

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

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8 **ABSTRACT**

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

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

25 **INTRODUCTION**

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

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

43 Litter addition caused lower root mass in the second year of our litter manipulation
44 experiment in the Gigante Peninsula of the Barro Colorado Nature Monument, Panama. In the

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

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

66

67 In our Litter Manipulation Experiment in lowland semi-evergreen forest in Panama, litter

68 manipulation has been continuous since January 2003. The plots are large (45 m x 45 m), they
69 were trenched to 50 cm, and trenches lined with plastic, to isolate the surface soil within the plots
70 from that in the surrounding forest. Soil nutrient concentrations have been changed by both litter
71 removal and litter addition and the effects are increasing over time - more nutrients became
72 significantly different and the depth to which differences were seen increased (Sayer and Tanner
73 2010, Tanner et al. 2016, Sheldrake et al. 2017). By nine years after the start of the experiment
74 litter removal plots had lower: $\text{NO}_3^- + \text{NH}_4^+$; 'available' P; exchangeable Ca and Mg; and litter
75 addition soils had higher: 'available' P and exchangeable Ca (Sheldrake et al. 2017). Litterfall
76 tended to decrease in litter removal plots compared to controls (in year six it was 10% lower)
77 and increase in litter addition plots (in year 6 it was 21% higher) though the differences were
78 significant (Sayer and Tanner 2010). Trunk growth did not differ significantly between the
79 treatments over the first six years of the experiment, 2003 - 2009 (Sayer and Banin 2016). To
80 date there have been no measures of fine root production, as opposed to standing crop; thus we
81 investigated fine root dynamics in 2013 - 2014, a decade after continuous litter removal and
82 addition started and after sufficient time had elapsed for significant differences to appear, and
83 after any transient effects caused by the initial transfer of all the existing litter standing crop from
84 litter removal to litter addition plots - for example a significantly higher litterfall in litter addition
85 plots cf controls in the rainy season in year one, which was absent in years two to six (Sayer and
86 Tanner 2010).

87 We predicted that that the pattern of lower fine root mass in litter removal and litter
88 addition treatments (Sayer et al. 2006), found in one month early in the wet season of 2004,
89 would be found over 12 months in 2013-2014. We were far from certain about this because
90 another litter manipulation experiment in old growth tropical rain forest in Costa Rica found

91 different patterns - no difference in fine root mass in litter removal plots cf controls but a 75%
92 higher fine root mass in litter addition plots (measured over the second year of the experiment,
93 Leff et al. 2012). Our predictions for the effects of litter removal on fine root mass *production*
94 were even less confident because there is only one other study, in 20-year-old secondary forest in
95 Para Brazil (Lima et al. 2010), which showed decreased fine root mass production in litter
96 removal plots. There is no published study of fine root survival in litter manipulation
97 experiments in tropical forest, and very few in fertilizer experiments in other ecosystems, so we
98 had no prediction for the effect of litter manipulation on fine root survival.

99 **METHODS**

100 *Study site*

101 We conducted the research in lowland (c. 70 m above sea level) semi-evergreen tropical
102 forest located on the Gigante Peninsula of the Barro Colorado Nature Monument in the Republic
103 of Panama (9°06'N, 79°54'W). This forest is more than 200 years old (Wright et al. 2011), it is
104 composed of c. 30 m canopy trees with up to 40 m emergents; understory palms and woody
105 lianas are abundant. Annual rainfall averages 2,600 mm with a strong dry season from January to
106 April. Annual mean temperature is 27°C (Leigh 1999). The soil is moderately acidic Oxisol, pH
107 in water c. 5.0 with low 'availability' of P and exchangeable K, moderate inorganic N and high
108 exchangeable Ca and Mg (Sayer and Tanner 2010). Rainfall data was collected daily at Barro
109 Colorado field station which c. 5 km nearby the study site (Smithsonian Tropical Research
110 Institute Panama).

