General response to reviewers on the manuscript: "*Ideas and perspectives: patterns of soil CO_2, CH_4 and N_2O fluxes along an altitudinal gradient - a pilot study from an Ecuadorian Neotropical montane forest*"

Paula Alejandra Lamprea Pineda[1], Marijn Bauters[2, 3], Hans Verbeeck[3], Selene Baez[4], Matti Barthel[5] and Pascal Boeckx[2]

Firstly, we would like to thank the reviewers and the editor for reviewing and providing constructive comments to improve the manuscript.

Below, a point-by-point response to the reviews is shown, with the comments of the reviewers first, followed by our response in *italic* font. The manuscript has been adapted as noted below.

As a relevant change, it is important to mention that a flux correction has been carried out, considering the effect of local pressure and temperature. Previously, the fluxes had been calculated at normal temperature and pressure conditions (i.e. P: 1 atm, T: 293 K), and we feel that these pressure-corrected values are more correct than non pressure-corrected. However, the changes in the flux quantification is negligible and does not in any way change the message of this paper. In this sense, the GHG fluxes have been updated throughout the manuscript, including tables, graphs and supplementary material.

Reviewer # 1

Klaus Butterbach-Bahl (Referee)

Dear authors,

thanks for the revision and restructuring of the manuscript. Looks more convincing to me now. I still find the discussion of results on soil CO_2 fluxes highly speculative as no details on site, stand properties are provided. However, the point on N_2O uptake, and the support of negative N_2O flux data by evidence from isotopic signatures pointing towards N_2O consumption is convincing and very interesting. I am surely supporting your lea for more research on tropical mountainous forest ecosystems under climate change, but this might need to go along as well with capacity building in the respective countries.

The discussion on soil CO_2 fluxes have been shortened and the fact that we did not measure, nor estimated root biomass is clearly stated (see answer below for line Line 95-112). On the other hand, we agree that more research on tropical montane forests must go along with capacity building in the respective countries, thus, this has been clearly stated in the conclusions (L223).

Here are a few additional points:

Line 37 while the argument is correct for CH_4 and N_2O, soil CO_2 fluxes are highly dependent on root (autotrophic) respiration too. Revise sentence (and see your line 40 too).

Line 37 & 40 have been revised and restructured from: "In general, soil CO_2, CH_4 and N_2O production or consumption depend on microbiological processes driven by a wide range of abiotic and biotic characteristics. The combination of these processes ultimately determines if a soil is a net source or sink of GHGs. Under aerobic conditions, CO_2 is emitted to the atmosphere by autotrophic and heterotrophic respiration (Dalal and Allen, 2008)" to "In general, soil CO_2 is produced mainly by root respiration, microbial respiration, litter decomposition, and oxidation of soil organic matter (Dalal and Allen, 2008). CH_4 is consumed by methanotrophic bacteria (Jang et al., 2006), however, *forest soils prone to inundation emit CH_4 by methanogenic microorganisms (Archaea domain). N_2O is emitted through denitrification or a number of alternative pathways (e.g. nitrification, nitrifier-denitrification, chemodenitrification, etc. (Butterbach-Bahl et al., 2013; van Cleemput, 1998; Clough et al., 2017)), but can also be consumed during complete denitrification (Butterbach-Bahl et al., 2013).”*

Line 44 tropical forest soils

Done

Line 46 Provide a reference for these numbers/ estimates of NEE

Reference provided

Line 57 I would suggest to include Table S1 in the main text

Although Table S1 gives an overview of all the studies that have been carried out on GHGs in South America, including it in the main text may deviate the main focus of the manuscript. The table is relatively long, and it would be more suitable for a review paper. Therefore, we refrain from including it to the main text.

Lines 95-112 I find the discussion on the soil CO_2 emissions extremely speculative as there is no information on vegetation biomass, root biomass, vegetation type etc. Given the acknowledged importance of root respiration and the known high temporal variability of soil respiration, and the missing stand information, I think that little can be learned out of this and would suggest to further shorten this part.

We agree on this. Therefore, the discussion on CO_2 emissions has been reduced and it has been clearly stated that root biomass was not measured nor estimated.

Line 115 just mention here the number of observations day

Done

Line 139 check numbers for C_2200. If 20 fluxes should result in net emissions the range should have a positive value too

Indeed, the range has been corrected.

Line 144 typo with “-and”

Done

Line 154-158 see also Denk et al. 2017, The nitrogen cycle: A review of isotope effects and isotope modelling approaches. Soil Biol. Biochem. 105, 121-137

Thanks for the suggestion, we have revised the paper and included Denk et al. 2017 in our discussion.

Line 177 mention that root biomass was not measured nor estimated

Done

Line 195-200 Possibly also mention the importance for capacity building on biogeochemistry and GHG research in the target regions. Given that these regions will be severely hit by climate change, but so far, knowledge mainly comes from outside, such a network approach would also be helpful to create a local knowledge base on how climate (and landuse change) might affect ecosystems (and people).

We agree and included the suggestions in L223.

Reviewer # 2

Anonymous Referee #2

This is an interesting work on soil GHG fluxes in an underrepresented region; it does not only provide a good (and short) dataset but also some insights into soil N_2O uptake. I have had a look to the previous version and the comments of the reviewers and I agree with them that the experimental set up and the dataset fits better into a “preliminary data” paper. The paper is nicely and clearly written, and it is honest with regard to its limitations; at the same time, serves in suggesting ways forward for increasing our understanding on the patterns of GHG fluxes in tropical montane forest soils.

Probably one of the most relevant changes I suggest is with regard to the title. “Varying sources and sinks” seems to me that you look at different components within the forest, but this is not the case. I suggest to make explicit mention to the soil (e.g. –only a suggestion- “Ideas and perspectives: patterns of soil CO_2, CH_4 and N_2O fluxes along ….”) and probably to montane forests.

