1  **Title:**

2  **Changes in soil carbon and nutrients following six years of litter removal and addition in a tropical**
3  **semi-evergreen rain forest.**

4

5  **Authors**

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11  **Abstract**

12  Increasing atmospheric $CO_2$ and temperature may increase forest productivity, including litterfall,
13  but the consequences for soil organic matter remain poorly understood. To address this, we
14  measured soil carbon and nutrient concentrations at nine depths to 2 m after six years of continuous
15  litter removal and litter addition in a semi-evergreen rain forest in Panama. Soils in litter addition
16  plots, compared to litter removal plots, had higher pH and contained greater concentrations of: KCl-
17  extractable nitrate (both to 30 cm); Mehlich-III extractable phosphorus and total carbon (both to 20
18  cm); total nitrogen (to 15 cm); Mehlich-III calcium (to 10 cm); Mehlich-III magnesium and lower bulk
19  density (both to 5 cm). In contrast, litter manipulation did not affect ammonium, manganese,
20  potassium or zinc, and soils deeper than 30 cm did not differ for any nutrient. Comparison with
21  previous analyses in the experiment indicates that the effect of litter manipulation on nutrient
22  concentrations and the depth to which the effects are significant are increasing with time. To allow
23  for changes in bulk density in calculation of changes in carbon stocks, we standardized total carbon
24  and nitrogen on the basis of a constant mineral mass. For 200 kg m$^{-2}$ of mineral soil (approximately
25  the upper 20 cm of the profile) about 0.5 kg C m$^{-2}$ was 'missing' from the litter removal plots, with a
26  similar amount accumulated in the litter addition plots. There was an additional 0.4 kg C m$^{-2}$ extra in
27  the litter standing crop of the litter addition plots compared to the control. This increase in carbon in
28  surface soil and the litter standing crop can be interpreted as a potential partial mitigation of the
29  effects of increasing $CO_2$ concentrations in the atmosphere.

30

31  **1  Introduction**

32  Tropical forests and their soils are an important part of the global carbon (C) cycle, because they
33  contain 692 Pg C, equivalent to 66 % of the C in atmospheric $CO_2$ (Jobbagy and Jackson 2000).
34  Carbon in tropical forest soils is dynamic: Schwendenmann and Pendall (2008) reported a turnover
35  time of 15 years for the 'slow' pool of soil C, comprising 38% of the total soil C, in the top 10 cm of
36  soil in semi-evergreen rain forest on Barro Colorado Island, Panama (61% of total soil C was 'passive'
37  with a turnover time of the order of a thousand years). Turner et al. (2015) reported an approximate
38  25% increase in soil C from one dry season to the next wet season in the top 10 cm of soil on the
39  Gigante Peninsula in Barro Colorado Nature Monument, Panama, at a site close to the current litter
40  manipulation experiment. Thus, there is the potential for the amount of C in tropical soils to change
41  over only a few years, with potentially important consequences for atmospheric $CO_2$ concentrations.

42        Atmospheric $CO_2$ concentrations have been steadily increasing for decades and, one of the
43    effects of this could be widespread increases in forest growth (Nemani et al. 2003) and, as a result,
44    increased litterfall. There are few experimental studies of the effects of elevated $CO_2$ on forest
45    growth.  Körner (2006) reported that elevated $CO_2$ caused increased litterfall in one of three studies
46    in steady-state tree stands in temperate forests, but there have been no such studies in the tropics.
47    Thus the potential exists for increased $CO_2$ to increase forest growth and litterfall – though we do not
48    know how widespread and how large any increase in litterfall might be, especially in the tropics.

49        Soil C has been shown to respond to experimental changes in litter inputs. In three studies in
50    temperate forests in the USA, litter removal always resulted in lower soil organic carbon, but litter
51    addition had much more variable effects, increasing in one (Lajtha et al. 2014a), not changing in the
52    second (Bowden et al. 2014) and decreasing in the third (Lajtha et al. 2014b). The single study from
53    the tropics, in lowland rain forest in Southwestern Costa Rica, reported decreased soil C in litter
54    removal plots and increased soil C in litter addition plots (Leff et al. 2012). It is therefore likely that
55    soil C will increase in many, but not all, forests as a result of increased litter input.

56        The relative importance of aboveground or below ground inputs as sources of soil organic
57    matter has been reassessed in the last decade (Schmidt et al. 2011). Recently it was shown that 50-
58    70 % of the soil organic matter in boreal coniferous forest is from roots and root associated micro-
59    organisms (Clemmensen et al. 2013). The origin of the soil organic matter is thus a question of the
60    relative contributions of above-ground and below-ground inputs. Litter manipulation experiments
61    can provide insights into this issue by controlling one source of C input – aboveground litterfall.