111 *Experimental design*

112 Gigante Litter Manipulation Project (GLiMP) was set-up from 2000 to 2003 with fifteen
113 45 m x 45 m plots. Plots were assigned to three treatments as litter removal, litter addition and
114 controls by stratified random design according to litterfall in 2002, a pretreatment year. Each
115 experimental plot was trenched to 0.5 m lined with plastic and backfilled to minimize nutrient
116 transfer between plots and the surrounding forest, the outer 7.5 m of each plot is treated as a
117 buffer zone. Starting in January 2003 litterfall on the forest floor has been removed monthly by
118 hand raking in litter removal plots and transferred immediately to the litter addition plots.

119

120 *Fine root biomass*

121 During the 10th year of litter manipulation from March 2013 to February 2014, fine roots
122 (≤ 2 mm diameter) were collected monthly, using a 2-cm diameter soil core sampler, over one
123 year at two depths in the mineral soil (0-5 cm and 0-10 cm). We used separate 0-5 cm and 0-10
124 cm cores because these soils compressed differentially – the 0-5 cm compressed more than 5-10
125 cm, so simply cutting a 0-10 cm core into equal halves would not have sampled 0-5 and 5-10 cm.
126 A previous study in the same forest reported that fine root mass from 0-10 cm of soil was 70% of
127 the fine root mass from 0-25 cm in the soil (Cavelier 1989). The sampling points were assigned
128 systematically in the inner 30 m x 30 m of each plot. The soil cores were sampled 1 m westward
129 from the initial sampling points each month. Fine roots growing in the litter layer were separated
130 from litter standing crop collected from the same points as the soil cores. All samples were
131 carried back to the laboratory on Barro Colorado Island and stored in a fridge at about 5°C then
132 processed within two weeks. Fine roots were washed in a 0.5-mm sieve with tap water. Fine
133 roots were not separated into different species, or live or dead roots, for practical reasons; with
134 more than 120 tree species present in the plots often with different colored roots it was

135 practically impossible to categorize the fine roots. Fine root samples were oven-dried to constant
136 mass at 60°C and weighed to ± 0.1 mg. Mean fine root biomass (g m^{-2}) was calculated as the
137 average of fine root biomass in each month for each litter treatment ($n=5$ per treatment).

138 *Fine root mass production from ingrowth cores*

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

149 *Fine root length, production and survivorship*

150 We installed 43 root windows in the study plots in April 2013 (three windows per plot
151 except one plot with one window because of the dense coarse roots present in the shallow soil
152 that made it impossible to install a root window panel). The root window panels (3-mm thick
153 clear acrylic sheet; 10 cm wide, 15 cm deep) were placed in a stratified random design within 3-
154 m of the trunks of individual trees of the five most abundant species. We dug a small soil pit (15
155 cm wide x 20 cm long x 10 cm deep) and carefully installed the acrylic sheet vertically against
156 the side adjacent to the tree with two stainless steel bars fixed against the acrylic panel. An area
157 of 10 cm x 10 cm from below the litter layer was marked permanently on the panel to determine

158 an observation area. We prevented disturbances from sunlight and air temperature by placing a
159 5-mm thick insulation sheet against each window and back filling the hole with soil wrapped
160 with plastic.

161 After installation the root windows were left undisturbed for two months (April to May
162 2013), then we took a photograph of each using a digital camera (5 megapixels, Sony cyber-shot
163 DSC-RX100 and iPhone 4) at 1-month intervals (June 2013 to May 2014). We minimized light
164 reflection by taking the photos between 8am to 12pm.

165 Each photo was prepared for root tracing using Gimp (GNU image manipulation
166 program, version 2.8.14); the photo was made into a 10 cm x 10 cm observed area in a format of
167 a 2500 x 2500 pixel image. We traced all fine roots (≤ 2 mm diameter) appearing in the image for
168 15 minutes per image using a computer tablet (Wacom Intuos pen, CTL-480) with a solid brush
169 head in Gimp (20-pixels). A 1-cm scale was inserted into a traced fine root image then the image
170 was saved in PNG format for fine root length analysis. Total fine root length per image was
171 evaluated from the traced fine root image using the ImageJ program (version 2.0.0) with
172 AnalyzeSkeleton plug-in (Arganda-Carreras et al. 2010, version 3.0.0).

