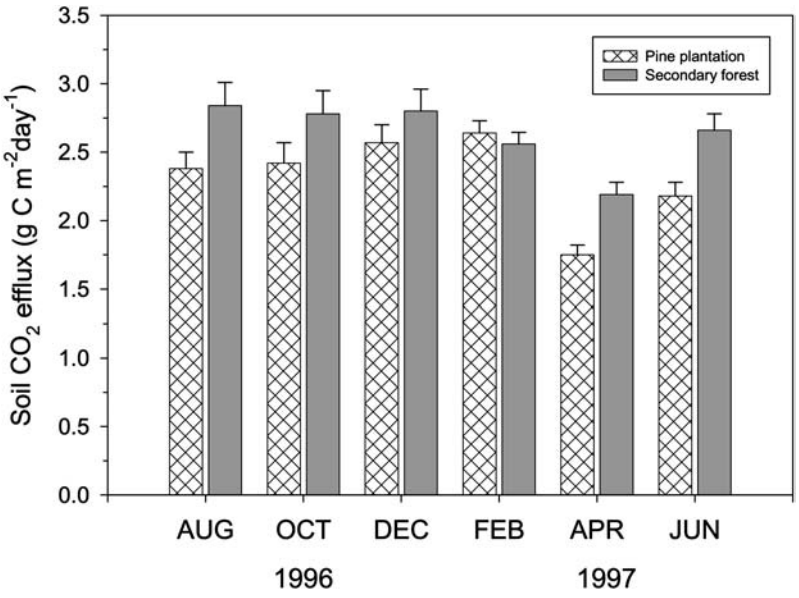

Figure 2. Soil CO_2 efflux (g C m^{-2} day^{-1}) and its seasonal variation in the control plots in the plantation and the secondary forests from August 1996 to June 1997.

CO_2 efflux in the plantation than the secondary forest with a reduction of 67.8% and 54.3%, respectively.

Root exclusion reduced soil CO_2 efflux by 63.5% by averaging the two forests after 7-year continuous root exclusion with a greater reduction in the in the secondary forest (69.5%) than in the plantation (56.2%). Soil CO_2 efflux decreased the most in the plots with both litter and root exclusion in both the plantation and the secondary forest. The rate of soil CO_2 efflux in the litter-and-root plots was reduced an average of 81.7% in these forests in comparison with the control plots.

Soil microbial biomass

Total soil microbial biomass (fungi + bacteria) in litter exclusion plots in both the plantation and the secondary forest were significantly ($P \leq 0.05$) reduced after 7-year litter exclusion. The total soil microbial biomass was reduced from 711 to 224 mg C kg^{-1} soil and from 618 to 202 mg C kg^{-1} soil, corresponding to a reduction of 68.5% and 67.3%, respectively, in the plantation and the secondary forest. Total bacterial biomass was significantly lower in the litter exclusion plots than the control plots in both the plantation and the secondary forest, but there was no significant ($P \leq 0.05$) difference in active bacteria biomass between the litter exclusion and the control plots in the plantation.

In the root exclusion plots, total soil microbial biomass was reduced on average from 711 to 489 mg C kg^{-1} soil and from 618 to 256 mg C kg−1 soil in the plantation and the secondary forest, respectively. These values of decrease accounted for a deduction of 31.2% and 58.6% of the total microbial biomass in these forests correspondingly. Root exclusion significantly ($P \leq 0.05$) reduced the total and active bacterial and total fungal biomass in both the plantation and the secondary forest. However, we did not find a significant ($P \leq 0.05$) difference in active fungi biomass between the root exclusion and the control plots in the secondary forest. Total soil microbial biomass in the litter-and-root exclusion plots decreased the most among all the treatments in both forests. Specifically, total fungal biomass was reduced 84.6% and 87.5% and total bacterial biomass was reduced 62.1% and 56.9%, respectively, in the plantation and the secondary forest. We found that microbial biomass demonstrated considerable seasonal variation in both the plantation and the secondary forest. Total microbial biomass, in general, was greater in the wet season than in the dry season with exceptions for the root exclusion and the litter-and-root exclusion plots in the secondary forest (Figure 5). Total and active fungal biomass was significantly ($P \leq 0.05$) higher in the plantation than in the secondary forest in all treatments during both the wet and dry seasons (Figures 4, 5). Total and active fungal biomass was significantly