62        Soil nutrients as well as C can change as a result of increasing or decreasing litter inputs and
63    are important because they will potentially affect soil fertility. In Panama, mineralization of organic
64    phosphorus (P) (inferred from the decrease in the concentration of organic P) in the top 2 cm of soil
65    during three years of litter removal was calculated to be sufficient to supply 20% of the P needed to
66    sustain forest growth – there were corresponding increases in organic P in litter addition plots, and
67    total nitrogen (N) showed a similar pattern (Vincent et al. 2010). 'Available' nutrients, including KCl-
68    extractable ammonium ($NH_4$) and nitrate ($NO_3$), and Mehlich-III extractable P, potassium (K), calcium
69    (Ca), magnesium (Mg), and micronutrients all changed over 4 years in the upper 2 cm of soil as a
70    result of litter manipulation (Sayer and Tanner 2010). After six years of litter manipulation surface
71    soils (0-10 cm) had lower $NO_3$ and K in litter removal plots, and higher $NO_3$ and Zn in litter addition
72    plots; other nutrients were not significantly affected (Sayer et al. 2012). In Costa Rica after 2.5 years
73    of litter manipulation surface soils (0-10 cm) had lower net nitrification in both litter removal and
74    addition treatments, while $NH_4$ concentrations were significantly lower in litter removal plots ($NH_4$
75    was 83-91% of the extractable N; Wieder et al. 2013). Thus, several soil nutrients in surface soils
76    change following litter manipulation, but there is no consistent pattern for N, very little data for P or
77    cations (the latter were not reported for the Costa Rican experiment), and no data for soils deeper
78    than 10 cm.

79        Here we report results from the Gigante Litter Manipulation Plots (GLiMP) experiment over
80    a much greater soil depth (0–200 cm) for total C, N, and P, and extractable ('plant-available') N,  P, K,
81    Ca, Mg, manganese (Mn), and zinc (Zn), measured after 6 years of continuous litter transfer. In
82    addition, we present a new way of expressing soil C (relative to the unchanging mineral mass), which
83    allows us to calculate overall changes in soil C and other elements independently of changes in bulk
84    density. Our objective was to describe changes in C and nutrient concentrations in the full soil profile
85    and to calculate C budgets to discover to the fate of the increased C input in litter addition plots. In
86    particular, we aimed to calculate the proportion of the added C that remains in the soil and the litter
87    standing crop, and can thus be considered as partial mitigation of atmospheric $CO_2$ accumulation

88  through increased forest productivity due to increased atmospheric $CO_2$ and temperature –
89  mitigation because C that is not in the soil will be in the atmosphere as extra $CO_2$. No other study has
90  tried to quantify the fate of C in organic matter added to tropical forest soils, though a study of
91  agricultural soil in temperate UK calculated that about 2.4% of organic matter in annual additions of
92  farmyard manure was still in the soil after 120 years (Powlson et al. 2011).

93  **2  Materials and methods**

94  The litter manipulation experiment is located in old-growth semi-evergreen lowland tropical forest
95  on the Gigante Peninsula (9°06′N, 79°54′W), part of the Barro Colorado Nature Monument in central
96  Panama. The experiment is located on the upper part of the landscape, where soils are Oxisols (Typic
97  Kandiudox). Surface soils have a pH of 4.5–5.0, low 'available' P concentrations, but high base
98  saturation and cation exchange capacity. Annual rainfall on nearby Barro Colorado Island (c. 5 km
99  from the study site) is 2600 mm and average temperature is 27°C. There is a strong dry season from
100 January to April, with approximately 90 % of the annual precipitation during the rainy season.

101         The experiment consists of fifteen 45-m x 45-m plots within a 40-ha area of old growth
102 forest. In 2001 all 15 plots were trenched to a depth of 0.5 m to minimize lateral nutrient and water
103 movement via the root/mycorrhizal network; the trenches were double-lined with plastic and
104 backfilled. Beginning in January 2003, litter (including branches <20 mm in diameter) was raked up
105 once a month in five plots, resulting in low, but not entirely absent, litter standing crop (litter
106 removal plots). The removed litter was immediately spread on five further plots (litter addition
107 plots), with five plots left as controls (CT plots). Treatments were assigned on a stratified random
108 basis using total litterfall per plot in 2002 (i.e. the three plots with highest litterfall were randomly
109 assigned to treatments, then the next three and so on) (Sayer et al. 2007).  The plots were
110 geographically blocked, litter from a particular litter removal plot was always added to a particular
111 litter addition plot and there was a nearby control plot.

