Title. Litter removal in a tropical rain forest reduces fine root biomass and production but litter addition has few effects

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ABSTRACT

Many old-growth tropical rain forests are potentially nutrient limited, and it has long been thought that many such forests maintain growth by recycling nutrients from decomposing litter. We investigated this by continuously removing (for ten years) freshly fallen litter from five (45 m x 45 m) plots, adding it to five other plots, there were five controls. From monthly measures over one year we show that litter removal caused lower: fine root mass, fine root length, fine root length production (three-month periods) and fine root length survivorship. Litter addition did not significantly change fine root mass or length or production. Nutrient concentrations in fine roots in litter removal plots were lower than those in controls for nitrogen (N), calcium (Ca) and magnesium (Mg), concentrations in fine roots in litter addition plots were higher for N and Ca. Overall the forest is responding to long-term litter removal, with lower fine root mass and length production, which together with decreasing litterfall (reported elsewhere) shows that chronic litter removal has resulted in decreased forest growth due to nutrient impoverishment, probably nitrogen. Conversely, long-term litter addition is having fewer effects than litter removal: it did not significantly change standing mass or production of fine roots.

Key words: fine root dynamics; litter manipulation; litterfall; tropical forest; Panama; litter-addition; litter-removal; nitrogen.

INTRODUCTION

Old-growth tropical rain forests grow on a wide range of soils and many are thought to be somewhat nutrient limited (Grubb 1977, Santiago 2015). It has long been reasoned that many such forests can maintain their growth by recycling nutrients from litterfall (Vitousek 1984) but there have been no long-term experimental tests of this in old-growth forest using plots large enough to study forest-scale effects; there is an interesting one-off litter removal and addition experiment in rain forest in Costa Rica (Wood et al 2009). We set out to experimentally test whether breaking into the nutrient cycle by continuously (for ten years) removing litter from, and adding litter to, forest plots would change root dynamics.

Addition of nutrients either in inorganic or organic form has increased aboveground forest growth in several experiments in the tropics (Cleveland et al. 2011). In contrast fine root production often decreased as a result of inorganic fertilization; a review of the responses of fine root production to fertilization in lowland tropical forests found that fertilization with N+P marginally reduced fine root production, fertilization with P alone significantly reduced fine root production and N alone had no effect (Yuan and Chen 2012). Fine root (≤2 mm diameter) production in tropical rain forests represents 37% of total net primary productivity (NPP) in Panama (Yavitt et al. 2011) and 36% in Amazonia (Aragão et al. 2009), so changes in fine root production could have an important effect on NPP.

Litter addition caused lower root mass in the second year of our litter manipulation experiment in the Gigante Peninsula of the Barro Colorado Nature Monument, Panama. In the same forest experimental fertilization with N+P+K (Wurzburger and Wright 2015) also reduced standing fine root mass. The removal of nutrients by litter removal also reduced fine root mass in our litter manipulation experiment (Sayer et al. 2006) as it did in 20-year old secondary rain forests in Para, Brazil (Lima et al. 2010); in contrast litter removal did not lower root mass in rainforest in Costa Rica (Leff et al. 2012). The finding of reduced fine root mass in less fertile conditions, caused by litter removal, is the seems to be the opposite of the generalization that plant mass allocation often shifts to fine roots in response to reduced nutrient availability (Poorter and Nagel 2000); and the general prediction that fine root mass ratio would increase under limiting nutrient conditions (Chapin et al. 1986, Poorter et al. 2012).

The finding that opposite treatments, litter removal and litter addition, both lower fine root mass (in general, and in our specific research site) suggests that different processes are happening. Broadly litter removal lowers overall forest growth and litter addition causes a partial switch from belowground to aboveground growth; these are the general questions we were investigating. Specifically we investigated whether the reduction of fine root mass in both litter removal and addition, measured in Panama in one month in the wet season of 2004, the second year of litter manipulation (Sayer et al. 2006), was generalizable to a whole year eight years later in the same experiment; in parallel we investigated whether any changes in fine root mass were due to changes in fine root production or fine root survival or both. From measurements of nutrient concentrations we inferred which, if any, nutrients limited growth in the litter removal (and control) plots. Fine root mass dynamics were measured by soil coring and fine root length dynamics by root windows.

In our Litter Manipulation Experiment in lowland semi-evergreen forest in Panama, litter manipulation has been continuous since January 2003. The plots are large (45 m x 45 m), they were trenched to 50 cm, and trenches lined with plastic, to isolate the surface soil within the plots from that in the surrounding forest. Soil nutrient concentrations have been changed by both litter removal and litter addition and the effects are increasing over time - more nutrients became significantly different and the depth to which differences were seen increased (Sayer and Tanner 2010, Tanner et al. 2016, Sheldrake et al. 2017). By nine years after the start of the experiment litter removal plots had lower: NO_3^-+NH_4^+; ‘available’ P; exchangeable Ca and Mg; and litter addition soils had higher: ‘available’ P and exchangeable Ca (Sheldrake et al. 2017). Litterfall tended to decrease in litter removal plots compared to controls (in year six it was 10% lower) and increase in litter addition plots (in year 6 it was 21% higher) though the differences were significant (Sayer and Tanner 2010). Trunk growth did not differ significantly between the treatments over the first six years of the experiment, 2003 - 2009 (Sayer and Banin 2016). To date there have been no measures of fine root production, as opposed to standing crop; thus we investigated fine root dynamics in 2013 - 2014, a decade after continuous litter removal and addition started and after sufficient time had elapsed for significant differences to appear, and after any transient effects caused by the initial transfer of all the existing litter standing crop from litter removal to litter addition plots - for example a significantly higher litterfall in litter addition plots cf controls in the rainy season in year one, which was absent in years two to six (Sayer and Tanner 2010).