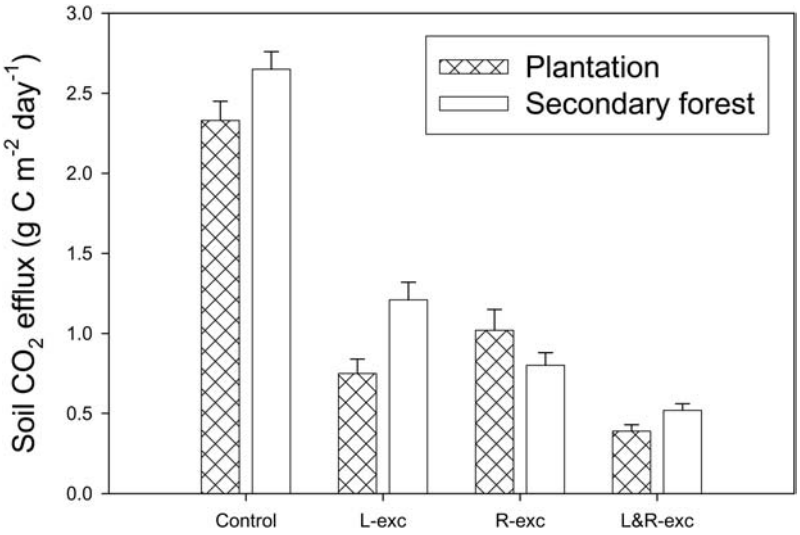

Figure 3. Mean soil CO_2 efflux (g C m−2 day^{-1}) in the control plot, litter exclusion (L-exc), root exclusion (R-exc) and both litter and root exclusion (L&R-exc) plots in the plantation and the secondary forest from August 1996 to June 1997.

greater in the wet season than in the dry season in both forests.

Microbial biomass was also significantly different among different treatments. The litter exclusion and the litter-and-root exclusion significantly reduced total fungal, total bacterial, active fungal, active bacterial biomass except for the active bacteria in the secondary forest during the wet season (Figures 4, 5). In most plots, the litter-and-root exclusion reduced microbial biomass the most in comparison with the control and the other treatments. While root exclusion significantly reduced total fungal biomass in both forests (Figure 5a, b), root exclusion did not significantly change total bacterial biomass except for the wet season in the secondary forest (Figure 5d). Root exclusion plots, in general, had the least reduction in both fungal and bacterial biomass among all the treatment plots.

Relationship between soil CO_2 efflux and microbial biomass

Soil CO_2 efflux was positively correlated with active fungal, total fungal, active bacterial and total bacterial biomass in both the plantation and the secondary forest and in both the wet and dry seasons (Table 1). However, a higher correlation between soil CO_2 efflux and active fungal biomass was found in the plantation than in the secondary forest in both the wet and the dry seasons. Using a simple linear regression model we found that active fungal biomass explained about 62% ($R^2 = 0.62$) and 51% ($R^2 = 0.51$) of the variance of soil CO_2 efflux in the wet and dry season, respectively, in the plantation and about 42% ($R^2 = 0.42$) in the secondary forest in both the wet and dry seasons. Meanwhile, active bacterial biomass explained 47% (wet season) and 35% (dry season) of the variance of soil CO_2 efflux in the plantation and 21% (wet season) and 64% (dry season) in the secondary forest (Table 1). Soil CO_2 efflux and active bacteria biomass were highly correlated in the secondary forest and weakly correlated in the plantation in the dry season, while a small difference was found between the two forests in the wet season. All the linear regressions between soil CO_2 efflux and active fungal and bacterial biomass are statistically significant ($P \leq 0.5$) except for the dry season in the plantation (Table 1). In addition, we examined the relationships between soil CO_2 efflux and total fungal and bacterial biomass and found similar results as using active fungal and bacterial biomass, though the corresponding R^2 values decreased slightly when the total fungal and bacterial biomass was used (Table 1).

We also conducted multiple linear regression analyses to estimate soil CO_2 efflux using fine root biomass, active or total fungal biomass, and active or total bacterial biomass. We found using active fungal and bacterial biomass gave was slightly better than using total fungal and bacterial biomass in estimating soil CO_2 efflux. Therefore, we just report our results of using the active fungal and bacterial biomass as follows:

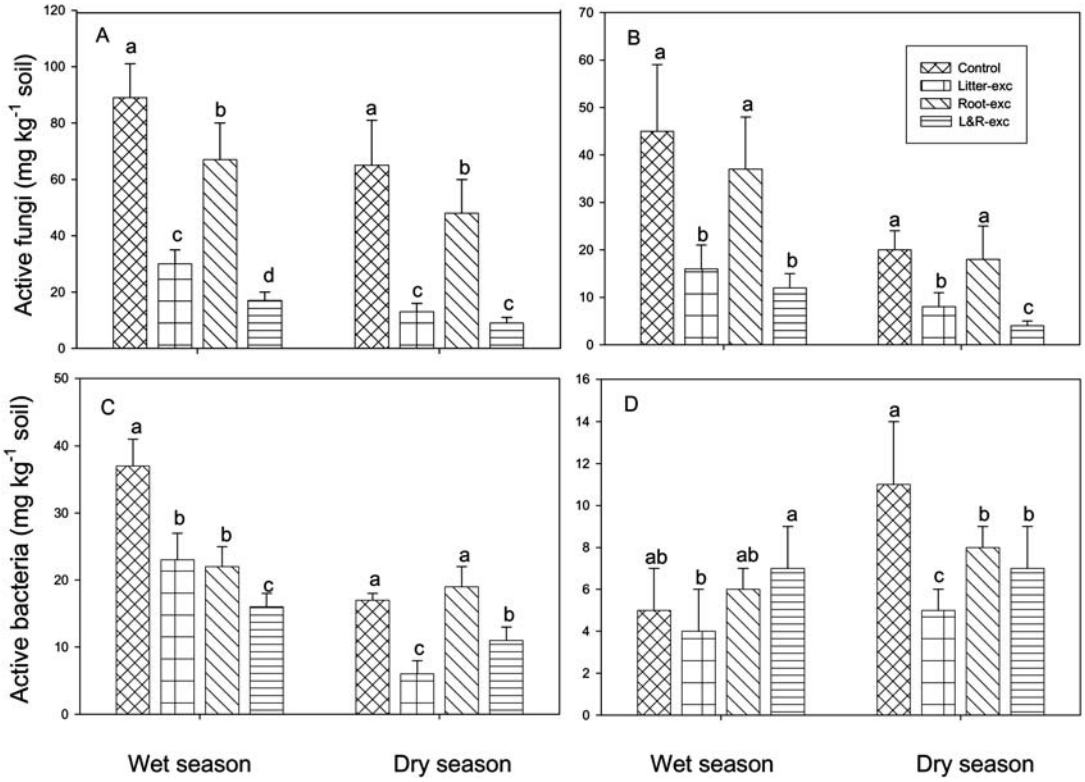

Figure 4. Active fungal biomass (mg C kg^{-1} soil) in the plantation (A) and the secondary forest (B) and active bacterial biomass (mg C kg^{-1} soil) in the plantation (C) and the secondary forest (D) in the wet (August) and dry (March) seasons. Common letters on the bars indicate no significant difference between the treatments at 95% confidence level.

Table 1. Linear regression between soil CO_2 efflux (g C m^{-2} day^{-1}) and fungal and bacterial biomass (mg C kg^{-1} soil) in the plantation and the secondary forest ($n = 12$)

Season	Ecosystem type	Microbial biomass	Regression equation	R^2	P
Wet	Plantation	Active fungi	$y = 1.45 + 0.011\times$	0.62	0.00
		Total fungi	$y = 1.62 + 0.001\times$	0.32	0.06
		Active bacteria	$y = 1.67 + 0.053\times$	0.47	0.01
		Total bacteria	$y = 1.97 + 0.014\times$	0.33	0.05
	Secondary forest	Active fungi	$y = 1.63 + 0.023\times$	0.42	0.02
		Total fungi	$y = 0.95 + 0.002\times$	0.51	0.01
		Active bacteria	$y = 2.17 + 0.012\times$	0.35	0.04
		Total bacteria	$y = 2.16 + 0.005\times$	0.37	0.03
Dry	Plantation	Active fungi	$y = 0.96 + 0.014\times$	0.51	0.00
		Total fungi	$y = 1.07 + 0.002\times$	0.41	0.02
		Active bacteria	$y = 1.59 + 0.033\times$	0.21	0.14
		Total bacteria	$y = 1.62 + 0.019\times$	0.12	0.27
	Secondary forest	Active fungi	$y = 1.58 + 0.033\times$	0.42	0.02
		Total fungi	$y = 1.58 + 0.002\times$	0.41	0.03
		Active bacteria	$y = 1.77 + 0.031\times$	0.64	0.00
		Total bacteria	$y = 1.78 + 0.013\times$	0.52	0.01