112         Soils samples were collected in January 2009, the early dry season, using a 7.6 cm diameter
113 constant volume corer for the top 20 cm of soil and 7 cm diameter auger from 20 – 200 cm. Fresh
114 soils were extracted for $NO_3$ and $NH_4$ within 2 hours of sampling in a 2 M KCl solution, with detection
115 by automated colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO). Phosphorus and
116 cations were extracted within 24 h in Mehlich III solution and analyzed by inductively coupled
117 plasma optical emission spectrometry (ICP-OES). Soil pH was measured on a 1:2 fresh soil solution in
118 distilled water.

119         Dried (22C x 10 d) and ground soil was analyzed for total C and N by combustion and gas
120 chromatography on a Flash 1112 analyzer (Thermo, Bremen, Germany). Total P was determined by
121 ignition at 550°C for 1 h and extraction for 16 h in 1 M H2SO4, with detection by automated
122 molybdate colorimetry at 880 nm using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO).

123         Nutrient data was analysed using mixed effects models, with 'litter treatment', 'depth', and
124 their interaction as fixed effects, and 'plot' as a random effect. Where nutrient concentrations varied
125 non-linearly with depth, we used splines with two or three knots. Some nutrients showed severe
126 heteroscedasticity, and we accounted for this in the model by using 'variance covariates', which
127 model the variance as a function of one or more of the effects in the model (Pinheiro and Bates
128 2000; Zuur et al. 2009). For all nutrients, depth was modelled as a numeric predictor and log
129 transformed prior to analysis. We performed model selection based on likelihood ratio tests and
130 Aikake Information Criterion with correction for small sample sizes (AICc, Burnham and Anderson
131 2002). We derived P-values for fixed effects by comparing null models to full models using likelihood

132 ratio tests. Final models were refitted using restricted maximum likelihood estimation (REML) (Zuur
133 2009). Where the treatment * depth term was significant, we refitted the model omitting either the
134 litter addition treatment or the litter removal treatment to assess the contribution of each of the
135 treatments (litter addition and litter removal) to the overall interaction term.  Analyses were done in
136 R version 3.1.2.

137       Amounts of soil total C and N were also calculated relative to soil mineral mass to allow
138 comparisons between the treatments where bulk density and soil depth was changing due to
139 removal and addition of litter; soil in litter removal plots was shrinking and had increasing bulk
140 density, soil in litter addition plots was increasing in depth and had lower bulk density. Expressing
141 potentially changing elements relative to unchanging mineral mass allows for change to be
142 expressed against an unchanging reference; it is analogous to expressing soil water relative to soil
143 dry mass rather than soil fresh mass. Soil organic C with depth was calculated for each plot by fitting
144 a line to cumulative soil organic C (Y) against cumulative soil mineral mass (X). Bulk density data
145 were measured for each plot only in the top 0-5 cm for soil. Below that we used bulk density data for
146 one pit only. Bulk density below 10 cm depth does not vary much across the site; data for four soil
147 pits (not in any of the plots) have a coefficient of variation of about 10 % for soils from 10 - 20 cm
148 deep and 3 % for soils from 20-50 cm deep), whereas coefficients of variation of bulk densities in
149 surface 0-5 cm soils were higher: control 12 %, litter addition 15 % and litter removal 4.9 %. Bulk
150 density data were used to estimate approximate soil depth for control plots in Figs. 3 and 4.
151 Statistical comparisons of modelled cumulative total C against cumulative mineral matter were
152 compared by bootstrapping, using R version 3.1.2.

153

## 3  Results

155 Soils in litter addition plots, compared to litter removal plots, had significantly lower bulk density
156 (both to 5 cm) and higher $NO_3$ and pH (to 30 cm), $P_{Meh}$ and total C (both to 20 cm), total N (to 15 cm),
157 Ca (to 10 cm), and Mg (to 5 cm) and (Fig. 1 and 2 and Tables S1 and S2). There were fewer
158 differences when compared to control soils:  litter addition soils had higher concentrations of $P_{Meh}$
159 (to 20 cm), $NO_3$ (to 15 cm), Ca (to 10 cm), and pH (to 10 cm). Nutrient concentrations in litter
160 removal soils were not significantly lower than those in controls. Nutrient concentrations in soils >
161 30 cm deep did not differ significantly for any nutrient. Thus, in some way total C, total N, $NO_3$, $P_{Meh}$,
162 Ca and Mg were significantly affected by litter removal or addition, but K, Mn, $NH_4$, Zn and were not;
163 effect sizes (log response ratio for 0-5 cm soils) decreased from 0.81 for $NO_3$, to 0.39 for Ca, 0.27 for
164 Zn, 0.20 for $P_{Meh}$, 0.20 for Mg, 0.15 for $C_{tot}$, 0.11 for $N_{tot}$.

