

**Response to the associate editor on the manuscript: “*Ideas and perspectives: patterns of soil CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes along an altitudinal gradient - a pilot study from an Ecuadorian Neotropical montane forest*”**

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We would like to thank the associate editor for the extraordinary revision of our manuscript after we found an inconsistency in the <sup>15</sup>N data. Since the analytical model employed (two-source mixing model) does not apply for our experimental conditions, and it is not clear how this might have consequences for the data interpretation, we have decided to leave out this section from the manuscript. The specific problem lies with the linear assumptions of the two-source mixing models: a constant atmospheric pool with constant <sup>15</sup>N signature, mixing with a sustained N-source with a constant <sup>15</sup>N signature. As the chamber headspace is in fact varying in concentration and <sup>15</sup>N signature, as the N<sub>2</sub>O consumption takes place, these conditions are not linear. We – unfortunately – only figured this out after the final acceptance of the paper. However, it seems incorrect to leave this in, as the consequence of the violation of these assumptions are unclear. After internal discussion, a much more complex model including a two-source mixing model, but also a Rayleigh-type equation to simulate both gross N<sub>2</sub>O production and consumption would be needed to truly constrain the fluxes in a correct way. However, we do not have these data (we would need much more timepoints, and at least also <sup>15</sup>N-NO<sub>3</sub>, along with considerable analytical model development).

As stated before, by omitting these data, the message of the manuscript does not change and the discussion and thus, conclusions obtained still remain. However, a small discussion has been included to indicate the analytical advances needed to disentangle gross consumption and production of N<sub>2</sub>O (see L152-165 final version).

On the other hand, regarding the following comment of the editor:

*One very minor suggestion which can be addressed during the proofing stage: I suggest to split the sentence L121-125 in two parts, e.g. by starting a new sentence on L124 (“Therefore, the observed...”). Right now, the sentence is ~80 words long, which seems a bit much of a good thing.*

L121-125 has been revised and shortened.

An updated version of the manuscript and supplementary information with track changes is added below:

# Ideas and perspectives: patterns of soil CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes along an altitudinal gradient - a pilot study from an Ecuadorian Neotropical montane forest

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## Abstract

20 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<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) 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  
25 campaign, our measurements showed 1) an unusual but marked increase of CO<sub>2</sub> emissions at high altitude, possibly linked to changes in soil pH and/or root biomass; 2) a consistent atmospheric CH<sub>4</sub> sink over all altitudes with high temporal and spatial variability; and 3) a transition from net N<sub>2</sub>O source to sink along the altitudinal gradient. 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  
30 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

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microclimate, and temporal (seasonality) and spatial (topographic positions) variability are needed, this in order to obtain more reliable estimates of the CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O source/sink strength of tropical montane forests.

### 1 The importance of tropical forests for GHG budgets

40 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<sub>2</sub>O, with an estimated contribution of 14-23% to the annual, global N<sub>2</sub>O budget (Werner et al., 2007). In general, soil CO<sub>2</sub> is produced mainly by root respiration, microbial respiration, litter decomposition, and oxidation of soil organic matter (Dalal and Allen, 2008). CH<sub>4</sub> is consumed by methanotrophic bacteria (Jang et al., 2006), however, forest soils prone to inundation emit CH<sub>4</sub> by methanogenic microorganisms (*Archaea* domain). N<sub>2</sub>O 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<sub>2</sub>-C ha<sup>-1</sup>y<sup>-1</sup> (heterotrophic and autotrophic respiration), slightly less than the net primary productivity (NPP) (12.5 t CO<sub>2</sub>-C ha<sup>-1</sup>y<sup>-1</sup>), i.e. the net C sink (below and aboveground) of tropical forests is ~ 0.4 t CO<sub>2</sub>-C ha<sup>-1</sup>y<sup>-1</sup> (Dalal and Allen, 2008; Grace et al., 2006). Under aerobic conditions, CH<sub>4</sub> fluxes vary from -0.7 to -30.0 kg CH<sub>4</sub>-C ha<sup>-1</sup>y<sup>-1</sup>, with an average consumption of -3.0 kg CH<sub>4</sub>-C ha<sup>-1</sup>y<sup>-1</sup>, while the mean rate of N<sub>2</sub>O emissions from tropical forest soils is 3.03±0.52 kg N<sub>2</sub>O-N ha<sup>-1</sup>y<sup>-1</sup> (Dalal and Allen, 2008), i.e. 2-3 times higher than the mean N<sub>2</sub>O emissions from temperate forest soils (1.0±0.36 kg N<sub>2</sub>O-N ha<sup>-1</sup>y<sup>-1</sup>; Chapui-Lardy et al., 2007; Van Groenigen et al., 2015).