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

181 *Fine root nutrient concentrations*

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

193 *Data analyses*

194 Linear mixed effects models were used to compare the effects of litter manipulation on
195 various fine root responses. The response variables were the plot-level means of fine root
196 standing crop (mass and length), fine root production (mass and length), fine root length
197 survivorship and fine root nutrient concentrations. We generated several models composed of
198 different fixed factors as litter treatments (litter removal, litter addition, control), seasons (wet
199 and dry, transition period only for ingrowth cores) and their interactions (treatment x season);
200 different random effects as plot and/or month. The best models were selected using *Akaike*
201 *Information Criterion* (AIC) then ANOVA was performed to compare between different fixed
202 factors. If the results were found to be significant using ANOVA ($P < 0.05$ or lower), post-hoc
203 Tukey test was used to compare the differences between the treatments. Mean annual standing

204 fine root length in all treatments was compared by using one-way ANOVA. All analyses were
205 performed in R 3.1.2 (R Core Team 2014) with linear mixed-effects models using the lme4
206 library.

207

208 **RESULTS**

209 *Fine root mass and production*

210 Wet season fine root mass in the soil was higher than in the dry season, both at 0-5 cm
211 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
212 there were no interseasonal differences in the fine root mass in the litter standing crop. Fine root
213 mass production, over 0-10 cm soil depth, was higher in the wet season than in the transition
214 (wet to dry) and dry seasons (Fig. 2; $F_{2, 87} = 52.6$, $P < 0.001$).

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

225 *Fine root length: standing crop, production and survivorship*

226 Seasonal changes in rainfall did not affect standing fine root length (in contrast to fine
227 root mass, which was higher in the wet season), despite the dry season in 2014 being the third
228 driest from 1971 to 2016 (Figs. 3a and 3b). Litter removal resulted in lower mean annual
229 standing fine root length than the controls at both 0-5 and 0-10 cm soil depth (one-way ANOVA,
230 $F_{2, 12} = 7.3$, $P < 0.01$ for 0-5 cm fine roots; $F_{2, 12} = 7.0$, $P < 0.01$ for 0-10 cm fine roots, data in
231 Supplementary Materials), litter addition did not significantly affect mean annual standing fine
232 root length at either 0-5 or 0-10 cm.

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

238 *Nutrient concentrations*

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

244 **DISCUSSION**

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

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

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

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

270 fine root survivorship in tropical rain forests. In summary, in lowland tropical rain forests lower
271 standing fine root length always seems to result from lower fine root production.

272 Litter addition did not significantly change fine root mass or length or production 10
273 years after litter manipulation started in Panama, in contrast after 1.5 years of litter addition in
274 the same experiment there was significantly lower fine root mass (Sayer et al. 2006). There was
275 probably a transient effect on fine root mass - a 29% reduction after 1.5 years (Sayer et al. 2006)
276 but an insignificant, 14%, reduction after ten years (see in Supplementary Materials). This
277 finding, of no significant reduction in fine root mass in soils with higher nutrient concentrations
278 caused by litter addition (Table 1), differs from the very significant, 50%, lower fine root mass in
279 the soils with higher nutrient concentrations caused by inorganic fertilization in the adjacent
280 Gigante Fertilization Experiment (Wurzburger and Wright 2015). The differences between the
281 two experiments – no significant effect in the litter addition experiment compared to a strong
282 effect in the fertilizer experiment may partly be caused by the much greater amount of P added
283 (c. ten times as much in the fertilizer as compared to the litter addition experiment) over a longer
284 time in the fertilizer experiment (13 years cf. 10 in the litter manipulation experiment), which
285 caused a much higher soil ‘available’ P in the fertilizer experiment – though the strongest
286 reduction in fine root mass in the fertilizer experiment was due to K (Wurzburger and Wright
287 2015). Thus a simple, and unsurprising, take home message could be that lower rates of
288 phosphorus addition (in the litter addition cf. N+P+K fertilization) caused smaller effects on fine
289 root mass, and thus that any effects of relatively small increases in nutrient input from, for
290 example, pollution are likely to have very small effects on fine root mass.