165       All nutrients decreased in concentration with increasing soil depth. In control soils,
166 concentrations at 50–100 cm compared to 0–5 cm were: $NH_4$ 50 %, Mg 37 %, $P_{tot}$ 36 %,  K 32 %, $P_{Meh}$
167 25 %, $NO_3$ 24 %, $N_{tot}$ 12 %, Ca 11 % and $C_{tot}$ 11 %;  $NO_3$ was only 24 % of the total inorganic N in
168 controls (mean over all depths) (Figs 1 and 2 and Table S1). Concentrations of most elements
169 continued to decrease below 100 cm deep in the soil; those from 150–200 cm were about half those
170 from 50–100 (ranging from 14% for Ca to 81% for $NH_4$, Table S1).

171       Soil bulk density in the top 5 cm was significantly lower in litter addition than litter removal,
172 though neither was significantly different from the controls. Soil C stocks standardized to a
173 consistent mineral mass (*i.e.* that in the control plots) was significantly greater in litter addition
174 compared to litter removal to about 10 cm deep in the soil (Fig. 3 and 4). Total N per mineral mass of
175 soil was also significantly greater in litter addition than litter removal in approximately the top 10 cm

176  of soil. In contrast, C:N ratios changed little with depth; in control soils, C:N was about 10.5 near the
177  surface and 10.0 at 150–200 cm, in litter removal plots, C:N was 10.5 at the surface and 10.3 at
178  depth, while litter addition soils were more variable, with C:N being 11.7 at the surface and about
179  10.0 at 150–200 cm deep.

180

181  **4  Discussion**

182  **4.1 Soil carbon dynamics**

183      The amount of C 'missing' from litter removal and 'extra' in the litter addition over about the
184  top 20 cm of soil (from calculations based on C per mineral matter), six years after (January 2009)
185  litter removal and addition started, was about 0.5 kg C m$^{-2}$ (Fig. 3). These changes are about c. 1%
186  per year; in contrast if we calculate the change based on a fixed depth of 20 cm, ignoring changes in
187  bulk density, we get a change of about 2% per year. Thus ignoring the changes in bulk density results
188  a misleading doubling of the estimated rate of change. The similarity of the losses from litter
189  removal and gains in litter addition probably has different causes: we speculate that losses from the
190  soil in the litter removal plots are due to respiration being greater than additions; we did not
191  physically remove organic matter from the mineral soil. We further speculate that increases in C in
192  the mineral soil in the litter addition plots are a result of infiltration of dissolved and particulate
193  organic matter draining from the litter standing crop, and/or changes in root exudates; increases in
194  root growth are not the explanation – root growth was lower in litter addition plots (Sayer et al.
195  2006).

196      In addition to the extra *soil* C in the litter addition plots, the litter standing crop was also
197  larger in litter addition plots. In September 2005 (2.8 years after litter manipulation started) there
198  was an additional 0.4 kg C m$^{-2}$ in the Oi and Oe layers compared to control plots (Sayer and Tanner
199  2010) and data from 2013 show that litter standing crop was at about this level (C. Rodtassana,
200  University of Cambridge, unpublished data). Together this extra 0.9 kg C m$^{-2}$ in the litter addition soil
201  and litter standing crop is about 30 % of the 3 kg C m$^{-2}$ in litter added to the litter addition plots over
202  6 years (litterfall is c. 1 kg m$^{-2}$ yr$^{-1}$, c. 45 % is C, times 6 years). This increase in C in surface soil and the
203  litter standing crop could be interpreted as *potential* partial mitigation of the effects of increasing
204  $CO_2$ concentrations in the atmosphere, though any increases in litterfall due to increased $CO_2$ will be
205  less than our experimental doubling. For example, a free air $CO_2$ experiment in 13-year old loblolly
206  pine plantation in North Carolina USA reported a 12% increase in litterfall over 9 years (Lichter et al.
207  2005, 2008).

208      The increases in soil C in our litter addition plots (c. 1% per year, of total C to c. 20 cm depth)
209  are much smaller than those reported in the other study of litter manipulation in tropical forest
210  (lowland rain forest in Southwestern Costa Rica) where two years of litter removal reduced soil C
211  concentration  in the top 10 cm of soil by 26 %, and doubling litter increased soil C by 31 % (Leff et al.
212  2012). In three temperate forest studies, rates of change in soil C were small, but they were
213  measured over much longer periods. In north central USA, soil C content decreased by 44 % in litter
214  removal plots and increased by 31 % in double litter plots over a 50-year period (Table 2 Lajtha et al.
215  2014a). In Pennsylvania, USA, 20 years of removing litter reduced soil C by 24%, although the
216  corresponding litter doubling had no effect (Bowden et al. 2014). In a deciduous forest in
217  Massachusetts, USA, 20 years of litter removal also reduced mineral soil C (by 19%), but litter
218  addition also resulted in lower mineral soil C (by 6%, Lajtha et al. 2014b). Differences between
219  forests in the effect of litter addition on soil organic matter could be partly due to differences in

220  priming of pre-existing soil organic C resulting in no, or small, increases in soil C in double litter plots.
221  Priming might be greater in N limited temperate forests remote from atmospheric N pollution,
222  because one cause of priming is mining of soil organic matter for N by microbes stimulated by
223  additions of litter with low N concentrations (relative to soil organic matter) (e.g. Nottingham et al.
224  2015). It is therefore likely that many, but not all, forests will show increased C in soils as a result of
225  increased litter input.

