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Title:

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

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Abstract

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

1 Introduction

Tropical forests and their soils are an important part of the global carbon (C) cycle, because they contain 692 Pg C (two thirds in evergreen and one third in deciduous forests), equivalent to 66 % of the C in atmospheric CO_2 (Jobbagy and Jackson 2000). Carbon in tropical forest soils is dynamic; Schwendenmann and Pendall (2008) reported a turnover time of 15 years for the 'slow' pool of soil C (38 % of the total soil C; 61% of total soil C was 'passive' with a turnover time of the order of a thousand years) in the top 10 cm of soil in semi-evergreen rain forest on Barro Colorado Island, Panama. Turner et al. (2015) reported an approximate 25% increase in soil C from one dry season to the next wet season in the top 10 cm of soil on the Gigante Peninsula in Barro Colorado Nature Monument, Panama; a site near where the current litter manipulation experiment was carried out.

Thus, there is the potential for the amount of C in tropical soils to change over only a few years, with potentially important consequences for atmospheric CO_2 concentrations.

Atmospheric CO_2 concentrations, and temperature, have been steadily increasing for decades, one of the effects of this could be widespread increases in forest growth (Nemani et al. 2003) and as a result increased litterfall. Few experimental studies of the effects of elevated CO_2 on forest growth have been done; Korner (2006) reported that elevated CO_2 caused increased litterfall in one of three studies in steady-state tree stands in temperate forests; there have been no such studies in the tropics. Thus the potential exists for increased CO_2 to increase forest growth and litterfall – though we do not know how widespread and how large any increase in litterfall might be, especially in the tropics.

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

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

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

Here we report results from the Gigante Litter Manipulation Plots (GLiMP) experiment over a much greater soil depth (0–200 cm) for total C, N, and P, and extractable ('plant-available') N, P, K, Ca, Mg, manganese (Mn), and zinc (Zn), measured after 6 years of continuous litter transfer. In addition, we present a new way of expressing soil C (relative to the unchanging mineral mass), which allows us to calculate overall changes in soil C and other elements independently of changes in bulk density. Our objective was to describe changes in C and nutrient concentrations in the full soil profile and to calculate C budgets to discover what happens to the increased C input in litter addition plots. In particular, we aimed to calculate the proportion of the added C that remains in the soil and the litter standing crop, and can thus be considered as partial mitigation for increased forest productivity due to increased atmospheric CO_2 and temperature – mitigation because C that is not in the soil will be in the atmosphere as extra CO_2. No other study has tried to quantify the fate of C in organic matter added to tropical forest soils; though a study of agricultural soil in temperate UK calculated that about 2.4% of organic matter in yearly-added farmyard manure was still in the soil after 120 years (Powlson et al. 2011).

2 Materials and methods

"The study was carried out as part of an ongoing long-term litter manipulation experiment to investigate the importance of litterfall in the C dynamics and nutrient cycling of tropical forests. The forest under study is an old-growth semi-evergreen lowland tropical forest, located on the Gigante Peninsula (9°06′N, 79°54′W) of the Barro Colorado Nature Monument in Panama, Central America. The soil is an Oxisol with a pH of 4.5–5.0, with low 'available' P concentration, but high base saturation and cation exchange capacity. Nearby Barro Colorado Island (c. 5 km from the study site) receives a mean annual rainfall of 2600 mm and has an average temperature of 27°C. There is a strong dry season from January to April with a median rainfall of less than 100 mm per month; almost 90 % of the annual precipitation occurs during the rainy season. Fifteen 45-m x 45-m plots were established within a 40-ha area (500 x 680-m) of old growth forest in 2000. In 2001 all 15 plots were trenched to a depth of 0.5 m in order to minimize lateral nutrient- and water movement via the root/mycorrhizal network; the trenches were double-lined with plastic and backfilled. Starting in January 2003, the litter (including branches <20 mm in diameter) in five plots was raked up once a month, resulting in low, but not entirely absent, litter standing crop (L- plots). The removed litter was immediately spread on five further plots (L+ plots); five plots were left as controls (CT plots). The assignment of treatments was made on a stratified random basis, stratified by total litterfall per plot in 2002, i.e. the three plots with highest litterfall were randomly assigned to treatments, then the next three and so on." (Sayer et al. 2007). The plots were geographically blocked, litter from a particular LR plot was always added to a particular LA plot and there was a nearby control plot.