Further, I understand the rationale of using GWP to compare between GHGs, but I strongly doubt the usefulness of including CO_2 fluxes since, as you mentioned in the paper, it is only a part of the story and do not reflect inputs to the soil.

Finally, you highlight quite prominently the topographic position as a key driver controlling spatial variability (e.g. L32, L186); while this is true, other sources of variability may be equally important (e.g. degradation/forest management, hydrological status at the catchment level, soil types, exposition and associated microclimate) and should be taken into account when proposing “broader studies”.

For the rest, I only have a couple of minor comments, which are depicted below.

We thank the Referee for this positive assessment. We agree that the title can give a wrong perception about the scope of the manuscript, therefore, the suggestion has been accepted indicating clearly that the research was carried out on “montane” tropical forests.

We agree with the Referee on the usefulness of including the CO_2*-eq emissions, since it does not add any extra information to the manuscript, we have decided to remove it from the text.*

Moreover, we agree that besides topographic position there are more drivers controlling GHG fluxes, therefore we have included them on L197-209 as well as in the abstract L32.

L41: Remove “(anaerobic)”

Done

L44-46. I am missing a citation here, so I can´t check on my own. I guess this is the C sink of the forest, and not of the soil; since you previously referred to the soil, it is misleading, unless you are able to infer what proportion is stored in the vegetation and in the soil (or below- and aboveground).

The citation has been added. The number indeed refers to the C sink of the forest, and since it is not possible to infer the proportion stored in the vegetation and in the soil, it has been clearly stated that the number refers to the below and aboveground sink.

L70: Probably merge the two paragraphs.

Done

L75: Remove "more" before "severe". Remove "even though forests can be managed to mitigate climate change as well", it is not really relevant here. Eventually, you can state that it is important to understand the feedbacks to come up with appropriate forest management options to mitigate climate change.

Done

L93: I suggest to change "budget" by "fluxes".

Done

L102: Zimmermann et al 2009(Eur J Soil Sci, doi: 10.1111/j.1365-2389.2009.01175.x) worked also on an altitudinal gradient in the Andes and pointed towards changing C allocation patterns. The paper might be also interesting for the summary table (I was not involved in this work).

Thanks for the recommendation. This paper has been included in the summary table.

L119: Remove "on the other hand". Reformulate the second sentence. The lowest flux is the closest to 0, and this is not what you want to point out.

"On the other hand" has been removed, and the statement has been reformulated to: "Only S_400 and M_1100 (both months) (i.e. plots located at the lower locations) acted as net sources of N_2O *(Fig. 1c and Table 1). Whereas the plots located at the highest stratum (P_3010 & C_2200) showed a general net* N_2O *consumption during August and September." It is important to mention that due to the flux correction (pressure and temperature), the data changed slightly and M_1100 in August showed a net emission of* N_2O *(0.8±6.9 µg N* m^{-2} h^{-1}*) instead of a net sink (-0.2±7.7 µg N* m^{-2} h^{-1}*) as it was reported before.*

L139: The range is wrong, I think, it should go from negative to positive.

The range has been revised and updated with the corrected fluxes.

L143: This is also similar to what Gerschlauer (Biogeosciences) found in a gradient in the Kilimanjaro. I don´t remember if they found relationships between N_2O fluxes and the isotopic signature in the soil, but may be worth looking at it (not involved either in the work).

This is indeed similar. No measurements regarding N_2O *fluxes were carried out, but the authors mention that soil* $\delta^{15}N$ *values suggest the tightest N cycling at high elevations (> 3000 m a.s.l.). Although they clearly claim that a conclusion about the nature for the N cycle (open or closed) should be made considering other processes as well (e.g. soil nitrate leaching). Nevertheless, we have cited this paper in the respective line.*

L152: Probably "support" or similar, rather than "confirm"

Agreed, support fits better the statement.

L186: Remove “For instance”

Done

L194: Why bi-weekly? To me, the sampling should cover seasonal fluctuations (dry vs. wet season), but also at a finer scale.

The main message of L194 was that long-term data that covers season fluctuations (i.e. dry vs wet) is needed, although “bi-weekly” was mentioned to give an example of the sampling intervals, we agree that sampling at a finer scale is important, as stated in line L169, thus, “bi-weekly” has been removed.

Fig 2: Note: “the dotted vertical line” instead of “the dotted x axis”

Done

Fig 3: Even if the three GHG are represented using CO_2-eq, consider an axe-break instead on the zoom-in view.

As stated before, the section on CO_2*-eq emissions has been removed.*

Table S1. This is a great compilation of information. However, I find difficult to compare the studies. I suggest to add more columns, in order to separate between measured and estimated annual, and also a field with comments/period of measurement). I also suggest not to use the publication as a basis, but the site (e.g. Garcia Montiel et al 2004 measured on different sites, so include a line for each, with the same reference). For me, manual and dynamic chambers are not mutually exclusive. Do you rather mean manual vs. automated? In any case, probably the frequency is more important than the method per se (e.g. manual chambers can have daily, or monthly frequency).

Extra columns to separate between measured and estimated annual fluxes has been added for each GHG. However, and extra column with comments/period of measurement was not added since the period of measurement sometimes differs between GHG and/or conditions (e.g. dry season vs wet season). Moreover, as suggested, a lined has been added when in the same study different sites were evaluated. Finally, the distinction between manual vs dynamic chambers has been removed, although the latter referred to automated; the frequency is indeed more important, but not always mention in the papers.