226  Soil C might on average originate predominantly from roots rather than shoots (Rasse et al.
227  2005) and that may be the case in our soils in Panama because although changes in litter inputs have
228  caused changes in soil C they are small – approximately 1% of total soil C per year, compared to the
229  'normal' turnover of C of 25% (0-10 cm soil) within 6 months (as calculated from changes in C
230  concentration from wet season to dry season; Turner et al. 2015) and an annual turnover of about
231  7% based on incorporation of $^{13}C$ into soils over decades (Schwendenmann and Pendall 2008).
232  Turnover rates of soil C are also high in other tropical forests; for example, in Eastern Brazil 40-50 %
233  of the C in the top 40 cm of soil had been fixed in about 32 years (Trumbore 2000). In Panama the
234  much greater rates of turnover of soil C as compared to changes caused by litter removal and
235  addition suggest that the main source of soil organic matter (over months to a few years) is roots,
236  root exudates and mycorrhizal fungi. Nevertheless, changes in above ground litter input are still
237  important, because they have resulted in overall decreases and increases in soil C.

238

239  **4.2 Litter manipulation - depth of effects.**

240  Effects of litter removal and addition differed among nutrients and were strongest near the soil
241  surface, with no significant differences below 30 cm. The strength of the effects and the depth to
242  which they were significant are increasing with time. Four years after the start of litter manipulation
243  six nutrients showed significant effects in the upper 2 cm of soil ($NO_3$, $NH_4$, $P_{Meh}$, K, Ca, Mg), whereas
244  only $NO_3$ and Ca showed significant effects from 0-10 cm (Sayer et. al 2010). After 6 years, in the
245  early dry season 2009 (current paper), effects were seen to greater depths: $NO_3$ was higher to 30 cm
246  and $P_{meh}$ to 20 cm in litter addition plots. Over time significant differences have become apparent
247  for more nutrients and to greater depth in the soil; these differences were caused by differences in
248  litter input.

249  The concentrations of $NH_4$ and $NO_3$ are usually only measured in surface soils in tropical rain
250  forests, perhaps because N is generally thought not to limit growth in such forests. However,
251  fertilization with N and K together increased growth of saplings and seedlings in the Gigante
252  Fertilization Project, which is adjacent to our litter manipulation experiment in Panama (Wright et al.
253  2011). Relevant concentrations of $NH_4$ and $NO_3$ are also difficult to measure since they change
254  rapidly over only a few hours (Turner and Romero 2009); extractions for the current paper were
255  done within two hours of collecting soils. In our litter manipulation experiment, $NH_4$ accounted for
256  76% of the sum of $NH_4$ and $NO_3$ (mean over all depths in controls plots) and decreased less with
257  depth than $NO_3$ (at 50-100 cm $NH_4$ was about 50 % of surface values whereas $N0_3$ was about 25 %).
258  In the nutrient addition experiment, Koehler et al. (2012) reported that $NH_4$ also deceased less with
259  depth (at 200 cm it was 41 % of surface soils) than $NO_3$ (to 17 % of surface soils), and that $NH_4$ was
260  the dominant form of total inorganic N (about 80 %) – the same patterns as in our litter
261  manipulation experiment. Nitrogen dynamics in soils have also been measured in a litter
262  manipulation experiment in Costa Rica (Wieder et al. 2013), where nitrification rates were lower in
263  both litter removal and litter addition plots and extractable $NH_4$ was significantly lower in litter
264  removal plots. This contrasts with our results of greater $NO_3$ in litter addition compared to litter

265 removal and no effect on NH$_4$; the differences between the experiments might be due in part to
266 different soils and a wetter climate in Costa Rica (c. 5 m rain per year, c.f. 2.6 in Panama). Thus, soil
267 N dynamics differ somewhat between the only two tropical litter manipulation experiments, but in
268 both NH$_4$ was the dominant form of inorganic N, and in both total inorganic N decreased in litter
269 removal plots and increased in litter addition plots (though differences were not always statistically
270 significant).