We predicted that that the pattern of lower fine root mass in litter removal and litter addition treatments (Sayer et al. 2006), found in one month early in the wet season of 2004, would be found over 12 months in 2013-2014. We were far from certain about this because another litter manipulation experiment in old growth tropical rain forest in Costa Rica found different patterns - no difference in fine root mass in litter removal plots cf controls but a 75% higher fine root mass in litter addition plots (measured over the second year of the experiment, Leff et al. 2012). Our predictions for the effects of litter removal on fine root mass *production* were even less confident because there is only one other study, in 20-year-old secondary forest in Para Brazil (Lima et al. 2010), which showed decreased fine root mass production in litter removal plots. There is no published study of fine root survival in litter manipulation experiments in tropical forest, and very few in fertilizer experiments in other ecosystems, so we had no prediction for the effect of litter manipulation on fine root survival.

METHODS

Study site

We conducted the research in lowland (c. 70 m above sea level) semi-evergreen tropical forest located on the Gigante Peninsula of the Barro Colorado Nature Monument in the Republic of Panama (9^o06'N, 79^o54'W). This forest is more than 200 years old (Wright et al. 2011), it is composed of c. 30 m canopy trees with up to 40 m emergents; understory palms and woody lianas are abundant. Annual rainfall averages 2,600 mm with a strong dry season from January to April. Annual mean temperature is 27^oC (Leigh 1999). The soil is moderately acidic Oxisol, pH in water c. 5.0 with low ‘availability’ of P and exchangeable K, moderate inorganic N and high exchangeable Ca and Mg (Sayer and Tanner 2010). Rainfall data was collected daily at Barro Colorado field station which c. 5 km nearby the study site (Smithsonian Tropical Research Institute Panama).

Experimental design

Gigante Litter Manipulation Project (GLiMP) was set-up from 2000 to 2003 with fifteen 45 m x 45 m plots. Plots were assigned to three treatments as litter removal, litter addition and controls by stratified random design according to litterfall in 2002, a pretreatment year. Each experimental plot was trenched to 0.5 m lined with plastic and backfilled to minimize nutrient transfer between plots and the surrounding forest, the outer 7.5 m of each plot is treated as a buffer zone. Starting in January 2003 litterfall on the forest floor has been removed monthly by hand raking in litter removal plots and transferred immediately to the litter addition plots.

Fine root biomass

During the 10th year of litter manipulation from March 2013 to February 2014, fine roots (≤ 2 mm diameter) were collected monthly, using a 2-cm diameter soil core sampler, over one year at two depths in the mineral soil (0-5 cm and 0-10 cm). We used separate 0-5 cm and 0-10 cm cores because these soils compressed differentially – the 0-5 cm compressed more than 5-10 cm, so simply cutting a 0-10 cm core into equal haves would not have sampled 0-5 and 5-10 cm. A previous study in the same forest reported that fine root mass from 0-10 cm of soil was 70% of the fine root mass from 0-25 cm in the soil (Cavelier 1989). The sampling points were assigned systematically in the inner 30 m x 30 m of each plot. The soil cores were sampled 1 m westward from the initial sampling points each month. Fine roots growing in the litter layer were separated from litter standing crop collected from the same points as the soil cores. All samples were carried back to the laboratory on Barro Colorado Island and stored in a fridge at about 5°C then processed within two weeks. Fine roots were washed in a 0.5-mm sieve with tap water. Fine roots were not separated into different species, or live or dead roots, for practical reasons; with more than 120 tree species present in the plots often with different colored roots it was practically impossible to categorize the fine roots. Fine root samples were oven-dried to constant mass at 60^{o}C and weighed to ±0.1 mg. Mean fine root biomass (g m^{-2}) was calculated as the average of fine root biomass in each month for each litter treatment (n=5 per treatment).

Fine root mass production from ingrowth cores

Ingrowth cores were made of HDPE-plastic (2-mm mesh size, 2-cm diameter, 10-cm depth; modified from the methods in Li et al. 2013) and installed systematically in each plot (total n=69 per time; five cores per plot except three plots with three cores because lianas and fallen trees obstructed installation in the designated points of these plots). We filled each ingrowth core with fine-root-free soil collected at the installation point using a 2-cm diameter soil core and using forceps to removed fine roots and small rocks from the soil. There were three separate sets of ingrowth cores set up in different seasons and collected three months after installation: wet season (May to August 2013), transition period (October 2013 to January 2014), and dry season (January to April 2014). Fine roots in the cores were washed carefully with tap water then oven-dried to constant weight at 60^{o}C and weighed to ±0.1 mg.

