Tanner responses to comments by W Wieder.

1) **Comment.**

“Was soil mineral mass measured in each plot, in each treatment, or in a single pit (like bulk density)”

Response

Soil mineral matter was calculated for each plot and soil depth from soil carbon concentration (mineral matter is total soil mass minus twice soil carbon content). Bulk density was measured for every plot for 0-5 cm depth; below 5 cm we used the bulk density from one soil pit (lines 146-151 in the manuscript).

2) **Comment.**

“More broadly, the emphasis placed on soil mineral mass to extrapolate findings seems somewhat surprising,”

Response

The emphasis on expressing soil carbon per mineral mass is to deal with the (general) problem that as soil organic matter changes the bulk density changes, so sampling to the same depth will not be comparing like with like. This is well known problem - Powlson et al 2011 say “The principle is that an equal mass of organic-matter-free mineral soil should be sampled between the treatments or times being compared.” For this reason in our study in Panama we expressed carbon relative to an unchanging mineral mass. It is also an easy calculation to make and can often be made retrospectively on published data. It was not done to get round a problem of non-significant results.

3) **Comment.**

“If data are available to make an extrapolation of Fig 3 with depth on the X axis it would be much more valuable for studies trying to quantify or model changes in soil C stocks, as information about mineral mass is typically lacking or not considered.”

Response

Fig 4 shows the cumulative (with depth) mineral matter and soil depth in the control plots, down to about 93 cm. An e mail exchange with the referee clarified that he wanted a second axis In Fig 3 showing the soil depth in the control plots – we have done this. We disagree with the comment that “information about mineral matter is typically lacking”, because if samples have data on soil carbon per dry soil mass, then the mineral matter is easily calculated (as total mass minus twice soil carbon - there will be a small error because soil organic matter is not exactly twice soil carbon, but the effect will be trivial.)

4) **Comment.**

“I recall publications from some of the temperate DIRT plots (e.g., Lajtha references in the paper) showed changes in different soil C fractions. I assume similar data are not available for this study, but I wonder if consideration of C stabilization mechanisms and soil mineralogical conditions could help explain some of the differences between temperate and tropical sites. Is it worth a brief discussion on this point (e.g. expanding / developing the paragraph that begins on line 202)?"

Response

Other researchers are working on this in the experiment. As we present no data on carbon fractions in this paper we think it best to leave discussion of that subsequent manuscripts.

5) **Comment.**

"The authors (justifiably) seem keen on their soil P results, which are interesting and relevant (line 262). Is it possible to extrapolate findings for P, similar to the soil C figure 3, making this a multi-panel figure?"

Response

It is not sensible to express cumulative Mehlich P per cumulative mineral mater (in an analogous way to cumulative carbon per cumulative mineral matter in Fig. 3) because a substantial (but unknown) amount of Mehlich P comes from organic matter. Soil matter is either organic or mineral and we plot one against the other in Fig. 3; Mehlich P is different - it comes from both mineral and organic matter.

6) **Comment.**

"The discussion starts off with the introduction of new results. I appreciate the authors wanting to focus readers' attention on these findings, but feel like results (Figs 3 & 4) are best introduced in the results, not discussion section of a paper"

Response

We disagree. The 'results' are concentrations of carbon per mass. We then use those results to calculate concentrations of carbon per mineral matter.

7) **Comment.**

"Finally, calling out the small plots from the Costa Rican study seems a bit unjustified in a single paragraph subsection of the discussion. Granted the authors make a good point about the appropriate size of experimental plots, but I think Leff and co-authors (2012, cited in the paper) acknowledge the limitation of their small plots. If the authors want this section to remain they should more broadly discuss other litter manipulation studies, not just the Costa Rican site."

Response

We are not making any personal points here, but we do think that there is a real issue about the size of experimental plots affecting the qualitative patterns of results. Specifically, small (3 x 3 m) litter removal and addition plots might be local cold spots and hot spots that will affect the responses. The pattern of results from small plots might be the OPPOSITE of those from large plots. For example, small litter addition plots might cause extra root growth into local patches of soil with extra nutrients, but large litter addition plots (45 x 45 m) might cause reduced root growth because the whole tree is receiving extra nutrients and 'can afford' to reduce root growth and put more into shoot growth, in other words, a completely opposite pattern of results caused by differences in experimental design. We simply want to point out that the design of these experiments might well affect the pattern of results. If there were lots of experiments like this we could look for patterns, but there aren't many.

To address the reviewer's comment, we have changed the last line to "small hot and cold spots may not represent what would happen in plots on the scale of the large trees - as pointed out by Leff et al 2012."

8) Technical corrections:
   Comment. Introduction: specific values for C pools, turnover times, and fractions seem unnecessarily detailed (lines 33, 36). More broadly the introductions reads a bit like a bullet point of disconnected ideas. This is a stylistic concern, not a scientific one.

   Response. As Wieder says this is stylist – we think this is clear and informative

   Comment. Throughout, check that abbreviations are defined before they are used in the text (eg. LR and LA line 55, GFP line 251).

   Response. We have changed all 'LR' to 'litter removal' and all 'LA' to 'litter addition'. We have reworded the text so that GFP is no longer used.

   Comment. Line 66-68, This is unclear P mineralization (0-2 cm) in LR plots met 20% of NPP needs, or the decline in P mineralization would have met this demand?

   Changed to "mineralization of organic phosphorus (P) (inferred from the decrease in the concentration of organic P) in the top 2 cm of soil during three years of litter removal was calculated to be sufficient to supply 20% of the P needed to sustain forest growth"

   Comment. Line 76. This study looked at net nitrification and should be Wieder et al. 2013 (i before e).

   Response. Added 'net' and corrected spelling of Wieder.

   Comment. Line 89. Awkward. Forest productivity isn't mitigated, but increases in terrestrial C storage can mitigate atmospheric CO2 accumulation.