55 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<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O 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<sub>4</sub> and N<sub>2</sub>O, 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, 65 quantitatively important in regional and global GHG budgets.

## 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).

To address these knowledge gaps, we present a pilot study of the soil-atmosphere exchange of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O 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 6<sup>th</sup> to September 28<sup>th</sup>, 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. Samples of soil were collected once during the whole field campaign for analysis of bulk density ( $\rho_b$ ), pH, nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) content, C and nitrogen (N) concentrations, stable N isotope signatures ( $\delta^{15}\text{N}$ ) and soil texture. Additionally, soil moisture (expressed as water-filled pore space (WFPS)) and soil temperature were measured daily. Specifically, we aimed to determine the magnitude of the soil-atmosphere exchange of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O during the dry season. 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.

## 3 What did we see in Ecuador?

Across our study sites, P\_3010 (the highest stratum) exhibited the highest soil CO<sub>2</sub> 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<sub>water</sub> 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<sub>2</sub> emissions with decreasing pH<sub>water</sub>. On the other hand, although not measured nor estimated in this study, an increase in fine root biomass

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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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>4</sub> (Fig. 1b and Table 1) (i.e. uptake of atmospheric CH<sub>4</sub> 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<sub>4</sub> (43.2 μg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup>). 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<sub>2</sub>O (Fig. 1c and Table 1). Whereas the plots located at the highest stratum (P\_3010 & C\_2200) showed a general net N<sub>2</sub>O consumption during August and September.

The N<sub>2</sub>O 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 the soil water content, temperature, and N availability observed at these sites (Fig. S2, S3 and Table 2). Firstly, N<sub>2</sub>O emissions in tropical forest soils are predominantly governed by WFPS, which influences microbial activity, soil aeration, and thus diffusion of N<sub>2</sub>O out of the soil (Davidson et al., 2006; Werner et al., 2007). Secondly, 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<sub>2</sub>O emissions. In fact, rising temperatures lead to a positive feedback in microbial metabolism, where the stimulation of mineralization and nitrification processes induces an increase in the availability of substrates for denitrification, and thus to an increase in N<sub>2</sub>O emissions (Butterbach-Bahl et al., 2013; Sousa Neto et al., 2011). Finally, the dependency of N<sub>2</sub>O emissions on WFPS and temperature is affected by substrate availability (NO<sub>3</sub><sup>-</sup>). High contents of NO<sub>3</sub><sup>-</sup> give an indication of an open or “leaky” N cycle with higher rates of mineralization, nitrification, and thus N<sub>2</sub>O emissions (Davidson et al., 2006). Moreover, NO<sub>3</sub><sup>-</sup> is normally preferred as an electron acceptor over N<sub>2</sub>O and it can also inhibit the rate of N<sub>2</sub>O consumption to N<sub>2</sub> (Dalal and Allen, 2008).