Soils samples were collected in January 2009, the early dry season, using a 7.6 cm diameter corer for the top 20 cm of soil and 2.5 cm diameter auger from 20 – 200 cm. Soil mineral concentrations. Fresh-soil extracts for mineral nutrients were prepared within 24 h of collection (except for NO_3 and NH_4, which were extracted within 2 hours of sampling in a 2 M KCl solution) and determined by automated colorimetry; soil P and cations were determined by Mehlich III extraction and analyzed by ICP-OES, soil pH was measured on a 1:2 fresh soil solution in distilled water. Dried and ground soil was analyzed for total C and N by combustion and gas chromatography on a Flash 1112 analyzer (Thermo, Bremen, Germany). Total P was determined by ignition at 550°C for 1 h and extraction for 16 h in 1 M H2SO4, with detection by automated molybdate colorimetry at 880 nm using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO).

Nutrient data was analysed using mixed effects models, with 'litter treatment', 'depth', and their interaction as fixed effects, and 'plot' as a random effect. Where nutrient concentrations varied non-linearly with depth, we used splines with two or three knots. Some nutrients showed severe heteroscedascicity, and we accounted for this in the model by using 'variance covariates', which model the variance as a function of one or more of the effects in the model (Pinheiro and Bates 2000; Zuur et al. 2009). For all nutrients, depth was modelled as a numeric predictor and log transformed prior to analysis. We performed model selection based on likelihood ratio tests and

Aikake Information Criterion with correction for small sample sizes (AICc, Burnham and Anderson 2002). We derived P-values for fixed effects by comparing null models to full models using likelihood ratio tests. Final models were refitted using restricted maximum likelihood estimation (REML) (Zuur 2009). Where the treatment * depth term was significant, we refitted the model omitting either the litter addition treatment or the litter removal treatment to assess the contribution of each of the treatments (litter addition and litter removal) to the overall interaction term. Analyses were done in R version 3.1.2.

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

3 Results

Soils in LA plots, compared to LR plots, had significantly higher: NO_3 and pH (to 30 cm); P_{Meh} and total C (both to 20 cm); total N (to 15 cm); Ca (to 10 cm); Mg and lower bulk density (both to 5 cm), (Figs 1 and 2 and Table S1). When compared to control soils, there were fewer differences, LA soils had higher concentrations of P_{Meh} (to 20 cm); NO_3 (to 15 cm); Ca (to 10 cm); and pH (to 10 cm). LR soil nutrient concentrations were not significantly lower than those in controls. Nutrient concentrations in soils > 30 cm deep did not differ significantly for any nutrient. Thus, in some way total C, total N, NO_3, P_{Meh}, Ca and Mg were significantly affected by litter removal or addition, but K, Mn, NH_4, Zn and were not; 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 Zn, 0.20 for P_{Meh}, 0.20 for Mg, 0.15 for C_{tot}, 0.11 for N_{tot}.

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

Soil bulk density in the top 5 cm was significantly lower in LA than LR, though neither was significantly different from the controls. Soil C stocks standardized to a consistent mineral mass (*i.e.* that in the control plots) was significantly greater in LA compared to LR to about 10 cm deep in the soil (Figs 3 and 4). Total N per mineral mass of soil was also significantly greater in LA than LR in approximately the top 10 cm of soil. In contrast, C:N ratios changed little with depth; in control soils, C:N was about 10.5 near the surface and 10.0 at 150–200 cm, in LR plots, C:N was 10.5 at the surface and 10.3 at depth, while LA soils were more variable, with C:N being 11.7 at the surface and about 10.0 at 150–200 cm deep.