271     The 'available' forms of P are also not often reported for the deeper horizons of tropical
272 forest soils, despite the fact that P is usually regarded as the most likely limiting nutrient in such
273 forests (Tanner et al. 1998 and Cleveland et al. 2011) and has been shown to limit fine litter
274 production in the adjacent nutrient addition experiment (Wright et al. 2011). Mehlich P and total P
275 both decreased with depth in control soils in our litter manipulation experiment (at 50-100cm
276 concentrations were 25 and 29 % of those at 0-5 cm); in litter removal soils the decrease was less
277 steep (37 % and 36 %). Litter addition increased Mehlich P in the surface soils (though total P was not
278 significantly greater), indicating increased P availability, which is consistent with the finding that
279 litter addition decreased the strength of phosphate sorption in these soils (Schreeg at al. 2013). Thus
280 for P, potentially the most commonly limiting nutrient in tropical rain forest soils, six-years of
281 continuous removal and addition of litter in our experiment has reduced and increased 'available' P
282 down to 20 cm in the soil.

283     The relative amounts of exchangeable cations and their change with depth in the control
284 plots of the Panamanian litter manipulation soils are similar to patterns in other tropical forest soils.
285 In our experiment, Ca concentrations (in centimoles of charge) are about twice those of Mg in
286 surface soils (though below 30 cm Mg to Ca ratios exceed 1); K concentrations are usually less than 5
287 % of the total exchangeable bases. With increasing depth, Ca, Mg and K concentrations all decrease,
288 with Ca decreasing more than Mg or K. Other tropical forest soils are similar: in 19 profiles
289 throughout Amazonia the sum of base cations (Ca, Mg, K) was usually dominated by exchangeable
290 Ca (11 cases) or Ca was equal to Mg (4 cases), and both Ca and Mg mostly decreased with depth,
291 while K was in low or in trace concentrations in all profiles (Quesada et al. 2011). In Hawaii (Porder
292 and Chadwick 2009), much younger soils (11,000 BP on lava), with much higher concentrations of
293 Ca, Mg and K than Panama and Amazonia, showed similar patterns: Ca was the dominant cation, K
294 was usually less than 5 % of the sum of exchangeable Ca, Mg and K, and all cations decreased with
295 depth at the wetter sites (but not in the drier sites). Thus, in most wet tropical forest soils, Ca is the
296 most abundant cation and most cations decrease with depth. Litter addition in Panama increased Ca
297 and Mg concentrations in the surface soils and thus steepened the depth gradient, whereas litter
298 removal decreased Ca and Mg and therefore decreased the gradient; K was at much lower
299 concentrations (as in Amazonia and Hawaii) and was not affected by litter addition and litter
300 removal even in 0-5 cm soils.

301 **4.3 Design of litter manipulation experiments**

302     The design of litter manipulation experiments needs to be carefully considered when
303 evaluating their results. The strength of the effect of litter manipulation on soil C in Panama was
304 much less than that in Costa Rica, but the Panama and Costa Rica experiments are very different in
305 spatial scale. Plots in Panama are large, 45 x 45 m, those in Costa Rica are small, 3 x 3 m. The small
306 plots are 'hot' and 'cold' spots relative to large individual tree crown areas (and likely tree root
307 areas); crowns of the largest trees in lowland rain forests are commonly 25 m in diameter, so a 3 x 3
308 m plot is 2 % of that area. These differences in experimental design and their effects on the pattern
309 of the results should be considered when trying to understand ecosystem level processes; small hot

310 and cold spots may not represent what would happen in plots on the scale of the large trees, as
311 pointed out by Leff et al. (2012).

312

## 5 Conclusions

314 The increase in C in the mineral soil and the litter standing crop following litter addition was
315 statistically significant in the top 20 cm of the soil, suggesting that any increased litterfall as a result
316 of increased atmospheric $CO_2$ and/or temperature could result in a substantial increase in soil C and
317 therefore partially mitigate the increase in atmospheric $CO_2$. However, the current experiment
318 added much more litter than might be produced by an increase in $CO_2$ of, say, 200 ppm, and added
319 more nutrients than might occur even in temperate polluted sites. Thus new experiments are
320 required to investigate the effects of more realistic increases in litterfall using litter with low nutrient
321 concentrations.