Ideas and perspectives: patterns of soil CO_2, CH_4 and N_2O fluxes along an altitudinal gradient - a pilot study from an Ecuadorian Neotropical montane forest

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Paula Alejandra Lamprea Pineda[1], Marijn Bauters[2, 3], Hans Verbeeck[3], Selene Baez[4], Matti Barthel[5] and Pascal Boeckx[2]

[1]Environmental Organic Chemistry and Technology - ENVOC, Department of Green Chemistry and Technology, Ghent University, Gent, 9000 Belgium
[2]Isotope Bioscience Laboratory – ISOFYS, Department of Green Chemistry and Technology, Ghent University, Gent, 9000 Belgium
[3]Computational and Applied Vegetation Ecology – CAVElab, Department of Environment, Ghent University, Gent, 9000 Belgium
[4]Departamento de Biología, Escuela Politécnica Nacional del Ecuador, Ladrón de Guevera E11-253 y Andalucía, Quito, Ecuador
[5]Department of Environmental Systems Science, ETH Zurich, Zurich, 8092, Switzerland

Correspondence to: Paula Lamprea (paulaalejandra.lampreapineda@ugent.be)

Co-authors: marijn.bauters@UGent.be, hans.verbeeck@UGent.be, selene.baez@epn.edu.ec, matti.barthel@usys.ethz.ch, pascal.boeckx@UGent.be

Keywords: Tropical Forests - Soil CO_2, CH_4 and N_2O fluxes - Altitudinal Gradients - N_2O Stable Isotopes.

Abstract

Tropical forest soils are an important source and sink of greenhouse gases (GHG) with tropical montane forests, in particular, poorly studied. The understanding of this ecosystem function is of vital importance for future global change and climate research. In this study, we explored soil fluxes of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) of four tropical forest sites located on the western flanks of the Andes in northern Ecuador. The measurements were carried out during the dry season from August to September 2018, and along an altitudinal gradient from 400 to 3010 m a.s.l. During this short-term campaign, our measurements showed 1) an unusual but marked increase of CO_2 emissions at high altitude, possibly linked to changes in soil pH and/or root biomass; 2) a consistent atmospheric CH_4 sink over all altitudes with high temporal and spatial variability; and 3) a transition from net N_2O source to sink along the altitudinal gradient, with bulk isotope ^{15}N-N_2O data indicating net N_2O reduction at high altitude. Our results provide arguments and insights for future and more detailed studies on tropical montane forests. Furthermore, they stress the relevance of using altitudinal transects as a biogeochemical open-air laboratory, with a steep *in-situ* environmental gradient over a limited spatial distance. Although short-term studies of temporal variations can improve our understanding of the mechanisms behind the production and consumption of soil GHGs, the inclusion of more rigorous sampling for forest management events, forest rotation cycles, soil type, hydrological conditions and drainage status, ground vegetation composition and cover, soil microclimate, and temporal (seasonality) and spatial

(topographic positions) variability are needed, this in order to obtain more reliable estimates of the CO_2, CH_4 and N_2O source/sink strength of tropical montane forests.

1 The importance of tropical forests for GHG budgets

Soils play a vital role in the global GHG budget. Tropical forest soils, in particular, represent a net sink of carbon (C) (Pan et al. 2011), but at the same time, they are the largest natural source of N_2O, with an estimated contribution of 14-23% to the annual, global N_2O budget (Werner et al., 2007). In general, soil CO_2 is produced mainly by root respiration, microbial respiration, litter decomposition, and oxidation of soil organic matter (Dalal and Allen, 2008). CH_4 is consumed by methanotrophic bacteria (Jang et al., 2006), however, forest soils prone to inundation emit CH_4 by methanogenic microorganisms (*Archaea* domain). N_2O is emitted through denitrification or a number of alternative pathways (e.g. nitrification, nitrifier-denitrification, chemodenitrification, etc. (Butterbach-Bahl et al., 2013; van Cleemput, 1998; Clough et al., 2017)), but can also be consumed during complete denitrification (Butterbach-Bahl et al., 2013). Overall, tropical forests soils emit on average 12.1 t CO_2-C ha^{-1}y^{-1} (heterotrophic and autotrophic respiration), slightly less than the net primary productivity (NPP) (12.5 t CO_2-C ha^{-1}y^{-1}) i.e. the net C sink (below and aboveground) of tropical forests is ~ 0.4 t CO_2-C ha^{-1}y^{-1} (Dalal and Allen, 2008; Grace et al., 2006). Under aerobic conditions, CH_4 fluxes vary from -0.7 to -30.0 kg CH_4-C ha^{-1}y^{-1}, with an average consumption of -3.0 kg CH_4-C ha^{-1}y^{-1}, while the mean rate of N_2O emissions from tropical forest soils is 3.03±0.52 kg N_2O-N ha^{-1}y^{-1} (Dalal and Allen, 2008), i.e. 2-3 times higher than the mean N_2O emissions from temperate forest soils (1.0±0.36 kg N_2O-N ha^{-1}y^{-1}; Chapui-Lardy et al., 2007; Van Groenigen et al., 2015).

Deleted: In general, soil CO_2, CH_4 and N_2O production or consumption depend on microbiological processes driven by a wide range of abiotic and biotic characteristics. The combination of these processes ultimately determines if a soil is a net source or sink of GHGs. Under aerobic conditions, CO_2 is emitted to the atmosphere by autotrophic and heterotrophic respiration

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The understanding of the mechanisms and processes underlying GHG flux variability has greatly improved during the last decades (Butterbach-Bahl et al., 2013; Heil et al., 2016; Müller et al., 2015; Sousa Neto et al., 2011; Su et al., 2019; Teh et al., 2014). However, there is still 1) considerable uncertainty about the overall balances of many ecosystems (Castaldi et al., 2013; Heil et al., 2014; Kim et al., 2016b; Pan et al., 2011; Purbopuspito et al., 2006), 2) a strong imbalance in field observations, skewed towards the Northern hemisphere (Jones et al., 2016; Montzka et al., 2011), and 3) a bias towards the quantification of emissions in lowland forests within the tropics (Müller et al., 2015; Purbopuspito et al., 2006; Wolf et al., 2011). For instance, based on a compilation made of CO_2, CH_4 and N_2O fluxes in South America (Table S1) from 1983 to 2019, there are only six studies carried out on tropical montane forests (i.e. > 2000 m. a.s.l.), while they represent more than 11% of the world's tropical forests (Müller et al., 2015; Teh et al., 2014). In fact, Teh et al (2014) and Spahni et al (2011) have argued that tropical upland soils are one potentially important source of CH_4 and N_2O, overlooked in both, bottom-up and top-down emissions inventories; their sink/source strength might be comparable or greater than their lowland counterparts, and therefore, quantitatively important in regional and global GHG budgets.