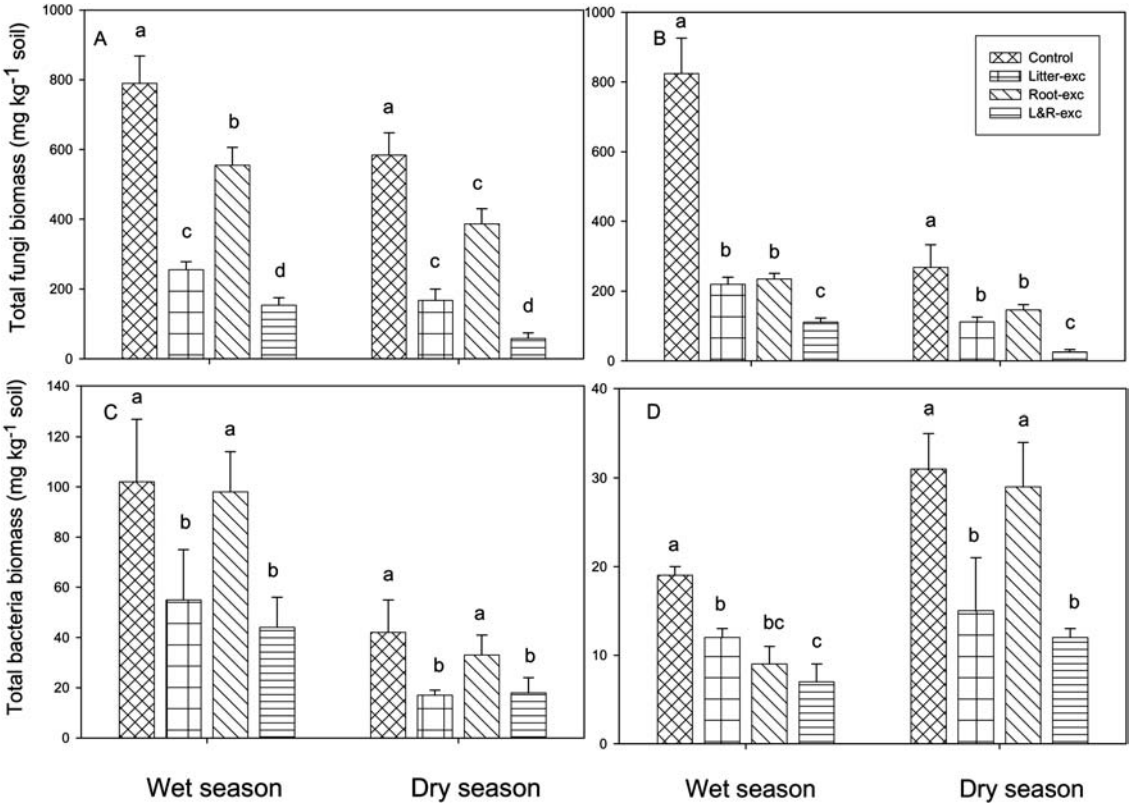

Figure 5. Total fungal biomass (mg C kg^{-1} soil) in the plantation (A) and the secondary forest (B) and total bacterial biomass (mg C kg^{-1} soil) in the plantation (C) and the secondary forest (D) in the wet (August) and dry (March) seasons. Common letters on the bars indicate no significant difference between the treatments 95% confidence level.

$$\text{Plantation (wet season)} : y = 1.138 + 0.025Mr + 0.0048Mf + 0.0275Mb(R^2 = 0.79, N = 12) \quad (1)$$

$$\text{Plantation (dry season)} : y = 0.686 + 0.0316Mr + 0.0104Mf + 0.0088Mb(R^2 = 0.61, N = 12) \quad (2)$$

$$\text{SF (wet season)} : y = 1.446 + 0.0134Mr + 0.0167Mf + 0.001Mb(R^2 = 0.79, N = 12) \quad (3)$$

$$\text{SF (dry season)} : y = 1.415 + 0.0112Mr + 0.0143Mf + 0.0182Mb(R^2 = 0.77, N = 12), \quad (4)$$

where y is soil CO_2 efflux in g C m^{-2} day^{-1}, Mr is fine root (diameter <5 mm) biomass in g dry-mass m^{-2}, Mf is active fungal biomass in mg C kg^{-1} soil, and Mb is active bacterial biomass in mg C kg^{-1} soil.