Fine root length, production and survivorship

We installed 43 root windows in the study plots in April 2013 (three windows per plot except one plot with one window because of the dense coarse roots present in the shallow soil that made it impossible to install a root window panel). The root window panels (3-mm thick clear acrylic sheet; 10 cm wide, 15 cm deep) were placed in a stratified random design within 3-m of the trunks of individual trees of the five most abundant species. We dug a small soil pit (15 cm wide x 20 cm long x 10 cm deep) and carefully installed the acrylic sheet vertically against the side adjacent to the tree with two stainless steel bars fixed against the acrylic panel. An area of 10 cm x 10 cm from below the litter layer was marked permanently on the panel to determine an observation area. We prevented disturbances from sunlight and air temperature by placing a 5-mm thick insulation sheet against each window and back filling the hole with soil wrapped with plastic.

After installation the root windows were left undisturbed for two months (April to May 2013), then we took a photograph of each using a digital camera (5 megapixels, Sony cyber-shot DSC-RX100 and iPhone 4) at 1-month intervals (June 2013 to May 2014). We minimized light reflection by taking the photos between 8am to 12pm.

Each photo was prepared for root tracing using Gimp (GNU image manipulation program, version 2.8.14); the photo was made into a 10 cm x 10 cm observed area in a format of a 2500 x 2500 pixel image. We traced all fine roots (≤2 mm diameter) appearing in the image for 15 minutes per image using a computer tablet (Wacom Intuos pen, CTL-480) with a solid brush head in Gimp (20-pixels). A 1-cm scale was inserted into a traced fine root image then the image was saved in PNG format for fine root length analysis. Total fine root length per image was evaluated from the traced fine root image using the ImageJ program (version 2.0.0) with AnalyzeSkeleton plug-in (Arganda-Carreras et al. 2010, version 3.0.0).

Fine root standing length (at 0-5 cm and 0-10 cm depth) was estimated from monthly observations (June 2013 to May 2014). Fine root length production was calculated from summing the fine roots that newly appeared at an observation time in every 1-month interval. Mean annual fine fine root length standing crop and production were calculated from the average of summations of mean length production in each month for each litter treatment (n=5) and presented in units of m m^{-2} (root window surface). Fine root length survivorship was estimated from the fine roots present at one observation and still present at subsequent observations (1-month interval).

Fine root nutrient concentrations

Fine root nutrient concentrations were analyzed from the samples from sequential coring at 0-5 cm soil depth (collected in March 2013 to February 2014). One composite sample per treatment per time, were made from pooling the samples from the five plots per treatment. Composite samples were made for each of six months – four in the wet season in June, September, October and December in 2013 and two in the dry season in March 2013 and February 2014. Fine root samples were ground and sent either to Forestry Research Alice Holt Lodge, Surrey, UK (June, September, December and February) or the University of Bern, Switzerland (March and October) to determine concentrations of nutrients including N, P, K, Ca and Mg. At Alice Holt N was determined using elemental analysers and the other elements were measured using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrophotometry) in Bern N and P were measured by colorimetry and cations by ICP-OES.

Data analyses

Linear mixed effects models were used to compare the effects of litter manipulation on various fine root responses. The response variables were the plot-level means of fine root standing crop (mass and length), fine root production (mass and length), fine root length survivorship and fine root nutrient concentrations. We generated several models composed of different fixed factors as litter treatments (litter removal, litter addition, control), seasons (wet and dry, transition period only for ingrowth cores) and their interactions (treatment x season); different random effects as plot and/or month. The best models were selected using *Akaike Information Criterion* (AIC) then ANOVA was performed to compare between different fixed factors. If the results were found to be significant using ANOVA ($P<0.05$ or lower), post-hoc Tukey test was used to compare the differences between the treatments. Mean annual standing fine root length in all treatments was compared by using one-way ANOVA. All analyses were performed in R 3.1.2 (R Core Team 2014) with linear mixed-effects models using the lme4 library.

RESULTS

Fine root mass and production

Wet season fine root mass in the soil was higher than in the dry season, both at 0-5 cm soil depth (Fig. 1; $F_{1, 178} = 10.2$, $P<0.01$) and at 0-10 cm soil (Fig. 1; $F_{1, 178} = 14.9$, $P<0.001$), but there were no interseasonal differences in the fine root mass in the litter standing crop. Fine root mass production, over 0-10 cm soil depth, was higher in the wet season than in the transition (wet to dry) and dry seasons (Fig. 2; $F_{2, 87} = 52.6$, $P<0.001$).

