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.**

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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

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

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

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

4.2 Litter manipulation - depth of effects.

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

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

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339

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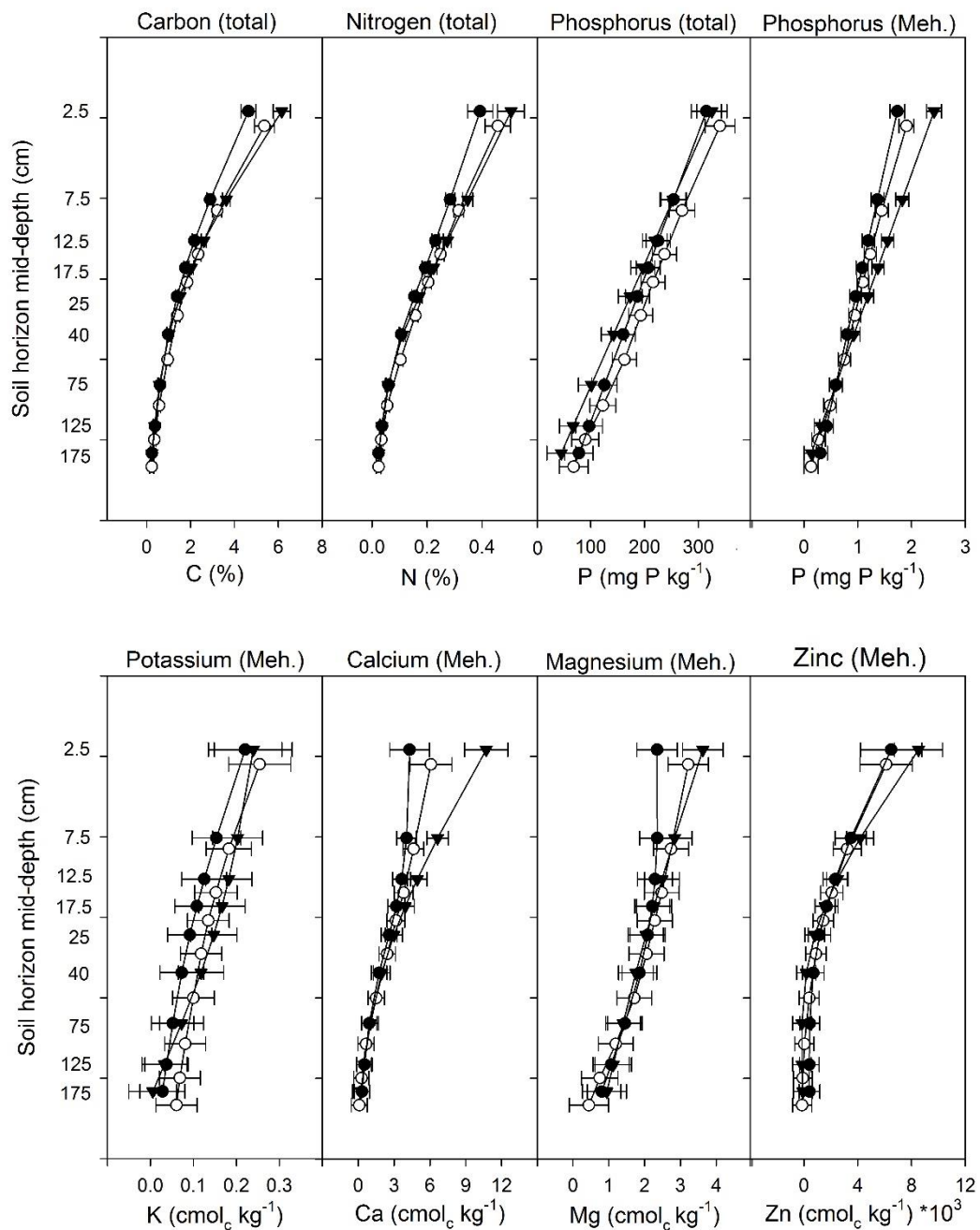
Fig. 1 Concentrations of soil C, N, P (various fractions) and cations (Mehlich extractions), plotted against the mid-point of the soil layers sampled (Zn values should be divided by 1000 to obtain actual means), control points are displaced below treatments. Data are fitted values of the mixed effects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