   Response. Changed to "can thus be considered as partial mitigation of atmospheric CO_2 accumulation through increased forest productivity"

   Comment. Line 210. Awkward, maybe insert 'a' here: In a deciduous forest in MA. . .

   Response. Changed to "In a deciduous forest in"

   Comment. Line 307. What is meant by 'polluted' sites? Is this sites receiving large amounts of N, P or micronutrient deposition (is the later actually a real a thing)? Is this just to say that litter manipulations aren't identical to CO2 enrichment alone, because they also serve as nutrient manipulations that modify ecosystem dynamics?

Response. This site is not receiving large amounts of N or P (though N input is increasing Hietz et al 2011 Science 334, 664). Our comparisons are based on N & P inputs in polluted sites in USA and Europe. We have added ‘temperate’.
We don’t mention micronutrients in the Conclusions – so we ignore that part of the comment.

Biogeosciences Discuss. of Tanner at al. “**Changes in soil carbon and nutrients”**
Author responses to reviewer 2

Comment “I would appreciate seeing a comparison of results using more traditional ways of measuring soil C (e.g., fraction of dry mass) and the approach utilized here. Given its novelty, mineral mass is of limited utility when comparing to other studies.”
Response
Tanner did a calculation (using the data in the supplementary material) of the changes in concentration over the top 20 cm of soil. Litter removal soil shows a 1.9% fall in concentration and litter addition a 2.0% increase in C concentration. This compares with 1% per year using the ‘new’ calculation based on the same amount of mineral matter. We put a sentence about this into the discussion in the revised ms. “These changes are about c. 1% per year; in contrast if we calculate the change based on a fixed depth of 20 cm, ignoring changes in bulk density, we get a change of about 2% per year. Thus ignoring the changes in bulk density results a misleading doubling of the estimated rate of change.”

Comment
Technical comments: Please clarify abbreviations: The LA and LR
Response. LA and LR now written out in full everywhere. L- and L+ now changed to litter removal and litter addition.

Comment
The sentence that begins on line 75 is awkward - perhaps a better way of saying this is that "After 2.5 years of litter manipulation in Costa Rica, surface soils (0-10 cm) had lower nitrification in both litter removal and addition treatments..."
Response
We ask to keep our original wording. We deliberately put “In Cost Rica” first in the sentence to mark the fact that we are moving on in the discussion from Panama to Costa Rica. If we start with “After 2.5 years of litter manipulation” it could be taken to mean that we are still discussing Panama.

Comment
“On line 89, the carbon that stays in soil and litter crop does not mitigate increased forest productivity”
Response.
I could not find this. Anyway, in our revised ms we use ‘mitigate’ only once
“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 CO2 and/or temperature could result in a substantial increase in soil C and therefore partially mitigate the increase in atmospheric CO2.”

Comment

I appreciated the improvements to the figures in response to previous comments. The figures could be strengthened by including notations to depict which litter effects were significantly different from controls. While this information is largely contained in the text, including this in the figures would help if the images were ever reproduced for other uses.

Response.

In Figs 1 & 2 we plot means and confidence errors if errors don't overlap means are significantly different; we say which are significant in the text. We make comparisons between litter removal and litter addition, as well as between each treatment and control, showing both types of comparison on the figure would clutter up the diagrams, so I drew a new figure for the supplementary material showing just those elements that were significantly different, and just down to 30 cm; I also added a second supplementary Table with the means and 95% confidence intervals for the data in Figs 1 & 2.

End of comments and responses.

Tanner responses to comments by W Wieder.

1) **Comment.**

“Was soil mineral mass measured in each plot, in each treatment, or in a single pit (like bulk density)”

Response

Soil mineral matter was calculated for each plot and soil depth from soil carbon concentration (mineral matter is total soil mass minus twice soil carbon content). Bulk density was measured for every plot for 0-5 cm depth; below 5 cm we used the bulk density from one soil pit (lines 146-151 in the manuscript).

2) **Comment.**

“More broadly, the emphasis placed on soil mineral mass to extrapolate findings seems somewhat surprising,”

Response

The emphasis on expressing soil carbon per mineral mass is to deal with the (general) problem that as soil organic matter changes the bulk density changes, so sampling to the same depth will not be comparing like with like. This is well known problem - Powlson et al 2011 say “The principle is that an equal mass of organic-matter-free mineral soil should be sampled between the treatments or times being compared.” For this reason in our study in Panama we expressed carbon relative to an unchanging mineral mass. It is also an easy calculation to make and can often be made retrospectively on published data. It was not done to get round a problem of non-significant results.

3) **Comment.**

“If data are available to make an extrapolation of Fig 3 with depth on the X axis it would be much more valuable for studies trying to quantify or model changes in soil C stocks, as information about mineral mass is typically lacking or not considered.”

Response

Fig 4 shows the cumulative (with depth) mineral matter and soil depth in the control plots, down to about 93 cm. An e mail exchange with the referee clarified that he wanted a second axis In Fig 3 showing the soil depth in the control plots – we have done this. We disagree with the comment that “information about mineral matter is typically lacking”, because if samples have data on soil carbon per dry soil mass, then the mineral matter is easily calculated (as total mass minus twice soil carbon - there will be a small error because soil organic matter is not exactly twice soil carbon, but the effect will be trivial.)

4) **Comment.**

“I recall publications from some of the temperate DIRT plots (e.g., Lajtha references in the paper) showed changes in different soil C fractions. I assume similar data are not available for this study, but I wonder if consideration of C stabilization mechanisms and soil mineralogical conditions could help explain some of the differences between temperate and tropical sites. Is it worth a brief discussion on this point (e.g. expanding / developing the paragraph that begins on line 202)?"

Response

Other researchers are working on this in the experiment. As we present no data on carbon fractions in this paper we think it best to leave discussion of that subsequent manuscripts.