Fine root mass was lower in litter removal soils at 0-5 cm (significant), and 0-10 cm soils (Fig. 1 and Supplementary Fig. 1; not significant). Litter addition did not significantly lower fine root mass in either 0-5 cm or 0-10 cm soils (Fig. 1 and Supplementary Fig. 1). Fine root mass in the litter standing crop was significantly higher in the litter addition plots than in the controls (Fig. 1). The sum of the fine root mass in the litter standing crop and 0-5 cm soils was significantly lower in litter removal than controls ($F_{2, 177} = 7.0$, $P<0.01$, data in Supplementary Materials), but not significantly different between litter addition and controls. Fine root mass in the litter standing crop was less than 10% of the total mass in the litter standing crop plus that in the top 5 cm of soil. Fine root mass production was not significantly affected by litter removal or litter addition.

Fine root length: standing crop, production and survivorship

Seasonal changes in rainfall did not affect standing fine root length (in contrast to fine root mass, which was higher in the wet season), despite the dry season in 2014 being the third driest from 1971 to 2016 (Figs. 3a and 3b). Litter removal resulted in lower mean annual standing fine root length than the controls at both 0-5 and 0-10 cm soil depth (one-way ANOVA, $F_{2, 12} = 7.3$, $P<0.01$ for 0-5 cm fine roots; $F_{2, 12} = 7.0$, $P<0.01$ for 0-10 cm fine roots, data in Supplementary Materials), litter addition did not significantly affect mean annual standing fine root length at either 0-5 or 0-10 cm.

Fine root length production (0-10 cm soils) was significantly lower in litter removal plots over the whole year (Fig. 3c; $F_{2, 162} = 5.3$, $P<0.01$); litter addition did not affect fine root length production (Fig. 3c). Fine root length survivorship was lower in litter removals than the controls over the whole year (Fig. 3d; $F_{2, 162} = 4.8$, $P<0.01$); survivorship in litter addition was not different from control (Fig. 3d).

Nutrient concentrations

Nutrient concentrations in fine roots in litter removal plots were lower than those in controls for N, Ca and Mg, concentrations were higher in litter addition than in controls for N and Ca. There were larger decreases in litter removal (20% over N, P, K, Ca, Mg) than increases in litter addition (11%); the difference between treatments and control varied by nutrient 3% in P, 8% in N, 12 % in K, 25% in Ca and 31% in Mg (Table 1).

DISCUSSION

Effect of litter manipulation and fertilization on fine root dynamics in tropical forests.

Ten years of continuous litter removal caused lower: fine root mass, fine root length, fine root length production and fine root length survivorship. This strengthens the trend, after 1.5

years, for lower fine root mass in litter removal plots (Sayer et al. 2006). Differences between the two sets of results are likely to be due to the fact that the earlier study was for one month only, whereas the current study was for 12 months; in addition effects may have strengthened over time, as litter is continuously removed, due to decreasing soil nutrient availability and increasing soil bulk density (Tanner et al. 2016). In Costa Rica in the second year of a litter manipulation experiment, litter removal did not affect fine root mass (Leff et al. 2012). The lack of effect in Costa Rica could be due to the relatively short duration of the experiment, or it could be due to differences in plot size – the small plots in Costa Rica (3 m x 3 m) are a small part of the fine root system of a large tree and so whole tree nutrient supply will hardly have been affected, in contrast in Panama the plots are large enough (45 m x 45 m) to affect the nutrient supply to whole trees, which are reducing their growth, both below and aboveground, in response to decreasing nutrient supplies.

Lower fine root mass and length can result from lower fine root production or lower survival or both. In the litter removal plots in Panama, the lower fine root length standing crop was associated with both lower production and lower survival. Other studies of fine root production are much less common than those of standing mass, especially in tropical forests. In Eastern Amazonian Brazil, in 20-year-old secondary forest, lower fine root mass in litter removal plots was caused by lower fine root mass production compared to the controls (Lima et al. 2010). Similarly in a study of primary productivity along a long elevation gradient in rain forests in Peru lower standing fine root mass was correlated with lower rates of fine root production measured in rhizotrons (r=0.48); though not with productivity measured in ingrowth cores (r=0.18) (Girardin et al. 2010, Girardin et al. 2013). There seem to be no other studies, besides ours, of fine root survivorship in tropical rain forests. In summary, in lowland tropical rain forests lower standing fine root length always seems to result from lower fine root production.

Litter addition did not significantly change fine root mass or length or production 10 years after litter manipulation started in Panama, in contrast after 1.5 years of litter addition in the same experiment there was significantly lower fine root mass (Sayer et al. 2006). There was probably a transient effect on fine root mass - a 29% reduction after 1.5 years (Sayer et al. 2006) but an insignificant, 14%, reduction after ten years (see in Supplementary Materials). This finding, of no significant reduction in fine root mass in soils with higher nutrient concentrations caused by litter addition (Table 1), differs from the very significant, 50%, lower fine root mass in the soils with higher nutrient concentrations caused by inorganic fertilization in the adjacent Gigante Fertilization Experiment (Wurzburger and Wright 2015). The differences between the two experiments – no significant effect in the litter addition experiment compared to a strong effect in the fertilizer experiment may partly be caused by the much greater amount of P added (c. ten times as much in the fertilizer as compared to the litter addition experiment) over a longer time in the fertilizer experiment (13 years cf. 10 in the litter manipulation experiment), which caused a much higher soil 'available' P in the fertilizer experiment – though the strongest reduction in fine root mass in the fertilizer experiment was due to K (Wurzburger and Wright 2015). Thus a simple, and unsurprising, take home message could be that lower rates of phosphorus addition (in the litter addition cf. N+P+K fertilization) caused smaller effects on fine root mass, and thus that any effects of relatively small increases in nutrient input from, for example, pollution are likely to have very small effects on fine root mass.