Comparing with the simple linear regressions listed in Table 1, we found multiple linear regression analysis significantly improved the estimation on soil CO_2 efflux as shown in Equations 1–4. Our further analysis revealed that the linear combination of root biomass, active fungal biomass and active bacterial biomass explained most of the variance in soil CO_2 efflux (data not shown), suggesting the interactions among these variables may play a critical role in determining soil CO_2 production.

Discussion

Our result that the soil CO_2 efflux dramatically decreased in the litter exclusion treatment indicated that aboveground litter plays an important role in regulating microbial activities and decomposition processes in the wet tropical forests. However, the larger percentage of reduction of soil CO_2 efflux in the plantation than in the secondary forest after 7-year litter exclusion suggests that soil CO_2 efflux is more sensitive to

above-ground litter input in the plantation than in the secondary forests. This finding also suggests that the response of soil CO_2 efflux to above-ground litter exclusion vary with vegetation types, which is consistent with previous studies conducted by Schlesinger (1977) and Raich and Schlesinger (1992). Higher sensitivity of soil CO_2 efflux to aboveground litter exclusion in the plantation and the higher sensitivity to root exclusion in the secondary forest suggest that aboveground litterfall is relative more important to belowground carbon cycle in the plantation, while the carbon input through the root system may be more important in the secondary forest. This is also supported by previous findings that aboveground littterfall was higher in the plantation, while root biomass was greater in the secondary forest, 246 g m^{-2} *versus* 156 g m^{-2}.

The proportion of root-derived respiration to the total soil surface CO_2 efflux found in the current study, 56% in the plantation and 67% in the secondary forest, was slightly higher than those reported in broad-leaved forests in temperate zones, 33–50% (Bowden et al., 1993; Nakane et al., 1983), but was within the range of 30–93% published in the literature across various ecosystems worldwide (Laudelout and Thierron, 1996; Ryan et al., 1997; Xu et al., 2001). By comparing root-derived respiration in different forest types, Nakane et al. (1996) concluded that the proportion of root-derived respiration to soil surface CO_2 efflux may converge to about 50% irrespective of forest types, when the cycle of soil carbon is near a dynamic equilibrium in a forest ecosystem.

The higher soil CO_2 efflux in the wet season than in the dry season suggests belowground microbial and root activities in these tropical forests are sensitive to soil moisture. This is also supported by our data that microbial biomass was greater in the wet season than in the dry season, which disagrees with previous finding that soil microbial biomass accumulated in the dry period and declined during the wet period when plant growth was rapid (Singh et al., 1989). Different relationships between the microbial biomass and soil CO_2 efflux in the plantation and the secondary forest as shown in our study, suggested the decomposition efficiency of microbes (mg C m^{-2} day^{-1} g C^{-1} microbe) is ecosystem/vegetation dependent. Moreover, microbial activity in the plantation may be more sensitive to the litter input than the secondary forest because soil CO_2 efflux decreased more significantly in the litter exclusion plots in the plantation than in the secondary forest. Soil CO_2 efflux had higher correlation with total fungal biomass but lower correlation with total bacterial biomass in the plantation than in the secondary forest, suggesting that the fungal communities may dominate soil heterotrophic respiration in the plantation, while bacterial communities may take the control in the secondary forest.

By separating the total fungal and bacterial biomass into active and inactive components, we found that active fungal and bacterial biomass demonstrated higher correlations with soil CO_2 efflux than their corresponding total fungal and bacterial biomass (Table 1). This conclusion suggests that the separation may enhance our understanding on the interactions between microbial communities and soil organic carbon cycle. However, our multiple regression analyses indicate that this separation provides little improvement on modeling soil CO_2 efflux, which may be partially attributed to the strong interactions and colinearity among root, fungal and bacterial biomass.

Removal of roots by trenching has often been used to separate root-derived respiration from heterotrophic soil CO_2 efflux, despite that decomposing root residue left in the trenching plots might contribute to total soil CO_2 efflux. Bowden et al. (1993) found that residue root decomposition had little contribution to belowground respiration when measurements were made nine months after the trenching treatment. Our measuring plots were trenched in 1990, six years before the measurements took place in this study, so the residue root effect on soil CO_2 efflux is minimal in our study. In addition, the alkali-trap method we used in this study might underestimate soil CO_2 efflux under high soil CO_2 efflux conditions and overestimate soil CO_2 efflux under very low soil CO_2 efflux conditions in comparison with the infrared gas analyzer (IRGA) technique (Jensen et al., 1996; Yim et al., 2002). We do not expect, however, that these biases will change our conclusion on the effects of litter and root exclusion under intermediate soil CO_2 efflux in these tropical forests.

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