5) **Comment.**

"The authors (justifiably) seem keen on their soil P results, which are interesting and relevant (line 262). Is it possible to extrapolate findings for P, similar to the soil C figure 3, making this a multi-panel figure?"

Response

It is not sensible to express cumulative Mehlich P per cumulative mineral mater (in an analogous way to cumulative carbon per cumulative mineral matter in Fig. 3) because a substantial (but unknown) amount of Mehlich P comes from organic matter. Soil matter is either organic or mineral and we plot one against the other in Fig. 3; Mehlich P is different - it comes from both mineral and organic matter.

6) **Comment.**

"The discussion starts off with the introduction of new results. I appreciate the authors wanting to focus readers' attention on these findings, but feel like results (Figs 3 & 4) are best introduced in the results, not discussion section of a paper"

Response

We disagree. The 'results' are concentrations of carbon per mass. We then use those results to calculate concentrations of carbon per mineral matter.

7) **Comment.**

"Finally, calling out the small plots from the Costa Rican study seems a bit unjustified in a single paragraph subsection of the discussion. Granted the authors make a good point about the appropriate size of experimental plots, but I think Leff and co-authors (2012, cited in the paper) acknowledge the limitation of their small plots. If the authors want this section to remain they should more broadly discuss other litter manipulation studies, not just the Costa Rican site."

Response

We are not making any personal points here, but we do think that there is a real issue about the size of experimental plots affecting the qualitative patterns of results. Specifically, small (3 x 3 m) litter removal and addition plots might be local cold spots and hot spots that will affect the responses. The pattern of results from small plots might be the OPPOSITE of those from large plots. For example, small litter addition plots might cause extra root growth into local patches of soil with extra nutrients, but large litter addition plots (45 x 45 m) might cause reduced root growth because the whole tree is receiving extra nutrients and 'can afford' to reduce root growth and put more into shoot growth, in other words, a completely opposite pattern of results caused by differences in experimental design. We simply want to point out that the design of these experiments might well affect the pattern of results. If there were lots of experiments like this we could look for patterns, but there aren't many.

To address the reviewer's comment, we have changed the last line to "small hot and cold spots may not represent what would happen in plots on the scale of the large trees - as pointed out by Leff et al 2012."

8) Technical corrections:

Comment. Introduction: specific values for C pools, turnover times, and fractions seem unnecessarily detailed (lines 33, 36). More broadly the introductions reads a bit like a bullet point of disconnected ideas. This is a stylistic concern, not a scientific one.

Response. As Wieder says this is stylist – we think this is clear and informative

Comment. Throughout, check that abbreviations are defined before they are used in the text (eg. LR and LA line 55, GFP line 251).

Response. We have changed all 'LR' to 'litter removal' and all 'LA' to 'litter addition'. We have reworded the text so that GFP is no longer used.

Comment. Line 66-68, This is unclear P mineralization (0-2 cm) in LR plots met 20% of NPP needs, or the decline in P mineralization would have met this demand?

Changed to "mineralization of organic phosphorus (P) (inferred from the decrease in the concentration of organic P) in the top 2 cm of soil during three years of litter removal was calculated to be sufficient to supply 20% of the P needed to sustain forest growth"

Comment. Line 76. This study looked at net nitrification and should be Wieder et al. 2013 (i before e).

Response. Added 'net' and corrected spelling of Wieder.

Comment. Line 89. Awkward. Forest productivity isn't mitigated, but increases in terrestrial C storage can mitigate atmospheric CO2 accumulation.

Response. Changed to "can thus be considered as partial mitigation of atmospheric CO_2 accumulation through increased forest productivity"

Comment. Line 210. Awkward, maybe insert 'a' here: In a deciduous forest in MA. . .

Response. Changed to "In a deciduous forest in"

Comment. Line 307. What is meant by 'polluted' sites? Is this sites receiving large amounts of N, P or micronutrient deposition (is the later actually a real a thing)? Is this just to say that litter manipulations aren't identical to CO2 enrichment alone, because they also serve as nutrient manipulations that modify ecosystem dynamics?

Response. This site is not receiving large amounts of N or P (though N input is increasing Hietz et al 2011 Science 334, 664). Our comparisons are based on N & P inputs in polluted sites in USA and Europe. We have added 'temperate'.
We don't mention micronutrients in the Conclusions – so we ignore that part of the comment.

Biogeosciences Discuss. of Tanner at al. "**Changes in soil carbon and nutrients"**
Author responses to reviewer 2

Comment "I would appreciate seeing a comparison of results using more traditional ways of measuring soil C (e.g., fraction of dry mass) and the approach utilized here. Given its novelty, mineral mass is of limited utility when comparing to other studies."
Response
Tanner did a calculation (using the data in the supplementary material) of the changes in concentration over the top 20 cm of soil. Litter removal soil shows a 1.9% fall in concentration and litter addition a 2.0% increase in C concentration. This compares with 1% per year using the 'new' calculation based on the same amount of mineral matter. We put a sentence about this into the discussion in the revised ms. "These changes are about c. 1% per year; in contrast if we calculate the change based on a fixed depth of 20 cm, ignoring changes in bulk density, we get a change of about 2% per year. Thus ignoring the changes in bulk density results a misleading doubling of the estimated rate of change."

Comment
Technical comments: Please clarify abbreviations: The LA and LR
Response. LA and LR now written out in full everywhere. L- and L+ now changed to litter removal and litter addition.

Comment
The sentence that begins on line 75 is awkward - perhaps a better way of saying this is that "After 2.5 years of litter manipulation in Costa Rica, surface soils (0-10 cm) had lower nitrification in both litter removal and addition treatments..."
Response
We ask to keep our original wording. We deliberately put "In Cost Rica" first in the sentence to mark the fact that we are moving on in the discussion from Panama to Costa Rica. If we start with "After 2.5 years of litter manipulation" it could be taken to mean that we are still discussing Panama.