291 In a litter doubling experiment in Costa Rica fine root mass was 75% higher in the second
292 year (Leff et al. 2012), completely the opposite effect to that in Panama. The difference between

293 the two experiments could be due to: a transient effect early in the experiment (as in the second
294 year of litter addition in Panama); or plot size, the small plots in Costa Rica (3 m x 3 m) are hot
295 spots relative to the size of the crowns of large trees, and if trees are limited by nutrients they
296 may concentrate fine root growth into these hot spots; something they would not need to do in
297 large plots. Thus whether or not increased fine root mass is seen in plots with doubled litter input
298 could be due to time since the treatment started and/or plot size.

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

309 The decrease in root growth in the litter removal plots in Panama, was paralleled by
310 lower root and soil available nitrogen concentrations (live leaf nitrogen concentrations were also
311 lower in trees in litter removal plots, Table 2). Soil available P was also lower in litter removal
312 plots – though root P concentrations were not; K concentrations in soils and roots were not
313 affected by litter removal (Table 2). In Brazil, in 20-year old secondary forest root mass and
314 growth was lower in (20 m x 20 m) litter removal plots (Lima et al. 2010) but soil resin
315 phosphorus was not lower and soil nitrogen not reported (Maia et al 2015). In Costa Rica in the

316 second year of litter removal (in 3 m x 3 m plots) fine root biomass was not lower than controls
317 though total soil nitrogen was significantly lower. There are too few studies to make
318 generalizations but the two studies with big plots both have lower root growth in litter removal
319 plots; and for Panama we conclude that the reduced root growth in litter removal plots may have
320 been caused by lower nitrogen availability.

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

334

335 *Conclusions*

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

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

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469 production and turnover rates than direct methods. *PLoS ONE* 7:e48989.

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472 **Table 1.** Nutrient concentrations in fine roots from GLiMP experiment in a lowland semi-
 473 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

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

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

	Litter removal				Litter addition				N+P+K		N+P	
	Soil ^a	Root	Litter	Leaf	Soil ^a	Root	Litter	Leaf	Soil ^a	Root	Litter	Leaf
	(1)	(3)	(7)	(5)	(1)	(3)	(7)	(5)	(2)	(4)	(7)	(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.

493 Notes: Down arrow symbols represent lower and up arrow symbols represent higher
 494 concentrations compared to the controls; n.s. means not significant and n.d. means no data. a)
 495 Soil N is $\text{NO}_3^- + \text{NH}_4^+$; soil P is resin extractable or Mehlich 3 extractable; soil K is Mehlich 3
 496 extractable. b) Litter K in LR significantly lower at five years ($P=0.03$), but not at three years
 497 ($P=0.70$). c) Litter N in N+P+K plots not significantly higher at five years ($P=0.083$, but was
 498 significantly higher at three years ($P<0.000$).

499 Sources and year of experiment in which effect measured:

500 (1) Sheldrake et al. 2017 (9 years); (2) Wright (unpublished data, 14 years); (3) This study (Table
501 1, 10 years); (4) Wurzburger and Wright 2015 (14 years); (5) Sayer and Tanner 2010 (5 years);
502 (6) Mayor et al. 2014 (13 years); (7) Sayer et al. 2012 (5 years)