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140 In contrast to the low elevation sites where net N<sub>2</sub>O 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<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>), whereas from 36 valid measurements, 19 resulted  
in net emissions at C\_2200 (range: -104.9 to 9.3 μg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>). Net N<sub>2</sub>O consumption is often related to N-limited  
ecosystems and it is presumably the cause in our case. At low NO<sub>3</sub><sup>-</sup> concentrations, atmospheric and/or soil gaseous N<sub>2</sub>O may  
145 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<sub>3</sub><sup>-</sup>, along with the lowest soil δ<sup>15</sup>N (Table 2), which clearly reflects the shift towards a more closed N cycle at  
higher elevations (Bauters et al., 2017; Gerschlaier 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<sub>2</sub>O emissions and thus the  
potential for N<sub>2</sub> production in soils at high elevations to differences in NO<sub>3</sub><sup>-</sup> availability. Moreover, Wolf et al. (2011) and  
150 Martinson et al. (2013) have indicated that N availability was 1) a dominant control on N<sub>2</sub>O fluxes and 2) inversely proportional  
to altitude. In addition, the low N<sub>2</sub>O fluxes could also be supported by the high content of clay (Table 2) and CO<sub>2</sub> emissions  
(Fig. 1a) (i.e. development of microsites for N<sub>2</sub>O reduction), along with the low soil water content (% of WFPS) (Fig. S3) (i.e.  
better diffusion of atmospheric N<sub>2</sub>O 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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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,  
160 the net N<sub>2</sub>O consumption with increasing altitude might be overlooked in an annual analysis, but equally important to 1)  
understand the mechanisms behind the production and consumption of N<sub>2</sub>O, and 2) have reliable estimates of the N<sub>2</sub>O  
source/sink strength of tropical forests for regional and even global GHG budgets. Moreover, in order to corroborate the net  
consumption observed at high altitudes and improve the understanding of N<sub>2</sub>O dynamics in terrestrial ecosystems,  
disentangling gross N<sub>2</sub>O production and consumption at field scale is needed. Although the most common used method to  
165 measure N<sub>2</sub>O fluxes via static chambers only allows the quantification of net fluxes, stable isotope techniques would greatly  
contribute to our mechanistic understanding of gross fluxes. For instance, enrichment and natural abundance approaches (<sup>18</sup>O,  
<sup>15</sup>N) can be used to identify and estimate the contribution of different microbial processes to N<sub>2</sub>O production/consumption  
(Butterbach-Bahl et al., 2013; Yu et al., 2020). Nevertheless, 1) the coupling of isotope techniques with molecular analyses of  
functional genes is paramount to fully understand the complexity of the microbial processes present, and 2) the improvement  
170 of measuring techniques for N<sub>2</sub>O reduction is needed to close N ecosystem balances (Butterbach-Bahl et al., 2013; Chapui-  
Lardy et al., 2007). In fact, microbial composition and diversity, as well as the presence or absence of important genes (e.g.  
N<sub>2</sub>O reductase *nosZ I* and *nosZ II* (Van Groenigen et al., 2015b)) can help to detect N<sub>2</sub>O consumption. Similarly, analytical

**Deleted:** Besides the soil isotope signatures, the bulk N<sub>2</sub>O isotope signatures (Fig 2) support the net N<sub>2</sub>O consumption at higher altitudes, and net N<sub>2</sub>O 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<sub>2</sub>O to N<sub>2</sub>, N<sub>2</sub>O-reductase fractionates against <sup>15</sup>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<sub>2</sub>O, leads to a <sup>15</sup>N enrichment of the residual N<sub>2</sub>O, and thus to higher δ<sup>15</sup>N<sub>Bulk</sub> values (Denk et al., 2017; Park et al., 2011) relative to the atmospheric bulk N<sub>2</sub>O composition (6.3‰ (Harris et al., 2017)). This in fact is reflected in the relatively enriched δ<sup>15</sup>N<sub>Bulk</sub> values measured during N<sub>2</sub>O consumption, while a relative depletion was observed during N<sub>2</sub>O 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 δ<sup>15</sup>N<sub>Bulk</sub> values between -22 and 2‰ in natural tropical forest soils to denitrification. ¶

techniques such as Raman gas spectroscopy could be used to detect and quantify N<sub>2</sub> fluxes from denitrification (Frosch et al., 2016), which is indeed a novel and simple approach compared to previously widely used techniques that may have led to underestimations (Fang et al., 2015).

#### 4 Conclusions and future directions

195 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<sub>2</sub> emissions at the highest altitude; probably explained by soil pH and root biomass, even  
though the latter was not measured nor estimated. Our CH<sub>4</sub> uptake fluxes exhibited a high temporal and spatial variability but  
reiterate the role of humid tropical forest soils as CH<sub>4</sub> sinks. Contrary to the net N<sub>2</sub>O emissions observed in the lowest strata,  
200 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. Our results highlight the importance of short-term variations in N<sub>2</sub>O  
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 implementation of isotope techniques, the coupling of microbial  
205 analysis with N<sub>2</sub>O 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; Jauhainen 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<sub>2</sub>O 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<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes (Koehler et al., 2012). Besides this, the effects of climate change in tropical regions (e.g. increases in temperature and CO<sub>2</sub> 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

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.