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2 Altitudinal gradients as a biogeochemical open-air laboratory

To further improve our understanding of the role of tropical forest ecosystems in the global GHG balance, environmental gradients (altitudinal, latitudinal, etc.) can offer great opportunities to study the influence of abiotic factors on biogeochemical processes under field conditions (Bauters et al., 2017; Jobbágy and Jackson, 2000; Kahmen et al., 2011; Laughlin and Abella, 2007); which complements the knowledge on short term responses from experimental approaches. In the case of altitudinal gradients, these responses are driven by abiotic variables that co-vary with elevation, which, amongst others, creates a distinctly strong climate gradient over a short spatial distance (Bubb et al., 2004; Killeen et al., 2007; Körner, 2007; Myers et al., 2000). Moreover, since altitudinal gradients reflect long-term adaptations based on a broad range of factors, they provide valuable insights into the influence that climate change may have on ecosystem processes (Malhi et al., 2010). There is indeed a growing concern regarding the sensitivity of tropical forests to climate change, mainly because species in the tropics have evolved with narrow thermal tolerances compared to their temperate counterparts; which makes them particularly vulnerable to changes in global climate (Fadrique et al., 2018; Perez et al., 2016). Therefore, the effects of global warming are expected to be severe in the tropics, and the understanding and integration of the magnitude of their feedbacks in the Earth system is important to come up with appropriate forest management options to mitigate climate change (Bonan, 2008; Li et al., 2020).

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To address these knowledge gaps, we present a pilot study of the soil-atmosphere exchange of CO_2, CH_4 and N_2O along an altitudinal gradient in a Neotropical montane forest located on the western flanks of the Andes in northern Ecuador. The sampling campaign took place from August 6th to September 28th, 2018. Four study sites (Fig. S1) were selected: Río Silanche at 400 m a.s.l (hereinafter: S_400), Milpe at 1100 m a.s.l. (hereinafter: M_1100), El Cedral at 2200 m a.s.l. (hereinafter: C_2200) and Peribuela at 3010 m a.s.l. (hereinafter: P_3010). Gas samples were taken using a static flux chamber method once per day during two weeks per stratum, with additional samples for bulk ^{15}N of N_2O ($\delta^{15}N^{Bulk}$). Samples of soil were collected once during the whole field campaign for analysis of bulk density (ρ_b), pH, nitrate (NO_3^-) and ammonium (NH_4^+) content, C and nitrogen (N) concentrations, stable N isotope signatures ($\delta^{15}N$) and soil texture. Additionally, soil moisture (expressed as water-filled pore space (WFPS)) and soil temperature were measured daily. Specifically, we aimed 1) to determine the magnitude of the soil-atmosphere exchange of CO_2, CH_4 and N_2O during the dry season, and 2) to support and couple the N_2O fluxes with their isotopic signatures. By working along this altitudinal gradient, we wanted to explore the potential effect of altitude on the GHG fluxes of the forest soils. Findings from this research could provide insights for future and more detailed studies on tropical montane forests.

Deleted: Finally, in order to compare the fluxes of the three GHGs measured (i.e. CO_2, CH_4 and N_2O) and to determine the overall budget of the soils per month, the CO_2-eq emissions for each gas were calculated (see supplementary material for methods).

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3 What did we see in Ecuador?

Across our study sites, P_3010 (the highest stratum) exhibited the highest soil CO_2 emissions (Fig. 1a and Table 1), probably due to a dominant role of soil pH and shifts in C allocation patterns. The highest soil pH_{water} was observed in this site (Table 2), and under acid conditions, Sitaula et al. (1995) and Persson & Wiren (1989) have reported a decrease in CO_2 emissions with decreasing pH$_{water}$. On the other hand, although not measured nor estimated in this study, an increase in fine root biomass is expected in tropical mountain forests compared to lowland forests -due to imbalances or limitations in resource (water and/or nutrients) availability at higher altitudes (Bauters et al., 2017; Leuschner et al., 2007), therefore, the observed increase in CO_2 emissions at P_3010 might be further driven by an increase in root biomass, as the latter has been shown to be positively correlated with soil respiration (Han et al., 2007; Luo and Zhou, 2006a; Reth et al., 2005; Silver et al., 2005).

In contrast to P_3010, the low CO_2 emissions observed at C_2200 could be attributed to 1) the lower WFPS (Fig. S3), 2) the lower contents of C and N (Table 2), and 3) the higher bulk density (Table 2). The lowest soil water content was observed at this site in August at 5 cm depth, and exactly in this month, the lowest emissions of CO_2 were obtained. The low contents of C and N exhibited in C_2200 (indeed, the lowest from all the sites), could also have hampered the CO_2 emissions (Dalal and Allen, 2008; Luo and Zhou, 2006a; Oertel et al., 2016). Additionally, this site had the highest soil bulk density (i.e. lowest porosity), which could have led to a decrease in soil respiration either by a physical impediment for root growth or by a decrease in soil aeration for microbial activities (Dilustro et al., 2005; Luo and Zhou, 2006b, 2006a).

All sites acted as net sinks for CH_4 (Fig. 1b and Table 1) (i.e. uptake of atmospheric CH_4 by soils). During the entire field campaign (10 days), only one chamber at one site (S_400) and a specific date (08/09/2018) showed a net source of CH_4 (43.2 µg CH_4-C m^{-2} h^{-1}). However, there were no statistical differences between months, and all sites exhibited indeed a high temporal and spatial variability.