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507 **FIGURE CAPTIONS**

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

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

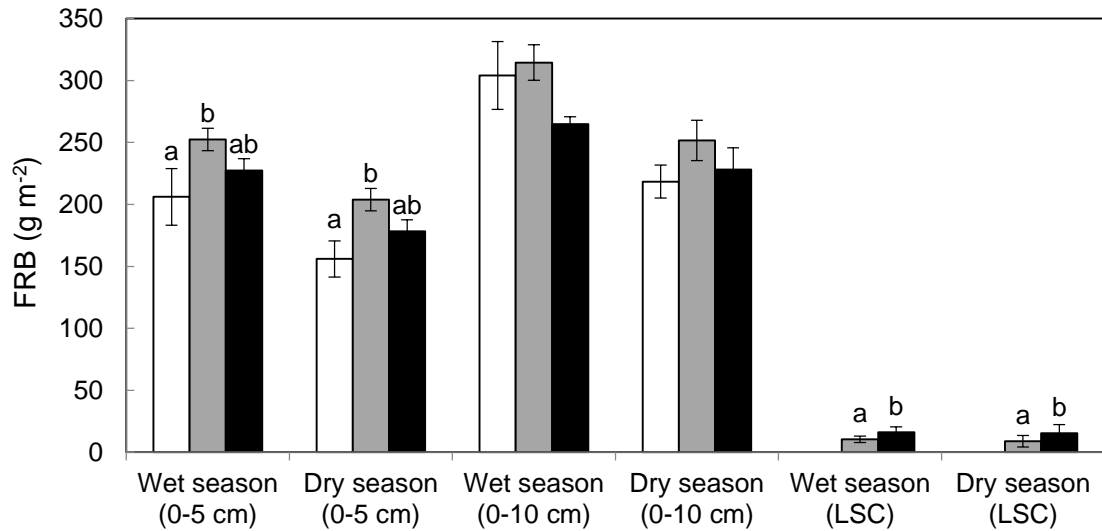
519 **Fig. 2.** Fine root mass production at 0-10 cm soil depth from 3-month interval ingrowth cores in
520 lowland semi-evergreen tropical forest Panama; open bars represent litter removals, gray bars

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

523 **Fig. 3.** Fine root length (FRL) in litter manipulation in lowland semi-evergreen forest in
524 Panama; (a) standing root length at 0-5 cm soil deep, (b) standing root length at 0-10 cm soil
525 deep, (c) root length production, (d) root length survivorship and (e) monthly rainfall; each bar
526 represents mean value with standard error (n=5) units are m of fine root per m² of rhizotron
527 surface; In panel a, b and c, open bars are litter removals, gray bars are controls, and black bars
528 are litter additions.