Comment
"On line 89, the carbon that stays in soil and litter crop does not mitigate increased forest productivity"
Response.
I could not find this. Anyway, in our revised ms we use 'mitigate' only once
"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 CO2 and/or temperature could result in a substantial increase in soil C and therefore partially mitigate the increase in atmospheric CO2."

Comment

I appreciated the improvements to the figures in response to previous comments. The figures could be strengthened by including notations to depict which litter effects were significantly different from controls. While this information is largely contained in the text, including this in the figures would help if the images were ever reproduced for other uses.

Response.

In Figs 1 & 2 we plot means and confidence errors if errors don't overlap means are significantly different; we say which are significant in the text. We make comparisons between litter removal and litter addition, as well as between each treatment and control, showing both types of comparison on the figure would clutter up the diagrams, so I drew a new figure for the supplementary material showing just those elements that were significantly different, and just down to 30 cm; I also added a second supplementary Table with the means and 95% confidence intervals for the data in Figs 1 & 2.

End of comments and responses.

Comparison of original ms submission and revised version

Title:

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

Authors

Edmund Vincent John Tanner[1,2], Merlin W. A. Sheldrake[1], and Benjamin L. Turner[2]

[1]Department of Plant Sciences, University of Cambridge, Downing St, Cambridge CB2 3EA, UK.

[2]Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama.

***Correspondence to*: E. V. J. Tanner (evt1@cam.ac.uk)**

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 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

Formatte

Deleted:

Moved d

Formatte

Deleted:

Deleted:

Deleted:

Deleted:

Deleted:
and lower

Deleted:

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, 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, comprising 38% of the total soil C, in the top 10 cm of soil in semi-evergreen rain forest on Barro Colorado Island, Panama (61% of total soil C was 'passive' with a turnover time of the order of a thousand years). 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, at a site close to the current litter manipulation experiment. 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 have been steadily increasing for decades and, one of the effects of this could be widespread increases in forest growth (Nemani et al. 2003) and, as a result, increased litterfall. There are few experimental studies of the effects of elevated CO_2 on forest growth. Körner (2006) reported that elevated CO_2 caused increased litterfall in one of three studies in steady-state tree stands in temperate forests, but 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 litter removal plots and increased soil C in litter addition plots (Leff et al. 2012). It is therefore likely that soil C will increase in many, but not all, forests 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. 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 phosphorus (P) (inferred from the decrease in the concentration of organic P) in the top 2 cm of soil during 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, and 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 P, potassium (K), calcium (Ca), magnesium (Mg), and micronutrients all changed over 4 years in the upper 2 cm of soil as a

Deleted: deciduous

Deleted:

Deleted:

Deleted:

Deleted: time of th

Deleted:

Deleted:

Deleted:

Deleted:

Formatte

Deleted: reassessed derived fr sources, e or via mic

Deleted:

Deleted: relative de

Deleted:

Deleted:

Deleted:

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 net 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; Wieder et al. 2013). Thus, several soil nutrients in surface soils change following 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 to the fate of 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 of atmospheric CO$_2$ accumulation through 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 annual additions of farmyard manure was still in the soil after 120 years (Powlson et al. 2011).

2 Materials and methods

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

The experiment consists of fifteen 45-m x 45-m plots within a 40-ha area of old growth forest. In 2001 all 15 plots were trenched to a depth of 0.5 m to minimize lateral nutrient and water movement via the root/mycorrhizal network; the trenches were double-lined with plastic and backfilled. Beginning in January 2003, litter (including branches <20 mm in diameter) was raked up once a month in five plots, resulting in low, but not entirely absent, litter standing crop (litter removal plots). The removed litter was immediately spread on five further plots (litter addition plots), with five plots left as controls (CT plots). Treatments were assigned on a stratified random basis using 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 litter removal plot was always added to a particular litter addition 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 constant volume corer for the top 20 cm of soil and 7 cm diameter auger from 20 – 200 cm. Fresh soils were extracted for NO$_3$ and NH$_4$ within 2 hours of sampling in a 2 M KCl solution, with detection

Deleted:
and K in li
addition p
(Sayer et a
manipulat
nitrificatio
while NH$_4$
removal p
Weider...i
surface so

Deleted:
much grea
years of c
new way o
mineral m
in soil C a
bulk densi
nutrient c
calculate C
the increa
aimed to c
remains in
be conside

Moved (

Deleted:
long-term
importanc
of tropical
old-growt
located...c
of the Bar
Central Ar
located on
Oxisols (Ty
with ...ow
high base
Annual rai
from the s
2600 mm
is a strong
rainfall of
approxima
during the

Deleted:
m) ...f old
trenched t
nutrient-...
network; t
backfilled.
(including
raked up o
entirely ab
The remov
plots (L+...

Deleted:
mineral co
nutrients v
NO$_3$ and N
within 2 h
determine

by automated colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO). Phosphorus and cations were extracted within 24 h in Mehlich III solution and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). Soil pH was measured on a 1:2 fresh soil solution in distilled water.

Dried (22C x 10 d) 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 heteroscedasticity, 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.

Amounts of soil total C and N 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 %, litter addition 15 % and litter removal 4.9 %. Bulk density data were used to estimate approximate soil depth for control plots in Figs. 3 and 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 litter addition plots, compared to litter removal plots, had significantly lower bulk density (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), Ca (to 10 cm), and Mg (to 5 cm) and (Fig. 1 and 2 and Tables S1 and S2). There were fewer differences when compared to control soils: litter addition soils had higher concentrations of P_{Meh}

(to 20 cm), NO_3 (to 15 cm), Ca (to 10 cm), and pH (to 10 cm). Nutrient concentrations in litter removal soils 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 litter addition than litter removal, 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 litter addition compared to litter removal to about 10 cm deep in the soil (Fig. 3 and 4). Total N per mineral mass of soil was also significantly greater in litter addition than litter removal 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 litter removal plots, C:N was 10.5 at the surface and 10.3 at depth, while litter addition 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 litter removal and 'extra' in the litter addition over about the top 20 cm of soil (from calculations based on C per mineral matter), six years after (January 2009) litter removal and addition started, was about 0.5 kg C m^{-2} (Fig. 3). These changes are about c. 1% per year; in contrast if we calculate the change based on a fixed depth of 20 cm, ignoring changes in bulk density, we get a change of about 2% per year. Thus ignoring the changes in bulk density results a misleading doubling of the estimated rate of change. The similarity of the losses from litter removal and gains in litter addition probably has different causes: we speculate that losses from the soil in the litter removal 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 litter addition plots are a result of infiltration of dissolved and particulate 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 litter addition plots (Sayer et al. 2006).