322 Supplementary material

323 R code for models used to estimate of means and confidence intervals

324 Supplementary Table S1 with full original data from soil analyses

325 Supplementary Table S2 Model estimates of concentrations (from Sheldrake)

326 Supplementary Figure 1. Expanded versions of parts of Figures 1 & 2 showing significant differences.

339

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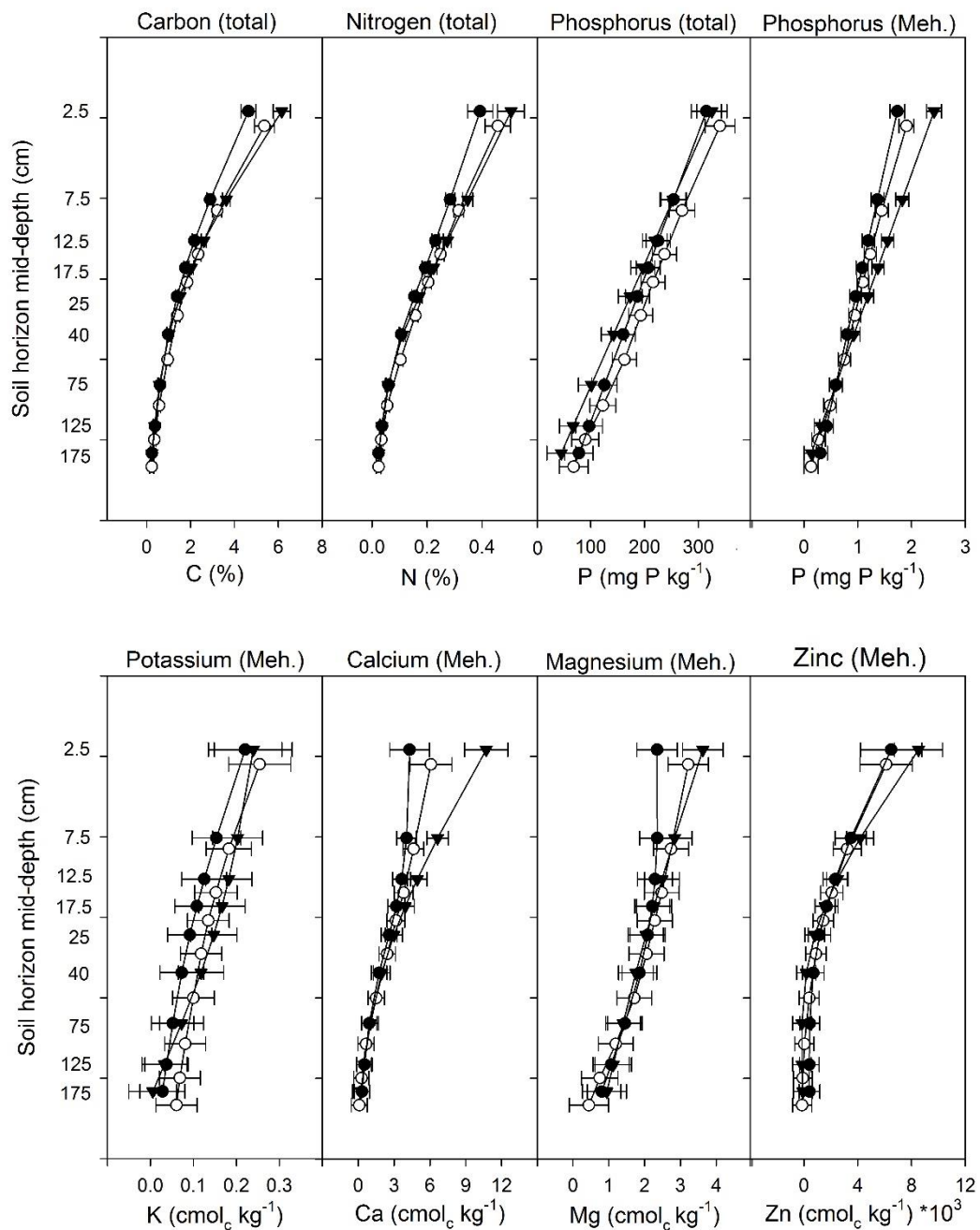428 98, 1616-1625, 2011.