Only S_400 and M_1100 (both months) (i.e. plots located at the lower locations) acted as net sources of N_2O (Fig. 1c and Table 1). Whereas the plots located at the highest stratum (P_3010 & C_2200) showed a general net N_2O consumption during August and September.

The N_2O emissions obtained at the lowest strata corroborate with literature data on lowland tropical forests (Butterbach-Bahl et al., 2004, 2013; Koehler et al., 2009), and could be mainly attributed to soil water content, temperature, and N availability observed at these sites (Fig. S2, S3 and Table 2). N_2O emissions in tropical forest soils are predominantly governed by WFPS, which influences microbial activity, soil aeration, and thus diffusion of N_2O out of the soil (Davidson et al., 2006; Werner et al., 2007). An increase in temperature leads to an increase in soil respiration and thus to a depletion of oxygen concentrations, which is indeed a major driver in N_2O emissions. Rising temperatures lead to a positive feedback in microbial metabolism; the stimulation of mineralization and nitrification processes induces an increase in the availability of substrates for denitrification, and thus to an increase in N_2O emissions (Butterbach-Bahl et al., 2013; Sousa Neto et al., 2011). Finally, the dependency of N_2O emissions on WFPS and temperature is affected by substrate availability (NO_3^-). High contents of NO_3^- give an indication of an open or “leaky” N cycle with higher rates of mineralization, nitrification, and thus N_2O emissions (Davidson et al., 2006).

Deleted: Moreover, Luo & Zhou (2006a), Oertel et al. (2016), Reth et al., (2005) and Wang et al., (2010) have reported positive correlations between soil pH$_{water}$ and CO_2 fluxes. On the other hand, shifts in C allocation could also give rise to shifts in CO_2 emissions. An increase in fine root biomass is expected in tropical mountain forests compared to lowland forests; either

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Moreover, NO_3^- is normally preferred as an electron acceptor over N_2O and it can also inhibit the rate of N_2O consumption to N_2 (Dalal and Allen, 2008).

In contrast to the low elevation sites where net N_2O emissions were observed, P_3010 and C_2200 (Fig. 1c and Table 1) presented net consumption (negative values, i.e. fluxes from the atmosphere to the soil). From 35 valid measurements only one resulted in net emission at P_3010 (range: -9.3 to 0.95 µg N_2O-N m^{-2} h^{-1}), whereas from 36 valid measurements, 19 resulted in net emissions at C_2200 (range: -104.9 to 9.3 µg N_2O-N m^{-2} h^{-1}). Net N_2O consumption is often related to N-limited ecosystems and it is presumably the cause in our case. At low NO_3^- concentrations, atmospheric and/or soil gaseous N_2O may be the only electron acceptor left for denitrification (Chapui-Lardy et al., 2007; Goossens et al., 2001). P_3010 had the lowest content of NO_3^-, along with the lowest soil $\delta^{15}N$ (Table 2), which clearly reflects the shift towards a more closed N cycle at higher elevations (Bauters et al., 2017; Gerschlauer et al., 2019). In fact, studies performed by Teh et al. (2014) and Müller et al. (2015) in the Southern Peruvian and Ecuadorian Andes, respectively, related the decrease in N_2O emissions and thus the potential for N_2 production in soils at high elevations to differences in NO_3^- availability. Moreover, Wolf et al (2011) and Martinson et al (2013) have indicated that N availability was 1) a dominant control on N_2O fluxes and 2) inversely proportional to altitude. In addition, the low N_2O fluxes could also be supported by the high content of clay (Table 2) and CO_2 emissions (Fig. 1a) (i.e. development of microsites for N_2O reduction), along with the low soil water content (% of WFPS) (Fig. S3) (i.e. better diffusion of atmospheric N_2O into the soil) and higher soil pH-value (Table 2) (i.e. less severe inhibition of the nitrous oxide reductase) observed at P_3010 (Chapui-Lardy et al., 2007).

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Besides the soil isotope signatures, the bulk N_2O isotope signatures (Fig 2) support the net N_2O consumption at higher altitudes, and net N_2O emission at lower altitudes, and rule out that our net consumption rates are due to sampling artifacts. Previous studies have indicated that during the reduction of N_2O to N_2, N_2O-reductase fractionates against ^{15}N (Barford et al., 1999; Butterbach-Bahl et al., 2013; Menyailo and Hungate, 2006; Pérez et al., 2000). Consequently, complete denitrification i.e. consumption of N_2O, leads to a ^{15}N enrichment of the residual N_2O, and thus to higher $\delta^{15}N_s^{Bulk}$ values (Denk et al., 2017; Park et al., 2011) relative to the atmospheric bulk N_2O composition (6.3‰ (Harris et al., 2017)). This in fact is reflected in the relatively enriched $\delta^{15}N_s^{Bulk}$ values measured during N_2O consumption, while a relative depletion was observed during N_2O production (two samples taken in September at S_400) (Fig. 2; Table S3). This is also in line with Park et al. (2011) and Pérez et al. (2000) who have attributed $\delta^{15}N_s^{Bulk}$ values between -22 and 2‰ in natural tropical forest soils to denitrification.

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It is important to mention that the region where these measurements were taken is characterized by a marked seasonality in rainfall. We measured at the end of the dry season; thus, it is expected to have fluctuations on net fluxes (sources vs sinks) depending on the season. Moreover, although our limited dataset did not allow us to corroborate the main drivers that controlled these fluxes, daily measurements as those carried out here, reflect the importance of evaluating short-term variations. As such, the net N_2O consumption with increasing altitude might be overlooked in an annual analysis, but equally important to 1)

Deleted: The differences in fluxes for each GHG are clearly visualized in Fig. 3. The high CO_2 emissions observed at P_3010 gave rise to the highest CO_2-eq emissions, and in terms of non-CO_2 GHG, this plot also exhibited the highest sink due to CH_4 and N_2O consumption. Although the calculated CO_2-eq emissions for CO_2 reflect only the impact of soil emissions (heterotrophic and autotrophic respiration) on the GHG budget and exclude photosynthesis and aboveground respiration, the fluxes here obtained during the dry season show a marked sink of non-CO_2 GHG in the upland soils. Nevertheless,

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understand the mechanisms behind the production and consumption of N_2O, and 2) have reliable estimates of the N_2O source/sink strength of tropical forests for regional and even global GHG budgets.