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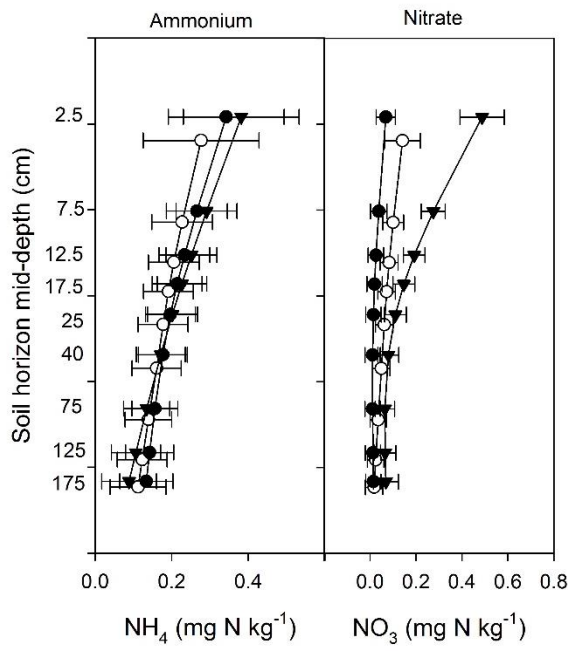
444 Fig. 2 Mean concentrations of ammonium and nitrate plotted against the mid-point of the soil layers
445 sampled, control points are displaced below treatments. Data are fitted values of the mixed effects
446 models with 95% confidence intervals (see Methods), in litter removal ●, control O and litter
447 addition ▼ plots.

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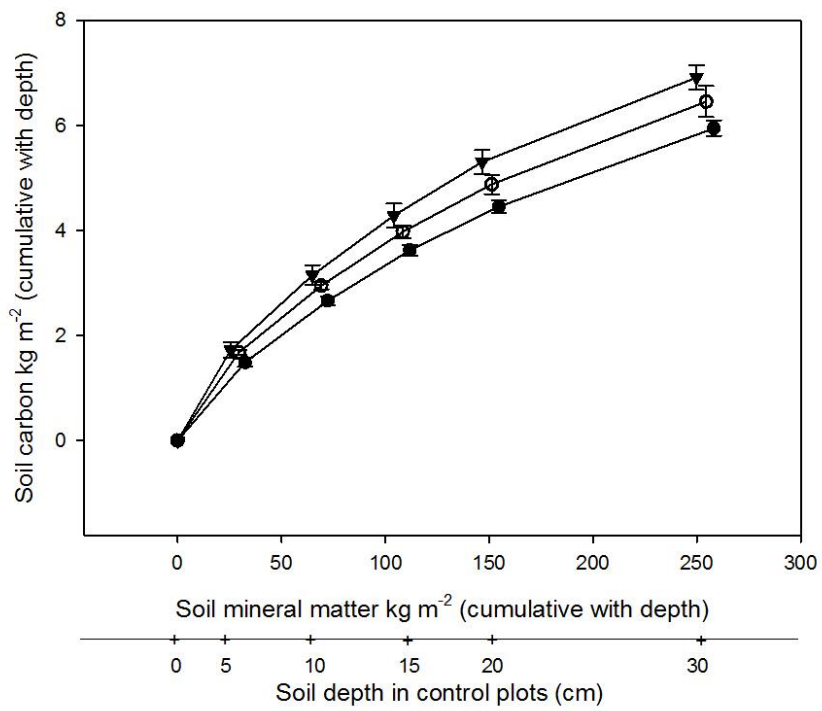
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452 Fig. 3 Soil carbon content and mineral content in litter addition, control, and litter addition

453 expressed as kg C m^{-2} cumulatively from 0 to 30 cm soil depth. Values are means for 5 plots per

454 treatment +/- SE, litter removal ●, control O, and litter addition ▼ .

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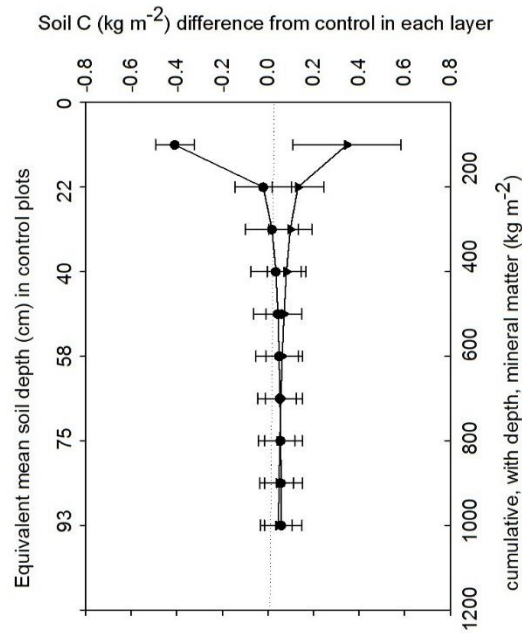
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459   Fig. 4 Differences in soil carbon content relative to control soils (mean and SE, n = 5), after 6 years of
460   litter manipulation, plotted for successive soil layers: 0-100 kg (mineral matter) m^{-2}, plotted at 100 kg
461   m^{-2} on right 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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