## 235 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 ( $^{\circ}\text{C}$ ) $\pm$ standard deviations (SD).

240 **Fig. S3.** Monthly average water-filled pore space (WFPS) $\pm$ standard deviations (SD).

**Table S1.** Measured and estimated  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  fluxes from tropical forest soils of South America.

**Table S2.** Characteristics of the study areas.

### 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  
245 [M. Barthel](#) and [S.B](#) provided technical and analytical support analyzing the gas and soil samples. 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<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O 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 <sub>2</sub> flux (mg C m <sup>-2</sup> h <sup>-1</sup> )	Average CH <sub>4</sub> flux (µg C m <sup>-2</sup> h <sup>-1</sup> )	Average N <sub>2</sub> O flux (µg N m <sup>-2</sup> h <sup>-1</sup> )
<b>August</b>	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
<b>September</b>	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

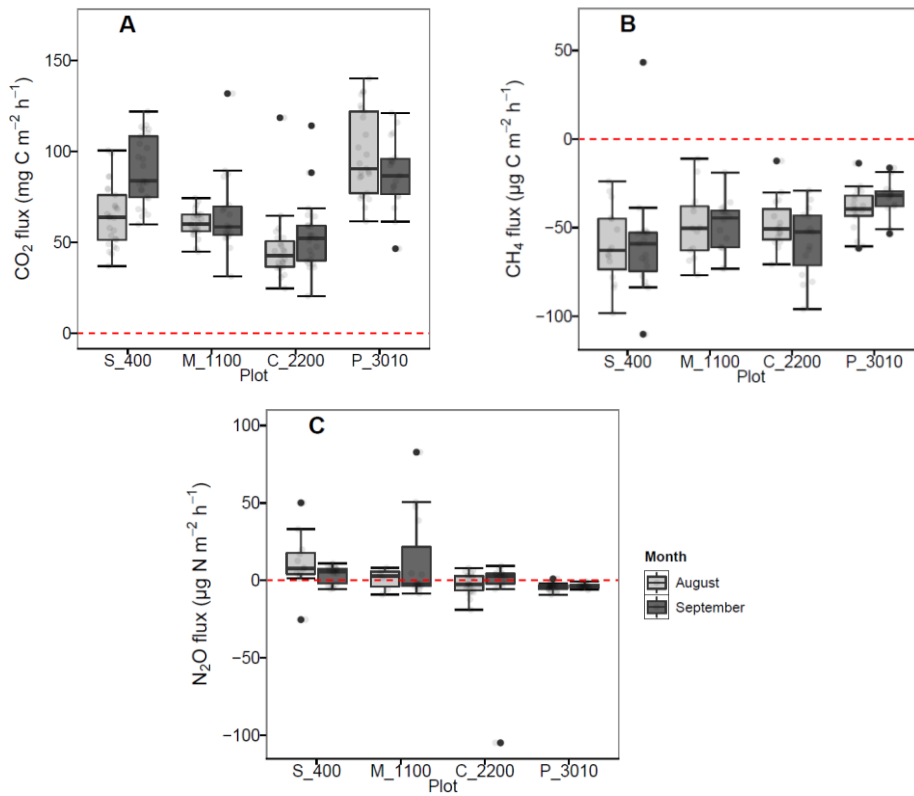
Note: flux values represent the mean of 5 chambers per site and measurement week, using four-point time series and considering the constraint set to evaluate linearity in each measurement cycle ( $R^2 > 0.65$ ).

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

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.

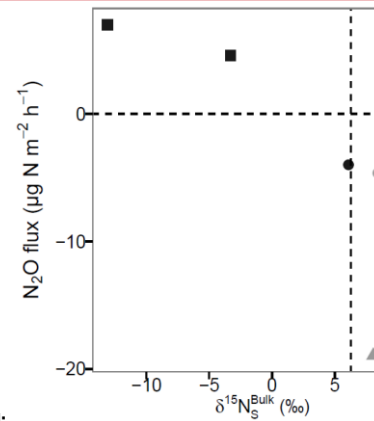
<sup>1</sup>Commonly known as *Andisol* in the USDA Soil Taxonomy; <sup>2</sup>expressed per gram of dry soil; <sup>3</sup>calculated by dividing C (%) by N (%) in each soil sample; and <sup>4</sup>expressed relative to the international standard AIR.