In addition to the extra *soil* C in the litter addition plots, the litter standing crop was also larger in litter addition plots. In September 2005 (2.8 years after litter manipulation started) there was an additional 0.4 kg C m^{-2} in the Oi and Oe layers compared to control plots (Sayer and Tanner 2010) and data from 2013 show that litter standing crop was at about this level (C. Rodtassana, University of Cambridge, unpublished data). Together this extra 0.9 kg C m^{-2} in the litter addition soil and litter standing crop is about 30 % of the 3 kg C m^{-2} in litter added to the litter addition 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. For example, a free air CO_2 experiment in 13-year old loblolly pine plantation in North Carolina USA reported a 12% increase in litterfall over 9 years (Lichter et al. 2005, 2008).

The increases in soil C in our litter addition 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 litter removal 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 small, but they were measured over much longer periods. In north central USA, soil C content decreased by 44 % in litter removal plots and increased by 31 % in double litter plots over a 50-year period (Table 2 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 a deciduous forest in Massachusetts, USA, 20 years of litter removal also reduced mineral soil C (by 19%), but litter addition 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 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

Deleted:
2006); ...h
CO_2 will be

Formatte

Deleted:
Carolina U
years (Lich

Deleted:
surface so
addition p
for. Soil re
were grea
Sayer et a
priming –
by the ad
soil organi
experimen
in the area
C has relea

Formatte

Deleted:
(Sayer et a
of total C
primed CO
addition a
standing c
of the prin
been subs
correspon
Increases
been...ma
forests. In
(lowland r
years of re
C concent
plots..., an
plots ...Lef
years, in...
studies, ra
measured

Deleted:
C...riginate
(Rasse et a
Panama b
caused ch

Formatte

Deleted:
turnover..
tropical fo
C in the to
(Trumbore

Deleted:

Deleted:
were stron
difference
depth to w
time. Four

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 removal and no effect on NH_4; the differences between the experiments might be due in part to 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 litter removal plots and increased in litter addition 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 nutrient addition experiment (Wright et al. 2011). Mehlich P and total P both decreased with depth in control soils in our litter manipulation experiment (at 50-100cm concentrations were 25 and 29 % of those at 0-5 cm); in litter removal soils the decrease was less steep (37 % and 36 %). Litter addition increased Mehlich P in the surface soils (though total P was not significantly greater), indicating increased P availability, which is consistent with the finding that litter addition 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 total exchangeable bases. 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

Deleted:
Deleted:
Deleted:
Formatte
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
Deleted:
'available'
Hawaii, bi
that in sur
% of that c
and Chadv
phosphate
approxima
shifting cu
logged for
Deleted:
third of th
substantia
Deleted:
Deleted:
Deleted:
Deleted:

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 litter addition and litter removal 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, but the Panama and Costa Rica 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, as pointed out by Leff et al. (2012).

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 temperate 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

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

Supplementary Table S1 with full original data from soil analyses

Supplementary Table S2 Model estimates of concentrations (from Sheldrake)

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

Acknowledgements. We thank J. Bee, L. Hayes, S. Queenborough, R. Upson and M. Vorontsova for surveying the plots, J Bee for setting up the experiment in 2000 and 2001; E. Sayer for running the experiment from 2001-2009; A Vincent for helping to maintain the experiment from 2003-2005. T. Jucker did the statistics to compare the effect of treatment on soil C relative to mineral matter. Funding for the project was originally from the Mellon Foundation (1999-2002); on-going costs were paid for by the Gates-Cambridge Trust (E Sayer); The University of Cambridge Domestic Research Studentship Scheme and the Wolfson College Alice Evans Fund (A. Vincent); The Drummond Fund of Gonville and Caius College and Cambridge University (E. Tanner). The whole of the experiment depended on the continuous raking of litter, which was done by Jesus Valdez and Francisco Valdez. We thank D. Agudo and T. Romero for doing the laboratory work and J. Rodriguez for collecting the samples in the forest. S.J. Wright has been a frequent source of help for many aspects of the experiment.

References

Bowden, R. D., Deem, L., Plante, A. F., Peltre, C., Nadelhoffer, K. and Lajtha, K.: Litter input controls soil carbon in temperate deciduous forest, Soil Sci. Soc. Am. J., s66-s75, 2014.

Burnham, K. P. and Anderson, D. R.: Information and likelihood theory: a basis for model selection and inference, in: Model Selection and Multimodel Inference, edited by: Burnham K. P., and Anderson, D. R., Springer New York, 49–97, 2002.

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J. Finlay, R. D., Wardle, D. A., and Lindahl, B. D.: Roots and associated fungi drive long-term carbon sequestration in boreal forest, Science 339, 1615-1618, 2013.

Cleveland, C. C., Townsend, A. R., Taylor, P., Alvrez-Clare, S., Bustamente, M. M. C., Chuyong, G., Dobrowski, S. Z., Grierson, P., Harms, K. E., Houlton, B. Z., Marklein, A., Parton, W., Porder, S., Reed, S. C., Sierre, C. A., Silver, W. L., Tanner, E. V. J., and Wieder, W. R.: Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis, Ecol. Lett., 14, 939-947, 2011.