4 Discussion

4.1 Soil carbon dynamics

The amount of C 'missing' from LR and 'extra' in the LA over about the top 20 cm of soil (from calculations based on C per mineral matter), six years after litter removal and addition started (January 2009), was about 0.5 kg C m^{-2} (Fig. 3). The similarity of the losses from LR and gains in LA probably has different causes: we speculate that losses from the soil in the LR plots are due to respiration being greater than additions; we did not physically remove organic matter from the mineral soil. We further speculate that increases in C in the mineral soil in the LA plots are a result of infiltration of dissolved and suspended organic matter draining from the litter standing crop, and/or changes in root exudates; increases in root growth are not the explanation – root growth was lower in LA plots (Sayer et al. 2006).

In addition to the extra *soil* C in the LA plots the litter standing crop (LSC) was also higher in LA plots; in September 2005 (2.8 years after litter manipulation started) there was 0.4 kg C m^{-2} extra in the Oi and Oe layers compared to control plots (Sayer and Tanner 2010) and data from 2013 show that LSC was at about this level (C. Rodtassana in prep.). Together this extra 0.9 kg C m^{-2} in the LA soil and litter standing crop is about 30 % of the 3 kg C m^{-2} in litter added to the LA plots over 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 litter standing crop could be interpreted as *potential* partial mitigation of the effects of increasing CO_2 concentrations in the atmosphere, though any increases in litterfall due to increased CO_2 will be less than our experimental doubling (a free air CO2 experiment in 13-year old loblolly pine plantation in North Carolina U.S.A reported a 12% increase in litterfall over 9 years (Lichter et al 2005 and 2008)).

The increases in soil C in our LA plots (c. 1% per year, of total C to c. 20 cm depth) are much smaller than those reported in the other study of litter manipulation in tropical forest (lowland rain forest in Southwestern Costa Rica) where two years of removing litter reduced soil C concentration, in the top 10 cm of soil, by 26 % and doubling litter increased soil C by 31 % (Leff et al. 2012). In three temperate forest studies rates of change in soil C were low; but they were measured over much longer periods. In north central USA soil C content decreased by 61 % in litter removal plots and increased by 33 % in double litter plots over a 50-year period (Lajtha et al. 2014a). In Pennsylvania, USA, 20 years of removing litter reduced soil C by 24%, although the corresponding litter doubling had no effect (Bowden et al. 2014). In deciduous forest in Massachusetts, USA, 20 years of LR also reduced mineral soil C (by 19%), but LA also resulted in lower mineral soil C (by 6%, Lajtha et al. 2014b). Differences between 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 may on average be composed more of C from roots 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 very small, c. 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). Other tropical forest soils also had high turnover rates of C; 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 higher 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 LA 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; though fertilization with N and K together increased growth of saplings and seedlings in the Gigante Fertilization Project (GFP), which was 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 GFP 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 LR and LA plots and extractable NH_4 was significantly lower in LR plots. This contrasts with our results of greater NO_3 in LA compared to LR and no effect on NH_4; the differences between the experiments may be partly due to somewhat different soils and a wetter climate in Costa Rica (c. 5 m rain per year c.f. 2.6 in Panama). Thus, soil N dynamics differ somewhat between the only two tropical litter manipulation experiments, but in both NH_4 was the dominant form of inorganic N, and in both total inorganic N decreased in LR plots and increased in LA plots (though differences were not always statistically significant).

The 'available' forms of P are also not often reported for the deeper horizons of tropical forest soils, despite the fact that P is usually regarded as the most likely limiting nutrient in such forests (Tanner et al. 1998 and Cleveland et al. 2011) and has been shown to limit fine litter production in the adjacent Gigante Fertilizer experiment (Wright et al. 2011). Mehlich P and total P both decreased with depth in control soils in our litter manipulation experiment (to 25 and 29 % of near surface values); in LR soils the decrease was less steep (37 % and 36 %). LA increased Mehlich P in the surface soils (though total P was not higher), indicating increased P availability, which is consistent with the finding that LA decreased the strength of phosphate sorption in these soils (Schreeg at al. 2013). Thus for P, potentially the most commonly limiting nutrient in tropical rain forest soils, six-years of continuous removal and addition of litter in our experiment has reduced and increased 'available' P down to 20 cm in the soil.