5 **Fig. 1.** A) Soil CO<sub>2</sub> (mg C m<sup>-2</sup> h<sup>-1</sup>), B) CH<sub>4</sub> (µg C m<sup>-2</sup> h<sup>-1</sup>) and C) N<sub>2</sub>O (µg N m<sup>-2</sup> h<sup>-1</sup>) 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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## Supplementary information

### 1 Materials and methods

#### 1.1 Study areas

The fieldwork was carried out along an altitudinal gradient from lowland (400 m a.s.l.) to upper montane evergreen forests (3010 m a.s.l.; Table S2). We selected areas of well-preserved natural forests located on the western flanks of the Andes in northern Ecuador; specifically, in the Sierra region in the provinces of Imbabura and Pichincha. 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). All sites experience two rainy seasons (March-April and October-November), with a mean annual precipitation (MAP) that varies on average between 900 and 3600 mm, and an adiabatic lapse rate of approx.  $-5^{\circ}\text{C}$  per 1000 m of altitude (Table S2) (Varela and Ron, 2018).

#### 1.2 Sampling strategy

The sampling campaign took place from August 6<sup>th</sup> to September 28<sup>th</sup>, 2018, corresponding to the end of the dry season. One plot (20x20 m) was selected for each site, and within each plot, five polyvinyl chloride (PVC) collars were installed to allow *in-situ* measurements using a static flux chamber method. The collars were inserted at random locations within the plots but guaranteeing at least 7 m distance between each one. The insertion of the collars was performed at least 12 h before the first measurements, by applying even pressure across all points to minimize effects caused by soil disturbance. The chambers consisted of a PVC pipe hermetically sealed on top with a rubber-sealed lid. The chamber area was  $0.0191\text{ m}^2$  and the internal volume ranged between 3.63 and 3.98 L. Each chamber was equipped with sampling ports mounted with three-way valves, and a vent tube was installed to reduce pressure interferences.

Gas samples were collected mid-morning to avoid extreme temperatures and consider them as representative of a whole day (Collier et al., 2014; Luo and Zhou, 2006). Measurement cycles on each site consisted of four consecutive gas measurements once per day for one hour and during five contiguous days. For these measurements, the collars were left in place for the duration of each measurement cycle; thus, the analysis per stratum lasted 1 week (i.e. 1 month for all measurements in the 4 strata). However, in order to assess both short-term and long-term variations mainly related to weather conditions, the gas measurements were done first in August and consequently repeated in the next month (September).

Adjacent to each chamber ( $\sim 1\text{ m}$ ), one pit was dug for soil sampling, and intact soil cores were collected using stainless steel cylinders (diameter: 5.08 cm, height: 5.11 cm). The samples were taken at 5 and 20 cm depth once during the first month (August) of measurements. Each soil core was immediately packed into airtight zip-lock bags and once the sampling campaign was over, they were sent to Belgium (Ghent University) for physicochemical soil analysis. Bulk density ( $\rho_b$ ) was measured by oven drying ( $75^{\circ}\text{C}$  for 48 h) and weighing the soil samples. Soil porosity was derived from Eq. (1), assuming a particle density of  $2.65\text{ g cm}^{-3}$ . pH was measured by a potentiometric method using a pH-sensitive glass electrode, a standard reference electrode (HI 4222; Hanna Instruments, Bedfordshire, UK), and a volumetric ratio soil:liquid of 1:5 for  $\text{pH}_{\text{water}}$  (distilled water) and  $\text{pH}_{\text{KCl}}$  (1M KCl).  $\text{NO}_3^-$  and  $\text{NH}_4^+$  content was determined colorimetrically (Auto Analyzer 3; Bran and Luebbe, Norderstedt, Germany) after extractions performed with 1M KCl at room temperature and neutral pH. C and N concentrations (%C, %N), along with the stable N isotope signatures ( $\delta^{15}\text{N}$ ) of the soil samples, were determined at natural abundance by a Continuous Flow Element Analyzer (Automated Nitrogen Carbon Analyzer), interfaced with an Isotope-Ratio Mass Spectrometer (Sercon 20-20; Sercon, Cheshire, UK). Moreover, the soil samples taken at 5 and 20 cm depth were combined to produce one composite sample associated with each site, and by means of the method described by the International