In a litter doubling experiment in Costa Rica fine root mass was 75% higher in the second year (Leff et al. 2012), completely the opposite effect to that in Panama. The difference between the two experiments could be due to: a transient effect early in the experiment (as in the second year of litter addition in Panama); or plot size, the small plots in Costa Rica (3 m x 3 m) are hot spots relative to the size of the crowns of large trees, and if trees are limited by nutrients they may concentrate fine root growth into these hot spots; something they would not need to do in large plots. Thus whether or not increased fine root mass is seen in plots with doubled litter input could be due to time since the treatment started and/or plot size.

Fine root mass and fine root length were not well correlated in the litter manipulation experiment in Panama, as was true in some other studies in various kinds of vegetation (e.g. in Appalachian forests in the U.S.A., Davis et al 2004). In our experiment this was probably caused by the fact that we recorded length in, fixed, root windows and separately mass from cores, which were in the same plots but necessarily in different places each time and probably different in their species composition. In Brazilian rain forest, when length and breadth were measured for roots from the same cores, there was a strong positive correlation (Metcalfe et al. 2007). Notwithstanding the lack of correlation between root mass and root length on a month by month basis, over a whole year we found similar patterns due to litter treatment – lower root mass and root length in litter removal plots.

The decrease in root growth in the litter removal plots in Panama, was paralleled by lower root and soil available nitrogen concentrations (live leaf nitrogen concentrations were also lower in trees in litter removal plots, Table 2). Soil available P was also lower in litter removal plots – though root P concentrations were not; K concentrations in soils and roots were not affected by litter removal (Table 2). In Brazil, in 20-year old secondary forest root mass and growth was lower in (20 m x 20 m) litter removal plots (Lima et al. 2010) but soil resin phosphorus was not lower and soil nitrogen not reported (Maia et al 2015). In Costa Rica in the second year of litter removal (in 3 m x 3 m plots) fine root biomass was not lower than controls though total soil nitrogen was significantly lower. There are too few studies to make generalizations but the two studies with big plots both have lower root growth in litter removal plots; and for Panama we conclude that the reduced root growth in litter removal plots may have been caused by lower nitrogen availably.

Although root growth was not significantly affected by litter addition, nitrogen concentrations in roots and live leaves (but not soil) were higher in litter addition plots (Table 2), whereas phosphorus and potassium concentrations were not different from controls in roots, live leaves or soil. While we suggest that lower nitrogen in litter removal plots lowered root growth, we think that there was sufficient nitrogen in control soils and that adding more nitrogen (in litter), although it increased nitrogen concentrations in roots and leaves did not change root growth because it was already in sufficient supply in control soil. Our finding of no change in root mass or length in litter addition plots differs from the effect in the adjacent Gigante Fertilizer Experiment where the addition of nitrogen, phosphorus and potassium together reduced root mass by 50% and root length by 20% (Wurzburger and Wright 2015); the different patterns in the different experiments are likely due to much higher rates of P input in the Gigante Fertilizer Experiment and the different chemical forms of the nutrients – inorganic, and therefore more available - in the fertilizer experiment and organic in the litter manipulation experiment.

Conclusions

Overall the lowland semi-evergreen forest in Panama is responding to long-term litter removal, with significantly lower fine root mass production and a trend for lower fine litterfall

(Rodtassana 2016); mycorrhizal composition was also changed in the litter removal plots (Sheldrake et al 2017). Trunk growth was not significantly lower by the ninth year of litter removal (Sayer and Banin 2016). The decrease in growth in litter removal plots was probably caused by decreases in N. Long-term litter addition is having fewer effects than litter removal; after 10 years of litter addition fine root mass dynamics were not significantly different from the controls. We conclude that total forest production will become lower in litter removal plots (though it was not significantly different after 10 years), because fine root production was significantly lower, and litterfall was decreasing with time; removing nutrients, particularly N, by removing litter is slowing forest growth.

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Table 1. Nutrient concentrations in fine roots from GLiMP experiment in a lowland semi-evergreen forest in Panama

Treatment	Litter removal	Control	Litter addition	F-value	P-value
N (%)	1.57 ± 0.07^{a}	1.74 ± 0.03^{b}	1.90 ± 0.04^{c}	24.811	<0.001
P (%)	0.11 ± 0.04^{a}	0.10 ± 0.03^{b}	0.11 ± 0.04^{c}	20.425	<0.001
K (%)	0.11 ± 0.02	0.13 ± 0.03	0.14 ± 0.03	-	-
Ca (%)	0.69 ± 0.05^{a}	1.07 ± 0.05^{b}	1.27 ± 0.05^{c}	54.732	<0.001
Mg (%)	0.10 ± 0.02^{a}	0.15 ± 0.03^{b}	0.17 ± 0.03^{b}	14.321	<0.01

Notes: The values are mean ± SE (n=6). The letters indicate the differences between each treatment from Tukey test. For K nutrient concentrations, ANOVA and Tukey tests were not done because of an insignificant model in LME.