5 Conclusions and future directions

GHG fluxes from tropical montane forests in South America are particularly scarce, with limited spatial coverage and seasonal fluctuation in rainfall, but important to be considered in future field measurements and modeling research. Overall, we found an unusual but marked increase of CO_2 emissions at the highest altitude; probably explained by soil pH and root biomass, even though the latter was not measured nor estimated. Our CH_4 uptake fluxes exhibited a high temporal and spatial variability but reiterate the role of humid tropical forest soils as CH_4 sinks. Contrary to the net N_2O emissions observed in the lowest strata, the net consumption at higher elevations seems to be quite unique, and it might reflect the shift towards a more closed N cycle at higher altitudes reported previously in tropical regions. This net N_2O uptake was in fact confirmed independently by soil and N_2O ^{15}N isotope signatures. Our results highlight the importance of short-term variations in N_2O fluxes, but it calls for more and broader studies especially in tropical montane forests, including the impact of spatial and temporal variability, forest management events and forest rotation cycles, ground vegetation composition and cover, soil microclimate and hydrological conditions, as well as the coupling of microbial analysis with N_2O fluxes, and the response of tropical forests to current and future changes in N content.

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In terms of spatial variation, GHG fluxes may vary between lower slope, mid-slope and/or ridge (see Table S1) (Courtois et al., 2018; Teh et al., 2014; Wolf et al., 2011, 2012). Fluctuations of net fluxes can be observed depending on the season and the transition between them (see Table S1) (Butterbach-Bahl et al., 2013; Kim et al., 2016a). Management events (e.g. thinning, clear cutting, fertilization, draining improvements) and/or forest composition and growth stage (e.g. young vs mature forest) may influence e.g. forest vegetation, soil characteristics, hydrology, and nutrient management among others, and ultimately lead to changes on soil GHG fluxes (Barrena et al., 2013; Jauhiainen et al., 2019; Kim et al., 2016a). Moreover, soil hydrology (runoff, evapotranspiration, soil moisture, etc.) may affect biogeochemical cycles (Kim et al., 2016a). Microbial composition and diversity could be a key to understand the variability of N_2O fluxes (Butterbach-Bahl et al., 2013). Changes in N content -due to e.g. urban development and increasing use of agricultural land- could cause shifts on soil N cycling and thus CO_2, CH_4 and N_2O fluxes (Koehler et al., 2012). Besides this, the effects of climate change in tropical regions (e.g. increases in temperature and CO_2 concentrations, as well as changes in rainfall patterns and drought events) may also affect soil GHG fluxes. Therefore, a strategic plan must be implemented. Long-term data with at least one or two hydrological years is needed, with sampling intervals covering seasonal fluctuations and appropriate to the type of land (i.e. spatial variability across different topographical positions). The effect of N content and climate change on tropical forests could be evaluated using laboratory (e.g. incubations under controlled conditions) and/or field experiments (e.g. see Koehler et al., (2009, 2012), Hall and Matson (1999), and Martinson et al., (2013)); with the use of altitudinal gradients as biogeochemical open-air laboratories. Finally, although sampling conditions in tropical montane regions can be challenging, 1) establishing networks and collaborations with

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local communities (i.e. citizen science) could contribute not only in terms of data acquisition, but also in the development of local knowledge (e.g. how climate and land use change might affect ecosystems and people), and 2) modeling approaches for C and N biogeochemistry in forest ecosystems (e.g. Forest-DNDC (GRAMP, 2013)) could help to up-scale fluxes from site to regional level. Nevertheless, the cooperation and contribution between field researchers and scientific organizations e.g. in South America and across the world, as well as the capacity building in the respective countries, are crucial to improve our understanding of soil GHG fluxes from tropical regions, and paramount to get tangible datasets of remote regions such as montane forests.

Supplementary information

1 Materials and methods

2 Results

Fig. S1. Overview map with the location of the study areas.

Fig. S2. Monthly average soil temperature (°C)±standard deviations (SD).

Fig. S3. Monthly average water-filled pore space (WFPS)±standard deviations (SD).

Table S1. Measured and estimated CO_2, CH_4 and N_2O fluxes from tropical forest soils of South America.

Table S2. Characteristics of the study areas.

Table S2. $\delta^{15}N_S^{Bulk}$ values and N_2O fluxes.

Author contributions

M.Bauters, H.V., SB. and P.B. developed the project; P.L. and M. Bauters carried out the fieldwork and analyzed the data; and M. Barthel performed the bulk isotope ^{15}N-N_2O analysis. All authors contributed to the ideas presented and edited the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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Table 1. Average measurements±standard deviations (SD) of soil CO_2, CH_4 and N_2O fluxes at Río Silanche (400 m a.s.l.; S_400), Milpe (1100 m a.s.l.; M_1100), El Cedral (2200 m a.s.l.; C_2200) and Peribuela (3010 m a.s.l.; P_3010) per month.