Organization for Standardization (ISO 11277:2009), soil texture was determined. The classification of the soil was made according to the United States Department of Agriculture (USDA, 2017); and the soil class was determined based on the classification of FAO and UNESCO: World Reference Base for Soil Resources (WRB) (FAO, 2007).

$$Porosity [\%] = \left(1 - \frac{\rho_b [g\ cm^{-3}]}{2.65 [g\ cm^{-3}]}\right) \cdot 100\% \quad (1)$$

45 Daily measurements of soil moisture, expressed as water-filled pore space (WFPS), were taken per site at 5 and 20 cm depth using soil moisture sensors (EC-5, Meter Environment, Pullman, Washington, USA) and data loggers (ProCheck, Meter Environment, Pullman, Washington, USA). Finally, soil temperature was determined daily for each measurement cycle and per chamber, by means of a thermometer inserted at 5 cm depth and approximately 10 cm from each chamber.

### 1.2.1 Soil-atmosphere exchange

50 Each day, the chambers were closed for a period of 1 h. Samples of 20 mL were taken with disposable syringes from the headspace air every 20 minutes:  $T_1 = 0$ ,  $T_2 = 20$ ,  $T_3 = 40$  and  $T_4 = 60$  min;  $T_1$  or time-zero indicates the sample taken immediately after the chamber was closed. Before each sample collection, the syringe was flushed twice with the air of the chamber to mix the chamber headspace and to avoid any possible stratification of gases.

55 The 20 mL samples were injected in pre-evacuated 12 mL exetainer vials (over-pressurized), and once the sampling campaign was over, the samples were sent to Belgium (Ghent University) for gas chromatography analysis. For  $CH_4$  and  $CO_2$  analysis, a gas chromatograph (Finnigan Trace GC Ultra; Thermo Electron Corporation, Milan, Italy) equipped with a flame ionization detector (FID) and a thermal conductivity detector (TCD) was used, respectively. For  $N_2O$ , another gas chromatograph equipped with an electron capture detector (ECD) (Shimadzu GC-14B; Shimadzu Corporation, Tokyo, Japan) was used.

### 60 1.3 Data analysis

The fluxes for each gas ( $CO_2$ ,  $CH_4$  and  $N_2O$ ) were calculated by means of linear regressions using the four consecutive measurements of each measurement cycle. The slope of the regressions represented the flux. Thus, following the ideal gas law, and considering the headspace volume of the chamber and the chamber area, the net gas flux was calculated by Eq. (2) (Collier et al., 2014; Dalal et al., 2008; Kutzbach et al., 2007):

$$F_c = \left(\frac{\Delta c [ppm]}{\Delta t [min]}\right) \cdot \left(\frac{P [atm]}{R [L\ atm\ mol^{-1}\ K^{-1}] \cdot T [K]}\right) \cdot (MW [g\ mol^{-1}]) \cdot \left(\frac{V_{ch} [L]}{A_{ch} [m^2]}\right) \quad (2)$$

65 where  $F_c$  corresponds to the net gas flux ( $CO_2$ ,  $CH_4$  or  $N_2O$ ),  $\Delta c/\Delta t$  is the rate of change of the gas concentration within the chamber or the slope of the regression line [ $ppm\ min^{-1}$  or  $\mu L\ L^{-1}\ min^{-1}$ ],  $P/RT$  corresponds to the ideal gas law used to convert concentration from volumetric to mass fraction at local temperature and pressure,  $R$  = gas law constant ( $0.08206\ L\ atm\ mol^{-1}\ K^{-1}$ );  $MW$  is the molecular weight of the gas ( $CO_2$ -C and  $CH_4$ -C:  $12.01\ g\ mol^{-1}$ ,  $N_2O$ -N:  $14.01\ g\ mol^{-1}$ ),  $V_{ch}$  is the headspace volume of the chamber, and  $A_{ch}$  the area of the chamber. The goodness-of-fit was evaluated for every linear regression using the adjusted coefficient of determination ( $R^2$ ), and time series (concentration vs time) with  $R^2 < 0.65$  were excluded from further analysis.