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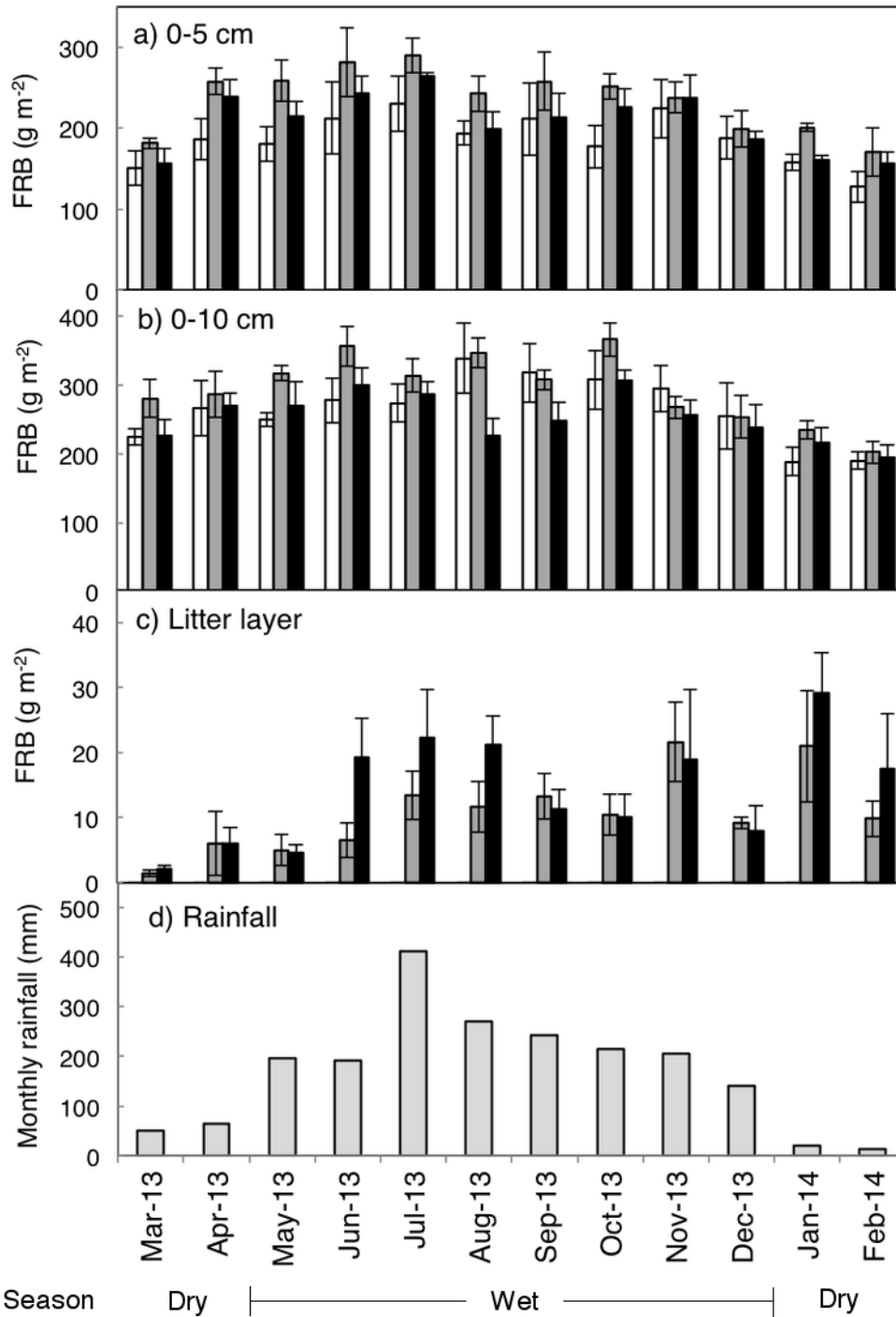
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533 **Fig. 1.** Fine root (≤ 2 mm diameter) biomass (FRB) in a litter manipulation experiment in lowland
534 semi-evergreen forest in Panama at 0-5 cm soil deep, at 0-10 cm soil deep and in litter standing
535 crop (LSC); each bar represents root biomass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