Jobbagy, E. G. and Jackson, R. B.: The vertical distribution of soil organic carbon and its relation to climate and vegetation, Ecol. Appl., 10, 423-436, 2000.

Koehler, B., Corre, M. D., Steger, K., Well, R., Zehe, E., Sueta, J. P., and Veldkamp, E.: An in-depth look into a tropical lowland forest soil: nitrogen-addition effects on the contents of N_20, $C0_2$ and CH_4 and N_20 isotopic signatures down to 2-m depth, Biogeochemistry, 111, 695-713, 2012.

Körner, C. Plant CO_2 responses: an issue of definition, time and resource supply, New Phytol., 172, 393-411, 2006.

Lajtha, K., Townsend, K. L., Kramer, M. G., Swanston, C., Bowden, R. B. and Nadelhoffer, K.: Changes to particulate versus mineral-associated soil carbon after 50 years of litter manipulation in forest and prairie experimental ecosystems, Biogeochemistry 119, 341-360, 2014a.

Lajtha K., Bowden R. D., Nadelhoffer K.: Litter and root manipulations provide insights into soil organic matter dynamics and stability, Soil Sci. Soc. Am. J., 78, s261–s269, 2014b.

Leff, J. W., Wieder, W. R., Taylor, P. G., Townsend, A. R., Nemergut, D. R., Grandy, A. S. and Cleveland, C. C.: Experimental litterfall manipulation drives large and rapid changes in soil carbon cycling in a wet tropical forest, Glob. Change Biol., 18, 2969-2979, 2012.

Lichter, J., Barron, S. H., Bevacqua, C. E., Finzi, A. E., Irving, K. F., Stemmler E. A., and Schlesinger, W. H.: Soil carbon sequestration and turnover in a pine forest after six years of atmospheric CO_2 enrichment, Ecology, 86, 1835-1847, 2005.

Lichter, J., Billings, S. A., Ziegler, S. E., Gaindh, D., Ryals, R., Finzi, A. C., Jackson, R. B., Stemmler, E. A. and Schlesinger, W. H.: Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment, Glob. Change Biol., 14, 2910-2922, 2008.

Deleted:

Deleted:
study of n
under vari
stores in b
Ecosyst. E

Deleted:

Deleted:
Nutrient r
tropical ra
¶

Deleted:

Moved d

Deleted:
J.: Long-te
Science, 3

Deleted:

Deleted:

Deleted:

Deleted:
earth syst

Moved d

Deleted:

Deleted:

Deleted:

Nemani, R. R., Keeling, C. D., Hashimoto, H., Jolly, W. M., Piper, S. C., Tucker, C. J., Myneni, R. B. and Running, S. W.: Climate-driven increases in global terrestrial net primary production from 1982 to 1999, Science, 300, 1560-1563, 2003.

Nottingham, A. T., Turner, B. L., Stott, A. W. and Tanner, E. V. J.: Nitrogen and phosphorus constrain labile and stable carbon turnover in lowland tropical forest soils, Soil Biol. Biochem., 80, 26-33, 2015.

Pinheiro, J. and Bates, D., Mixed-effects Models in S and S-PLUS, Springer, New York. 548 pp., 2000.

Porder, S and Chadwick, O. A.: Climate and soil-age constraints on nutrient uplift and retention by plants, Ecology, 90, 623-636, 2009.

Powlson, D. S., Whitmore, A. P. and Goulding, K. W. T.: Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false, Eur. J. Soil Sci., 62, 42-55, 2011.

Quesada, C. A., Lloyd J., Anderson L. O., Fyllas N. M., Schwarz M., and Czimczik C. I.: Soils of Amazonia with particular reference to the RAINFOR sites, Biogeosciences, 8, 1415–1440, 2011.

Rasse, D. P., Rumpel, C. and Dignac, M-F.: Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation, Plant Soil, 269, 341-356, 2005.

Sayer, E. J., Heard, M. S., Grant, H. K. Marthews, T. R. and Tanner E. V. J.: Soil carbon release enhanced by increased tropical forest litterfall, Nature Clim. Change, 1, 304-307, 2010.

Sayer, E. J., Powers, J. S. and Tanner, E. V. J.: Increased litterfall in tropical forests boosts the transfer of soil CO_2 to the Atmosphere, PLoS ONE 2(12): e1299, 2007

Sayer, E. J. and Tanner, E. V. J.: Experimental investigation of the importance of litterfall in lowland semi-evergreen tropical forest nutrient cycling, J. Ecol., 98, 1052-1062, 2010.

Sayer, E. J., Tanner, E. V. J. and Cheesman, A. W.: Increased litterfall changes fine root distribution in a moist tropical forest, Plant Soil, 281, 5-13, 2006.

Sayer, E. J., Wright, S. J., Tanner, E. V. J., Yavitt, J. B., Harms, K. E., Powers, J. S., Kaspari, M., Garcia, M. N., and Turner, B. L.: Variable responses of lowland tropical forest nutrient status to fertilization and litter manipulation, Ecosystems, 15, 387-400, 2012.

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., and Trumbore, S. E.: Persistence of soil organic matter as an ecosystem property, Nature 478, 49-56, 2011.

Schreeg, L. A., Mack, M. C. and Turner, B. L.: Leaf litter inputs decrease phosphate sorption in a strongly weathered tropical soil over two time scales, Biogeochemistry, 113, 507-524, 2013.

Schwendenmann, L. and Pendall, E.: Response of soil organic matter dynamics to conversion from tropical forest to grassland as determined by long-term incubation, Biol. Fert. Soils, 44, 1053-1062, 2008.

Tanner, E. V. J., Vitousek, P. M., and Cuevas, E.: Experimental investigation of nutrient limitation of forest growth on wet tropical mountains, Ecology, 79, 10-22, 1998.

Trumbore, S. E.: Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecol. Appl., 10, 399-411, 2000.