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

4.3 Design of litter manipulation experiments

The design of litter manipulation experiments needs to be carefully considered when evaluating their results; the strength of the effect of litter manipulation on soil C in Panama was much less than that in Costa Rica. The Panamanian and Costa Rican experiments are very different in spatial scale. Plots in Panama are large, 45 x 45 m, those in Costa Rica are small, 3 x 3 m. The small plots are 'hot' and 'cold' spots relative to large individual tree crown areas (and likely tree root areas); crowns of the largest trees in lowland rain forests are commonly 25 m in diameter, so a 3 x 3 m plot is 2 % of that area. These differences in experimental design and their effects on the pattern of the results should be considered when trying to understand ecosystem level processes; small hot and cold spots may not represent what would happen in plots on the scale of the large trees.

5 Conclusions

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

Supplementary material

Table S1 with full original data from soil analyses

Table S2 Model estimates of concentrations (from Sheldrake)

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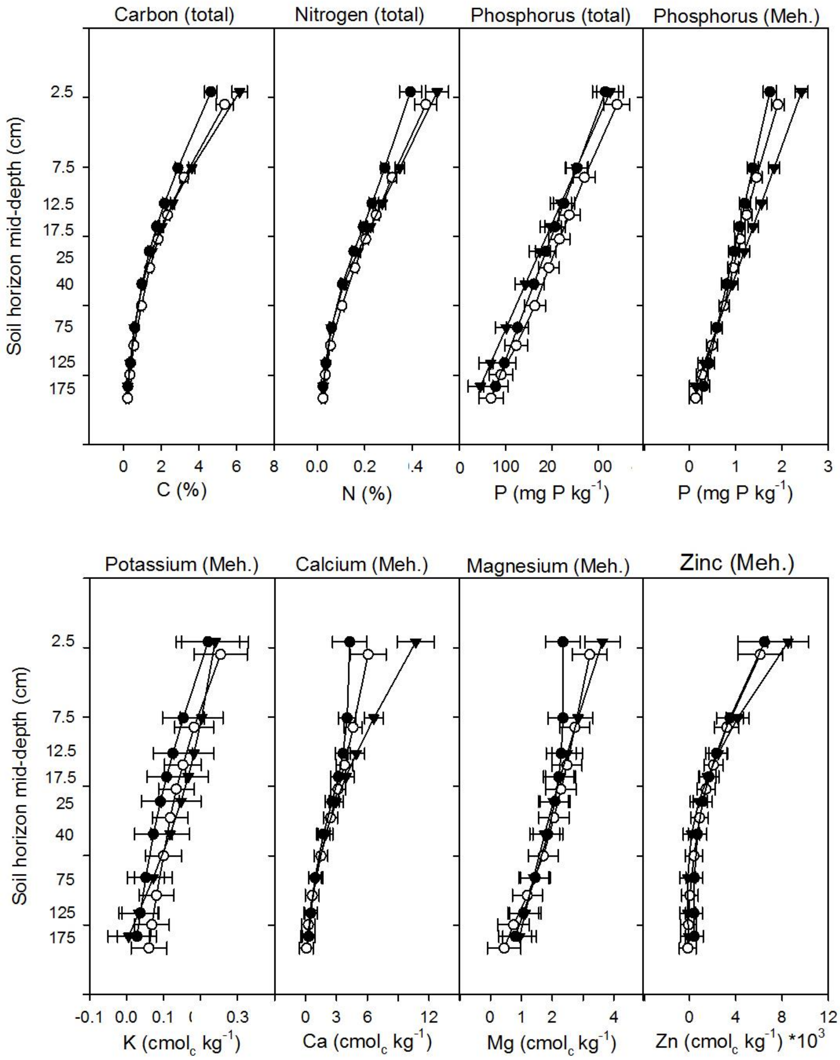