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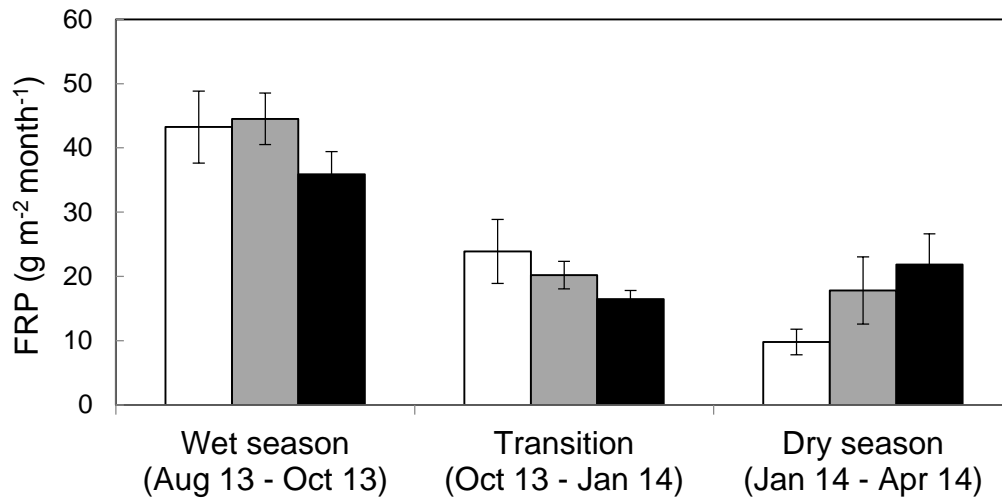
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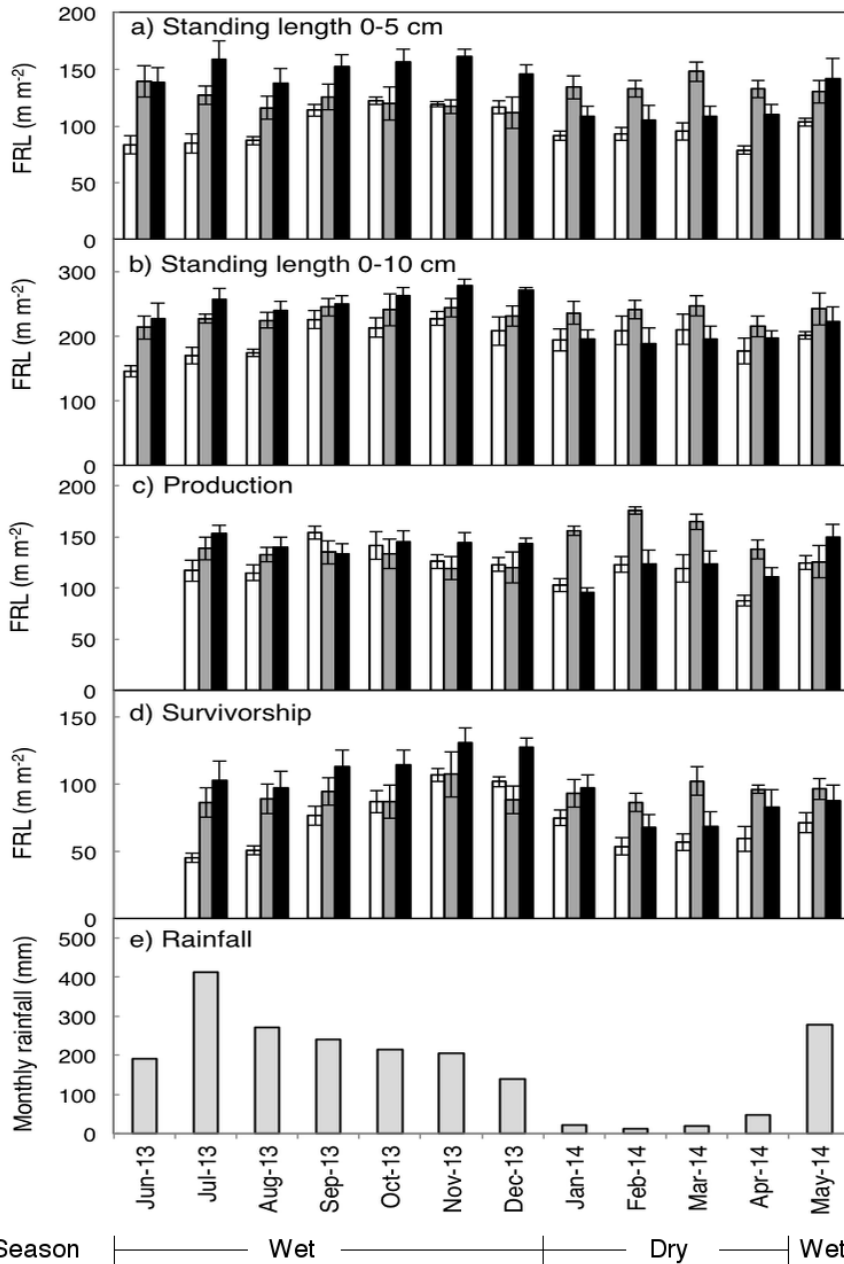
Supplementary Fig. 1. Fine root biomass (FRB, ≤ 2 mm diameter) in litter manipulation in lowland semi-evergreen forest in Panama (a) 0-5 cm soil deep, (b) 0-10 cm soil deep, (c) in litter layer and (d) monthly rainfall; each bar represents mean value (n=5) with standard error; In panel a, b and c, open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



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548 **Fig. 2.** Fine root mass production at 0-10 cm soil depth from 3-month interval ingrowth cores in
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 550 represent controls, black bars represent litter additions. Data are means and standard error of five
 551 plots per treatment; there was no significant difference in all litter treatments.

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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 m² of rhizotron surface; In panel a, b and c, open bars are litter removals, gray bars are controls, and black bars are litter additions.