Deleted:
M., Stott,

Moved d

Deleted:
lowland tr
Biogeoche

Deleted:

Deleted:

Moved (i

Deleted:

Moved d

Formatte

Moved (i

Deleted:

Deleted:

Moved (i

Moved u
Marthews
enhanced
Change, 1
¶
Sayer, E. J

Moved (i

Formatte

Formatte

Deleted:

Moved u
A. W.: Incr
moist trop

Deleted:
Sayer, E

Moved u

Deleted:
cycling in
experimen
Responses
G. and Sar
2016.¶

Deleted:

Deleted:

Moved (i

Moved (i

Formatte

Deleted:
dissolved
dissolved
ecosystem

Turner, B. L. and Romero, T. E.: Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils, Soil Sci. Soc. Am. J., 73, 1972-1979, 2009.

Turner, B. L., Yavitt, J. B., Harms, K. E., Garcia, M. and Wright, S. J.: Seasonal changes in soil organic matter after a decade of nutrient addition in a lowland tropical forest, Biogeochemistry, 123, 221-235, 2015.

Vincent, A. G., Turner, B. L. and Tanner, E. V. J.: Soil organic phosphorus dynamics following perturbation of litter cycling in a tropical moist forest. Eur. J. Soil Sci., 61, 48-57, 2010.

Wieder, W. R., Cleveland, C. C., Taylor, P. G., Nemergut, D. R., Hinkley, E-L., Philippot, L., Bru, D., Weintraub, S. R., Martin, M., and Townsend, A. R.: Experimental removal and addition of leaf litter inputs reduces nitrate production and loss in a lowland tropical forest, Biogeochemistry 113, 629-642, 2013.

Wright, S. J., Yavitt, J. B., Wurzburger, N., Turner, B. L., Tanner, E. V. J., Sayer, E. J. Santiago, L. S., Kaspari, M., Hedin, L. O., Harms, K. E., Garcia, M. N. and Corre, M. D.: Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest, Ecology, 98, 1616-1625, 2011.

Zuur, A.F., Leno, E. N., Walker, N., Saveliev, A.A. and Smith, G. M. Mixed Effects Models and Extensions in Ecology with R, Springer, New York. 574 pp., 2009

Moved (

Moved (

Deleted:

Deleted:

Deleted: above-gro
biological
manipulat
2013.¶

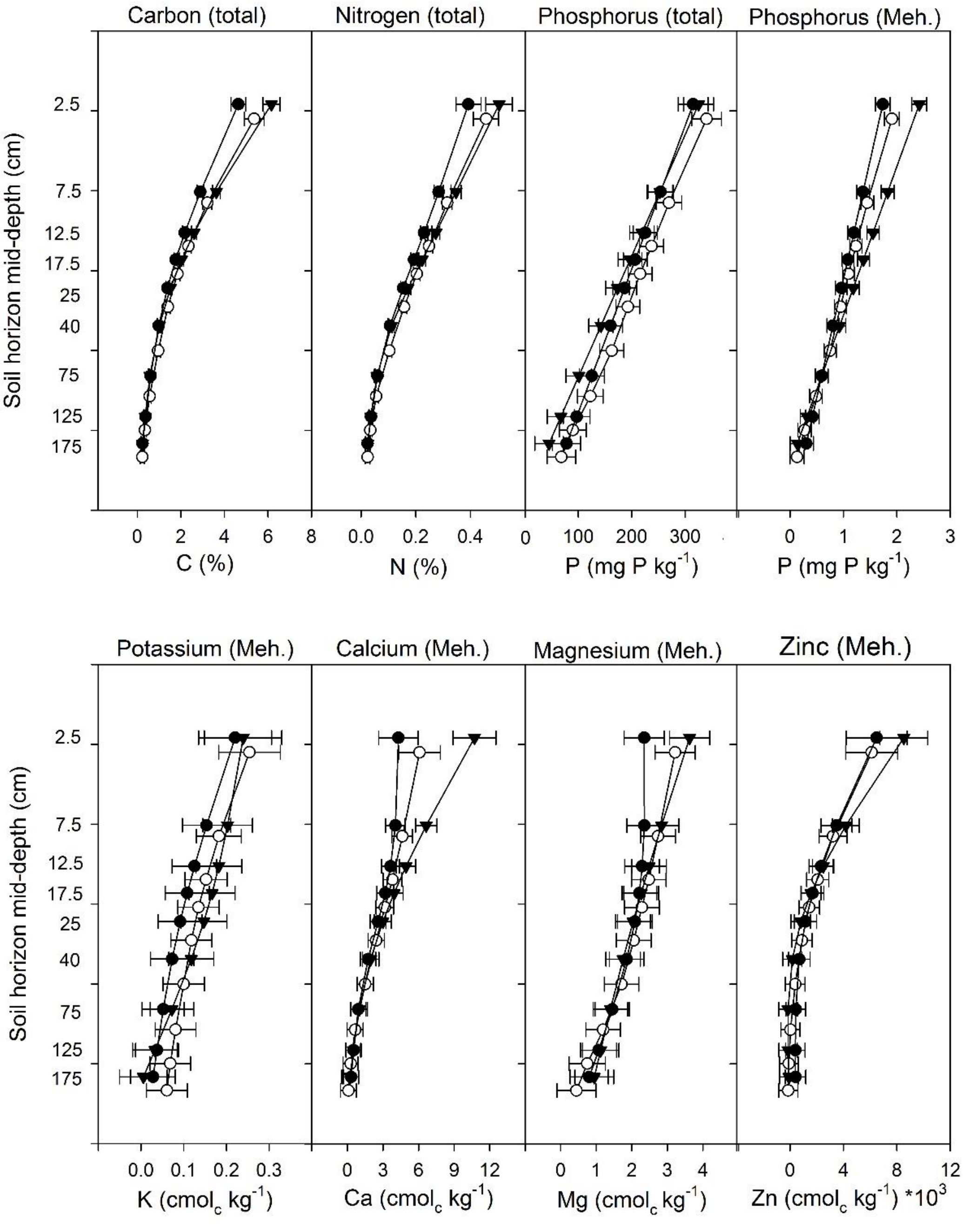

Fig. 1 Concentrations of soil C, N, P (various fractions) and cations (Mehlich extractions), plotted against the mid-point of the soil layers sampled (Zn values should be divided by 1000 to obtain actual means), control points are displaced below treatments. Data are fitted values of the mixed effects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