Month	Plot	Average CO_2 flux ($mg\ C\ m^{-2}\ h^{-1}$)	Average CH_4 flux ($\mu g\ C\ m^{-2}\ h^{-1}$)	Average N_2O flux ($\mu g\ N\ m^{-2}\ h^{-1}$)
August	S_400	64.5±17.2	-59.6±21.6	11.3±18.4
	M_1100	60.5±7.4	-48.7±19.6	0.8±6.9
	C_2200	46.4±18.3	-47.6±14.5	-2.3±6.8
	P_3010	98.6±23.5	-39.8±12.6	-4.4±2.4
September	S_400	89.9±18.8	-59.8±29.9	3.8±5.7
	M_1100	65.5±21.6	-48.9±14.6	12.9±27.8
	C_2200	53.4±19.3	-57.9±19.4	-4.7±27.0
	P_3010	87.7±18.9	-33.7±10.6	-3.7±1.4

Note: flux values represent the mean of 5 chambers per site and measurement week, using the four-point time series and considering the constraint set to evaluate linearity in each measurement cycle ($R^2 > 0.65$).

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Table 2. Physicochemical soil properties of the study areas Río Silanche (400 m a.s.l.; S_400), Milpe (1100 m a.s.l.; M_1100), El Cedral (2200 m a.s.l.; C_2200) and Peribuela (3010 m a.s.l.; P_3010) at 5 and 20 cm depth, including mean values±standard deviation (SD) of bulk density (ρb), porosity, pH in water (pH$_{water}$) and KCl suspension (pH$_{KCl}$), nitrate (NO$_3^-$) and ammonium (NH$_4^+$) concentration, bulk nitrogen (N) and carbon (C) content, carbon-to-nitrogen ratio (C/N) and δ^{15}N signatures from samples of soil taken in August. Similar lowercase letters in superscript and next to some values within one row and per depth (5 and 20 cm) indicate no significant difference at $P < 0.05$ between sites (S_400, M_1100, C_2200 and P_3010).

	S_400		**M_1100**		**C_2200**		**P_3010**	
	5 cm	**20 cm**	**5 cm**	**20 cm**	**5 cm**	**20 cm**	**5 cm**	**20 cm**
Soil class	Andosol[1]		Andosol[1]		Andosol[1]		Andosol[1]	
Soil texture	Loam	Loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Loam	Loam
Sand (%)	41.0	40.0	70.8	67.0	63.7	60.5	41.9	45.0
Silt%)	43.4	47.0	21.7	27.9	29.7	34.4	32.5	34.9
Clay (%)	15.6	13.1	7.6	5.0	6.6	5.2	25.6	20.1
ρb (g cm^{-3})	0.43±0.15^{b}	0.58±0.07^{b}	0.62±0.09^{a,b}	0.86±0.12^{a}	0.70±0.11^{a}	0.92±0.05^{a}	0.62±0.09^{a,b}	0.81±0.06^{a}
Porosity (%)	83.8±5.5^{a}	78.0±2.5^{a}	76.5±3.3^{a,b}	67.7±4.7^{b}	73.7±4.1^{b}	65.4±2.0^{b}	76.7±3.4^{a,b}	69.6±2.1^{b}
pH$_{water}$	4.6±0.7^{a,b}	5.2±0.5	4.6±0.8^{b}	5.5±0.4	4.8±0.4^{a,b}	4.8±0.6	5.7±0.5^{a}	5.6±0.5
pH$_{KCl}$	4.4±0.2^{b}	4.9±0.3^{a,b}	4.5±0.2^{b}	5.0±0.0^{a}	4.5±0.1^{b}	4.6±0.0^{b}	5.1±0.2^{a}	4.9±0.2^{a,b}
NO$_3$-N (µg g^{-1})[2]	71.9±39.5^{a}	35.7±29.5^{a}	23.1±15.9^{b}	6.7±7.7^{a,b}	30.6±19.4^{a,b}	7.3±4.3^{a,b}	0.8±0.3^{b}	3.6±7.1^{b}
NH$_4$-N (µg g^{-1})[2]	34.3±14.8	27.9±16.1^{a,b}	22.6±4.0	11.9±2.4^{b}	26.5±16.0	18.8±4.9^{b}	22.9±11.3	40.4±13.5^{a}
N (%)	0.8±0.2	0.5±0.1^{a}	0.6±0.2	0.2±0.1^{b}	0.6±0.2	0.3±0.0^{a,b}	0.6±0.0	0.4±0.2^{a,b}
C (%)	8.9±2.4	4.0±1.0^{a,b}	7.1±1.8	2.4±0.7^{b}	6.6±1.7	3.3±0.4^{a,b}	8.6±0.5	4.8±1.5^{a}
C/N[3]	10.6±0.4^{c}	8.9±0.4^{c}	11.9±0.6^{b}	10.6±0.7^{b}	11.8±0.8^{b}	10.4±0.5^{b}	14.6±0.5^{a}	12.8±1.3^{a}
δ^{15}N (‰)[4]	6.2±0.5^{a}	8.6±0.9^{a}	6.0±0.8^{a}	6.7±0.8^{b}	4.0±1.2^{b}	4.8±0.5^{c}	3.7±0.6^{b}	4.2±0.4^{c}

Notes: mean values±SD were calculated from soil samples taken adjacent to each soil chamber (n = 5), except for soil texture, where composites for each site at 5 and 20 cm depth were made from the soil samples taken from each chamber.

[1]Commonly known as *Andisol* in the USDA Soil Taxonomy; [2]expressed per gram of dry soil; [3]calculated by dividing C (%) by N (%) in each soil sample; and [4]expressed relative to the international standard AIR.