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Fig. 1 Concentrations of soil C, N, P (various fractions) and cations (Mehlich extractions), plotted against the mid-point of the soil layers sampled (Zn values should be divided by 1000 to obtain actual means), control points are displaced below treatments. Data are fitted values of the mixed effects models with 95% confidence intervals (see Methods), in litter removal ●, control O and litter addition ▼ plots.
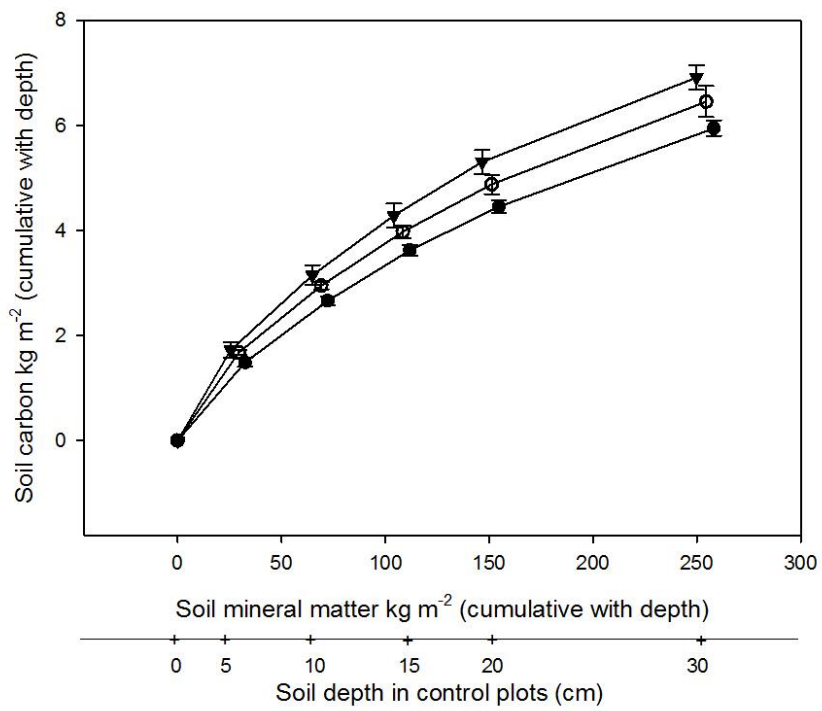
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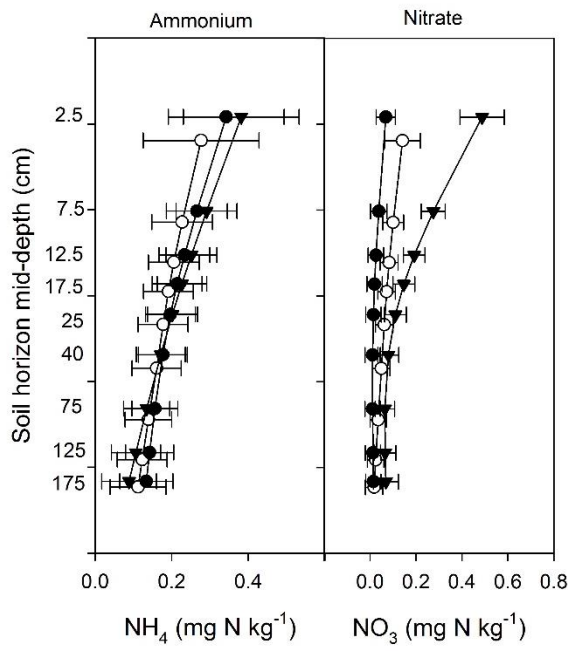


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444 Fig. 2 Mean concentrations of ammonium and nitrate plotted against the mid-point of the soil layers

445 sampled, control points are displaced below treatments. Data are fitted values of the mixed effects

446 models with 95% confidence intervals (see Methods), in litter removal ●, control O and litter
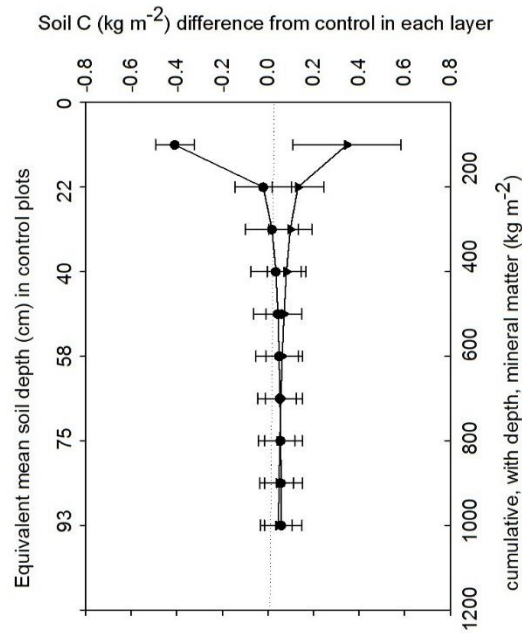
447 addition ▼ plots.

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Fig. 3 Soil carbon content and mineral content in litter addition, control, and litter addition expressed as kg C m$^{-2}$ cumulatively from 0 to 30 cm soil depth. Values are means for 5 plots per treatment +/- SE, litter removal ●, control O, and litter addition ▼.

459  Fig. 4 Differences in soil carbon content relative to control soils (mean and SE, n = 5), after 6 years of
460  litter manipulation, plotted for successive soil layers: 0-100 kg (mineral matter) m$^{-2}$, plotted at 100 kg
461  m$^{-2}$ on right y axis; 100-200 kg m$^{-2}$, plotted at 200 kg m$^{-2}$; and so on to 900-1000 kg m$^{-2}$, plotted at
462  1000 kg m$^{-2}$; in litter removal ● and litter addition ▼ plots. We calculated the soil C in the litter
463  removal and litter addition plots at the mineral mass equal to that at various depths in the control
464  plots (0-5 cm, 5-10 cm, etc), we then calculated the difference in C between each litter removal (or
465  litter addition) and its control plot for the same mineral mass. Approximate depth for cumulative soil
466  mineral mass in control plots is shown on left y axis.

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