Table 2. Summary of significant changes in nutrient concentrations (cf. controls) in litter manipulation (GLiMP) and fertilization (Gigante Fertilizer Experiment) in a lowland semi-evergreen forest in Panama

	Litter removal				Litter addition				N+P+K			N+P
	Soil[a] (1)	Root (3)	Litter (7)	Leaf (5)	Soil[a] (1)	Root (3)	Litter (7)	Leaf (5)	Soil[a] (2)	Root (4)	Litter (7)	Leaf (6)
N	↓	↓	n.s.	↓	n.s.	↑	↑	↑	n.s.	n.s.	n.s.[c]	n.s.
P	↓	n.s.	n.s.	n.s.	↑	n.s.	n.s.	n.s.	↑	↑	n.s.	↑
K	n.s.	n.s.	↓[b]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.d.

Notes: Down arrow symbols represent lower and up arrow symbols represent higher concentrations compared to the controls; n.s. means not significant and n.d. means no data. a) Soil N is $NO_3^- + NH_4^+$; soil P is resin extractable or Mehlich 3 extractable; soil K is Mehlich 3 extractable. b) Litter K in LR significantly lower at five years (*P*=0.03), but not at three years (*P*=0.70). c) Litter N in N+P+K plots not significantly higher at five years (*P*=0.083, but was significantly higher at three years (*P*<0.000).

Sources and year of experiment in which effect measured:

(1) Sheldrake et al. 2017 (9 years); (2) Wright (unpublished data, 14 years); (3) This study (Table 1, 10 years); (4) Wurzburger and Wright 2015 (14 years); (5) Sayer and Tanner 2010 (5 years); (6) Mayor et al. 2014 (13 years); (7) Sayer et al. 2012 (5 years)

FIGURE CAPTIONS

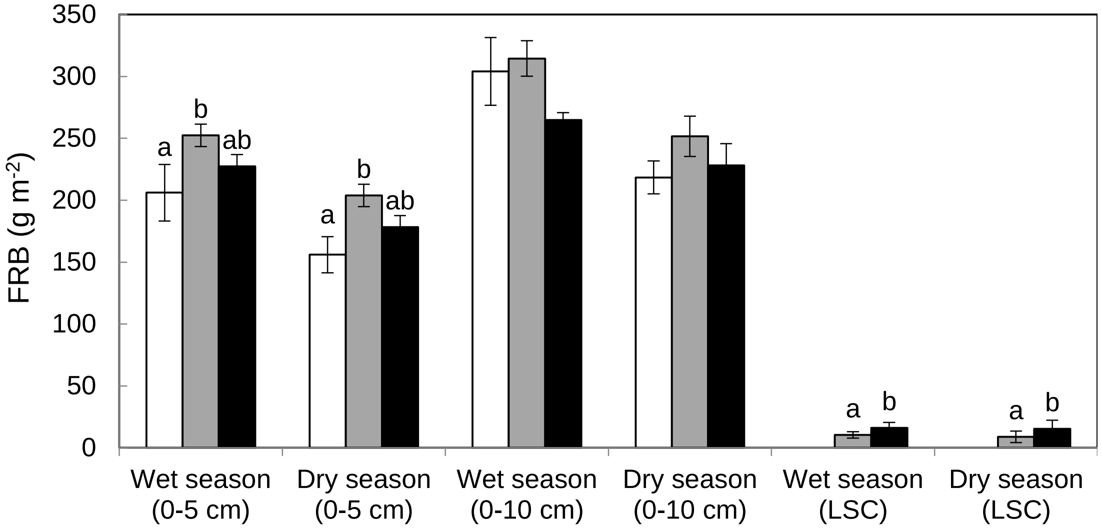
Fig. 1. Fine root (≤2 mm diameter) biomass (FRB) in a litter manipulation experiment in lowland semi-evergreen forest in Panama at 0-5 cm soil deep, at 0-10 cm soil deep and in litter standing crop (LSC); each bar represents root biomass (mean ± SE) from 5 plots per treatment; open bars represent litter removals, gray bars represent controls, black bars represent litter additions; the data was from monthly sampling between March 2013 to February 2014; wet season is from May 2013 to December 2013. Different letters show significant difference between litter treatments ($P<0.05$); there was no significant difference at 0-10 cm depth.

Supplementary Fig. 1. Fine root biomass (FRB, ≤2 mm diameter) in litter manipulation in lowland semi-evergreen forest in Panama (a) 0-5 cm soil deep, (b) 0-10 cm soil deep, (c) in litter layer and (d) monthly rainfall; each bar represents mean value (n=5) with standard error; In panel a, b and c, open bars are litter removals, gray bars are controls, and black bars are litter additions.