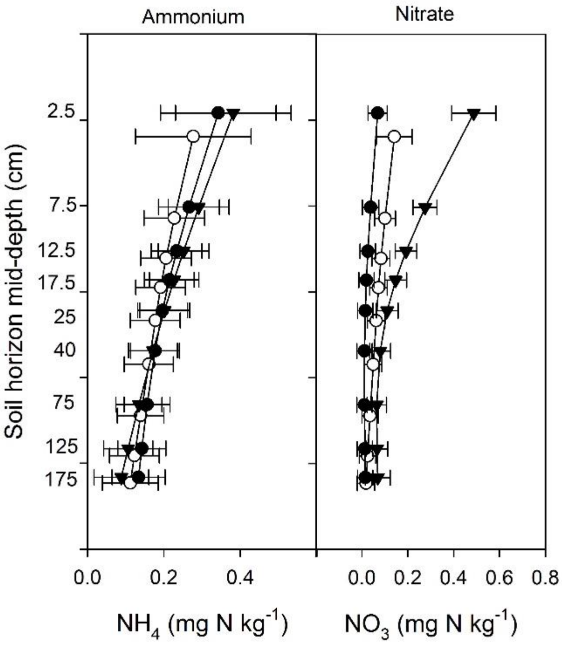

Fig. 2 Mean concentrations of ammonium and nitrate plotted against the mid-point of the soil layers sampled, control points are displaced below treatments. Data are fitted values of the mixed effects models with 95% confidence intervals (see Methods), in litter removal ●, control O and litter addition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

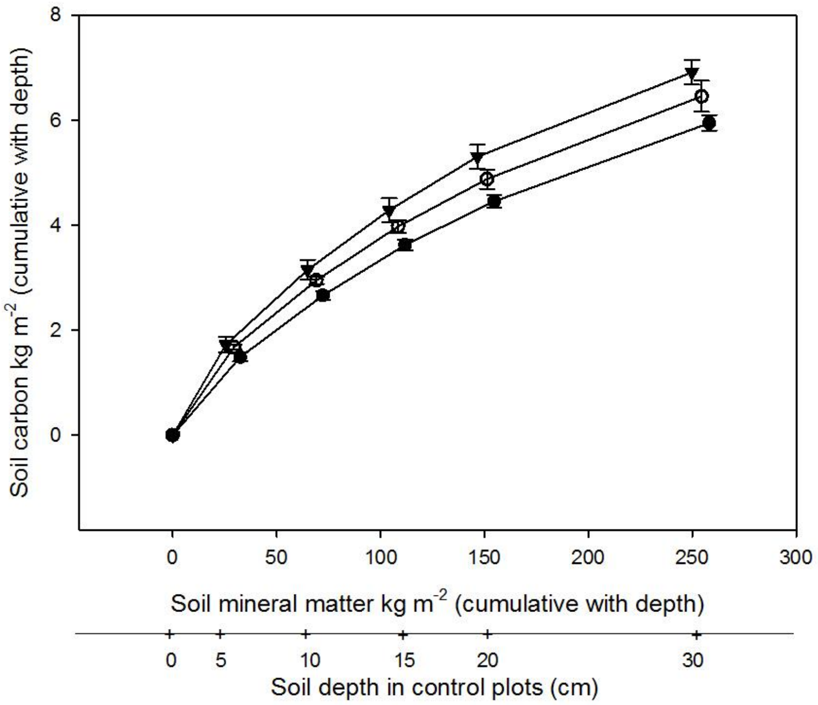

Fig. 3 Soil carbon content and mineral content in litter addition, control, and litter addition expressed as kg C m^{-2} cumulatively from 0 to 30 cm soil depth. Values are means for 5 plots per treatment +/- SE, litter removal ●, control **O**, and litter addition ▼ .

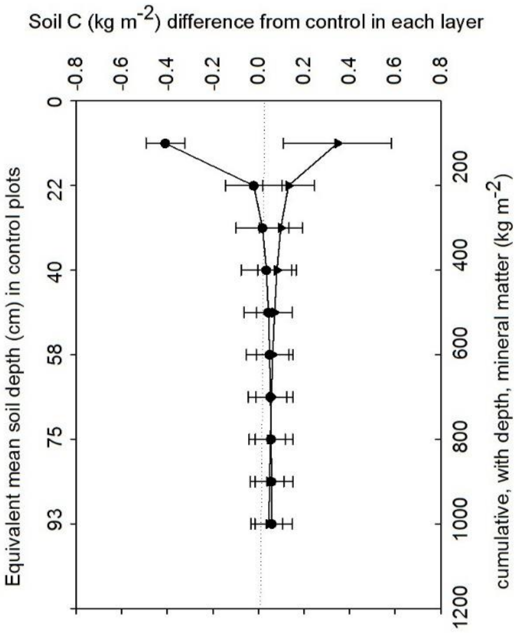

Fig. 4 Differences in soil carbon content relative to control soils (mean and SE, n = 5), after 6 years of litter manipulation, plotted for successive soil layers: 0-100 kg (mineral matter) m^{-2}, plotted at 100 kg m^{-2} on right y axis; 100-200 kg m^{-2}, plotted at 200 kg m^{-2}; and so on to 900-1000 kg m^{-2}, plotted at 1000 kg m^{-2}; in litter removal ● and litter addition ▼ plots. We calculated the soil C in the litter removal and litter addition 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.