Fig. 1 Concentrations of soil C, N, P (various fractions) and cations (Mehlich extractions), plotted against the mid-point of the soil layers sampled (Zn values should be divided by 1000 to obtain actual means), control points are displaced below treatments. Data are fitted values of the mixed effects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

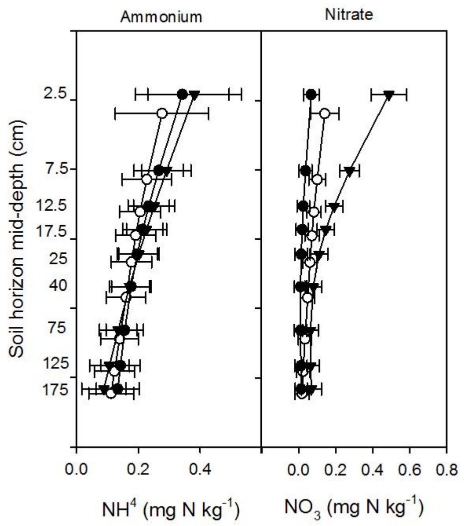

Fig. 2 Mean concentrations of ammonium and nitrate plotted against the mid-point of the soil layers sampled, control points are displaced below treatments. Data are fitted values of the mixed effects models with 95% confidence intervals (see Methods), in litter removal ●, control O and litter addition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

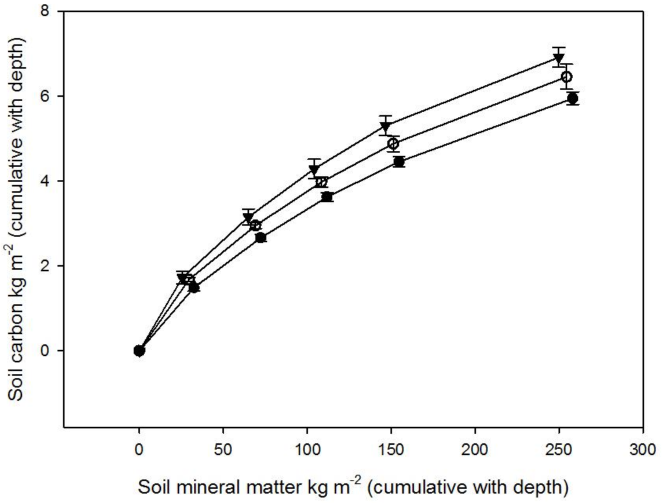

Fig. 3 Soil carbon content and mineral content in litter addition, control, and litter addition expressed as kg C m^{-2} cumulatively from 0 to 30 cm soil depth. Values are means for 5 plots per treatment +/- SE, litter removal ●, control **O,** and litter addition ▼ .

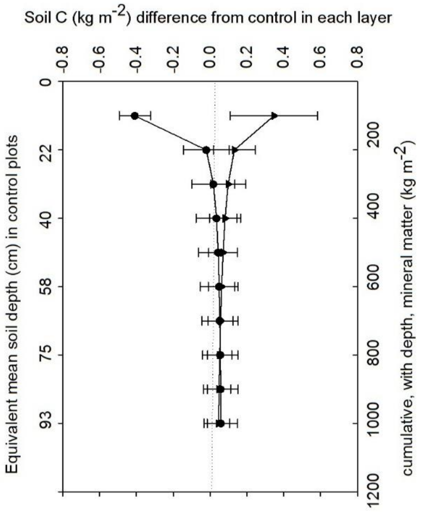

Fig. 4 Differences in soil carbon content relative to control soils (mean and SE, n = 5), after 6 years of litter manipulation, plotted for successive soil layers: 0-100 kg (mineral matter) m^{-2}, plotted at 100 kg m^{-2} on right y axis; 100-200 kg m^{-2}, plotted at 200 kg m^{-2}; and so on to 900-1000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