Fig. 2. Fine root mass production at 0-10 cm soil depth from 3-month interval ingrowth cores in lowland semi-evergreen tropical forest Panama; open bars represent litter removals, gray bars

represent controls, black bars represent litter additions. Data are means and standard error of five plots per treatment; there was no significant difference in all litter treatments.

Fig. 3. Fine root length (FRL) in litter manipulation in lowland semi-evergreen forest in Panama; (a) standing root length at 0-5 cm soil deep, (b) standing root length at 0-10 cm soil deep, (c) root length production, (d) root length survivorship and (e) monthly rainfall; each bar represents mean value with standard error (n=5) units are m of fine root per m^2 of rhizotron surface; In panel a, b and c, open bars are litter removals, gray bars are controls, and black bars are litter additions.



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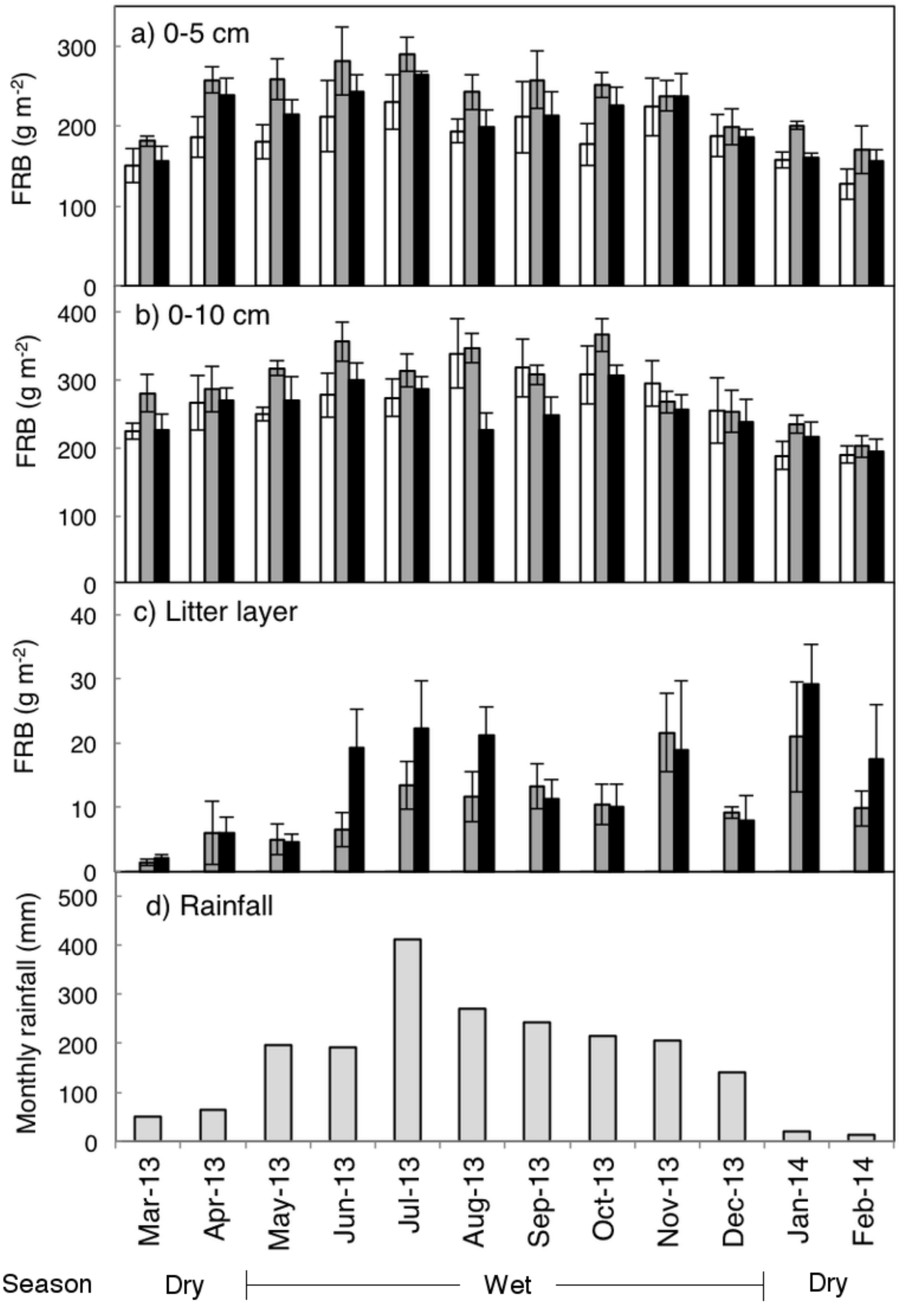