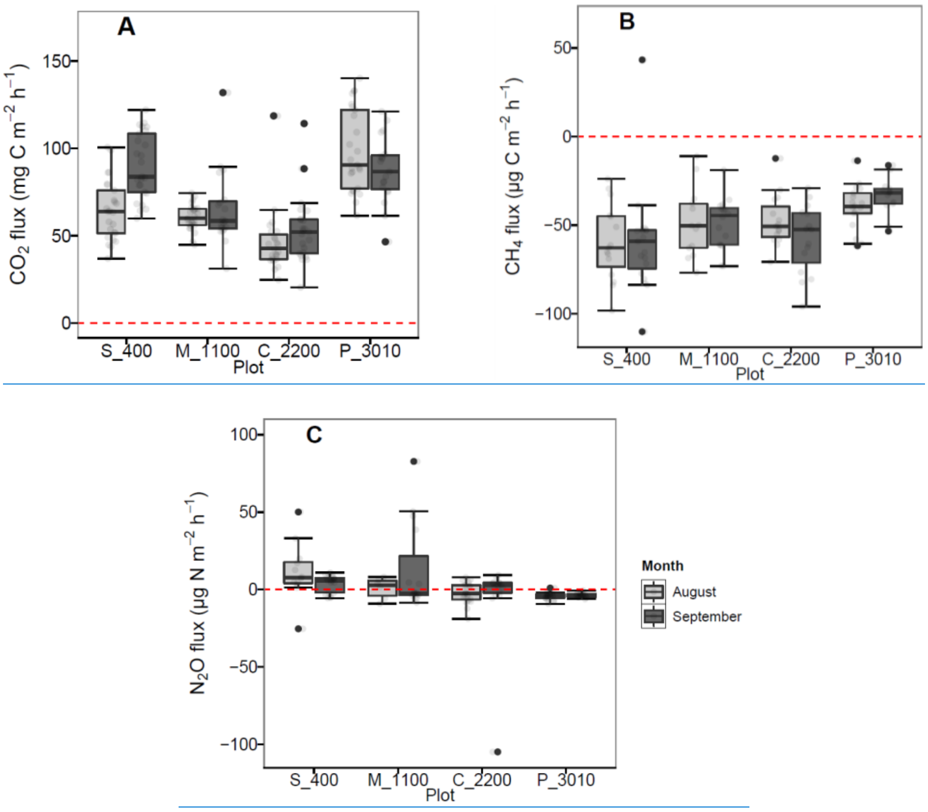
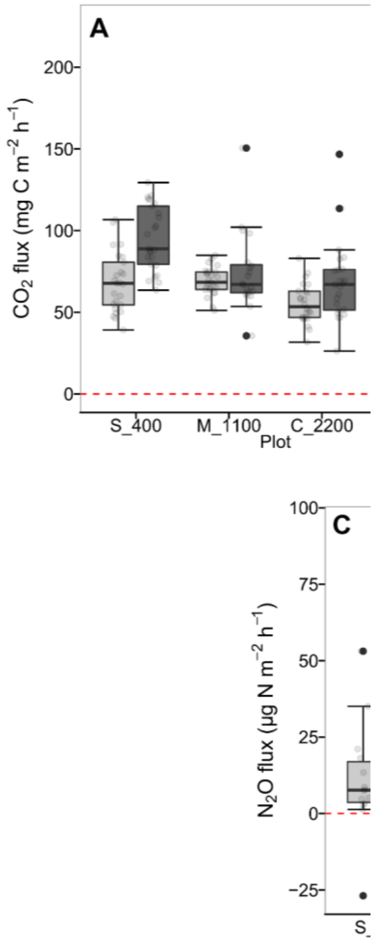


Fig. 1. A) Soil CO$_2$ (mg C m^{-2} h^{-1}), B) CH$_4$ (µg C m^{-2} h^{-1}) and C) N$_2$O (µg N m^{-2} h^{-1}) fluxes per month at Río Silanche (400 m a.s.l.; S_400), Milpe (1100 m a.s.l.; M_1100), El Cedral (2200 m a.s.l.; C_2200) and Peribuela (3010 m a.s.l.; P_3010). Light gray boxplots indicate the fluxes in August 2018, whereas dark gray boxplots, the fluxes in September 2018. Light gray dots in each boxplot represent the measurements taken each day; and black dots, outliers of the respective site. The red dotted line across the boxes indicates zero net flux.



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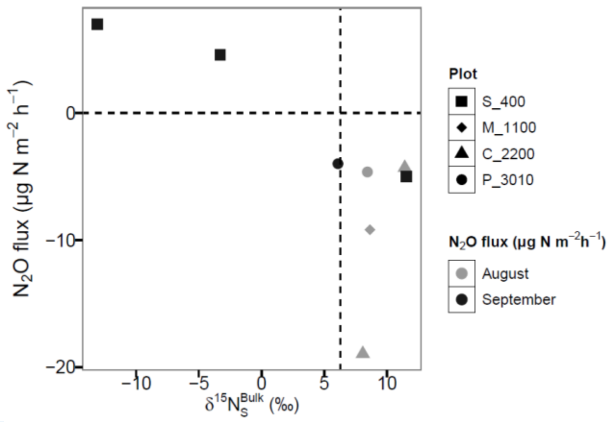
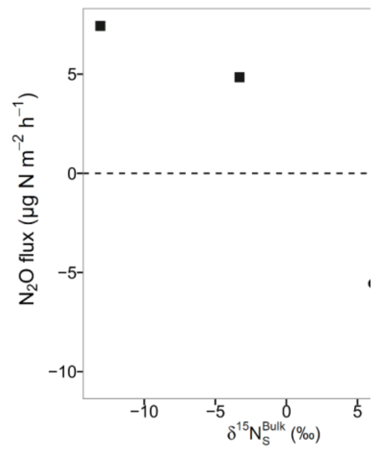


Fig. 2. N_2O fluxes plotted against the bulk isotopic signature of soil N_2O ($\delta^{15}N_S^{Bulk}$) for point samples taken at Río Silanche - squares (400 m a.s.l.; S_400), Milpe - diamonds (1100 m a.s.l.; M_1100), El Cedral - triangles (2200 m a.s.l.; C_2200) and Peribuela - circles (3010 m a.s.l.; P_3010) during August (grey) and September (black). Note: the dotted vertical line at 6.3‰ represents the atmospheric bulk N_2O composition (Harris et al., 2017); and $\delta^{15}N_S^{Bulk}$ values were calculated based on a two-source mixing model, considering a threshold of 20 ppb to exclude low fluxes and thus, avoid larger uncertainties in the source calculation.



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