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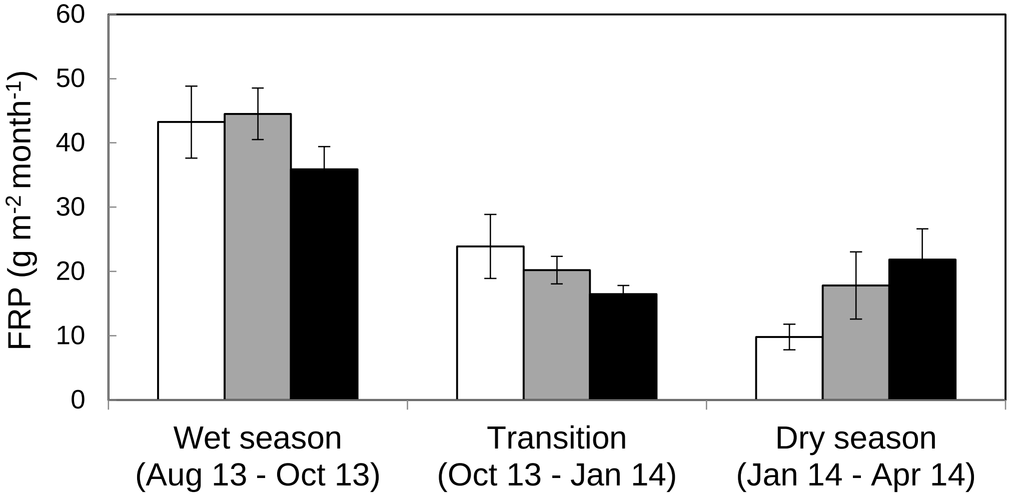

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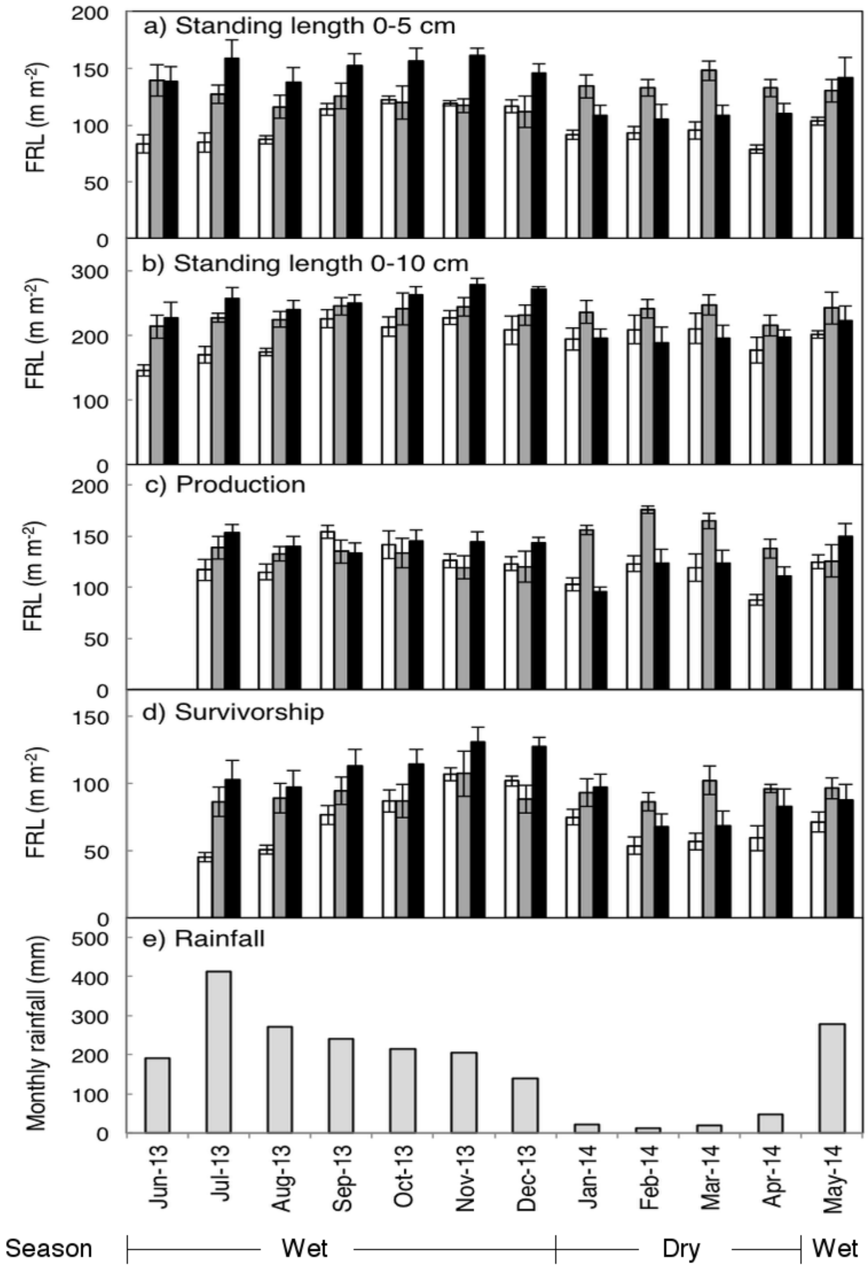

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