#### Deleted: 1.2.2 $N_2O$ bulk isotopic composition¶

Two extra gas samples were taken for stable isotope analysis at the start ( $T_1$ ) and at the end ( $T_4$ ) of a chamber closure. These samples were taken only once per site and in only one of the chambers. For this, in addition to the small exetainer vials, pre-evacuated big serum vials (110 mL) were used to inject gas samples of 180 mL (over-pressured). At the end of the field campaign, the samples were transported to Switzerland (ETH Zurich) and analyzed for bulk  $^{15}N$  of  $N_2O$  ( $\delta^{15}N^{bulk}$ ) using a gas preparation unit (Trace Gas, Elementar, Manchester, UK) coupled to an Isotope Ratio Mass Spectrometer (IRMS) (IsoPrime100, Elementar, Manchester, UK). For measurement and calibration details see Verhoeven et al. (2019). ¶

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The soil isotopic signature of  $N_2O$  was calculated using a two-source mixing model, based on the conservation of mass depicted in Eq. (3). The atmospheric concentration of  $N_2O$  ( $C_a$ ) reflects the background atmospheric concentration of the gas ( $C_b$ ), plus the amount added by the source(s) ( $C_s$ ); including the isotope ratios of each component (4), the soil isotopic signature of  $N_2O$  - or - can be calculated by Eq. (5). However, due to the nature of the data (very low  $N_2O$  concentrations), a minimum concentration difference of 20 ppb was defined as a threshold to remove super-low fluxes and thus, avoid larger uncertainties in the source calculation (Harris et al., 2020). ¶

$$\begin{aligned} &= + \quad (3)¶ \\ &= + \quad (4)¶ \\ &= \quad (5)¶ \end{aligned}$$

## 2. Results

### 105 2.1 Physicochemical soil properties

All soils along the altitudinal gradient are Andosols and the soil texture was classified (USDA) between loam and sandy loam at all sites (WRB; Table 2). All sites had a relatively acidic soil;  $\text{pH}_{\text{water}}$  ranged from strong to medium acidic (4.6 - 5.7), with an increase in acidity with depth, except at P\_3010 (Table 2). The most acidic soil was found at S\_400 at 5 cm, although not significantly different from M\_1100 and C\_2200; whereas the least acidic one at P\_3010 at 5 cm depth, and only significantly different from M\_1100. Except for P\_3010,  $\text{NO}_3\text{-N}$  concentrations were 2-4 times higher at 5 cm compared to 20 cm depth; the highest variability was observed at S\_400, and in comparison to the other sites, P\_3010 seems to be depleted in  $\text{NO}_3\text{-N}$  at both depths ( $0.8 - 3.6 \mu\text{g g}^{-1}$ ). In contrast, the highest concentration of  $\text{NH}_4\text{-N}$  was obtained at P\_3010 at 20 cm, followed by S\_400 at 5 cm. However, at all sites,  $\text{NH}_4\text{-N}$  concentrations at 5 cm were not significantly different from each other. Such as  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  also decreased with depth, except at P\_3010 where the increase at 20 cm with respect to 5 cm was almost doubling. Higher N contents were measured at 5 cm compared to 20 cm depth at all sites; and S\_400 exhibited the highest content at both depths, 1.3-1.4 times higher than any other N percentage at the same depth, and even 4 times higher than any other N percentage at 20 cm depth. The C content showed a general decrease with depth at all sites, with the highest percentage at S\_400 at 5 cm, and the lowest one at M\_1100 at 20 cm. Higher  $\delta^{15}\text{N}$  signatures were obtained at 20 cm compared to 5 cm depth; at S\_400 the soil was most enriched in  $^{15}\text{N}$ , and P\_3010 showed the most depleted one.

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Soil temperature decreased with altitude with a gradient of  $-4.2 \text{ }^\circ\text{C}$  per 1000 m, with no statistical difference between months (Fig. S2). WFPS increased significantly with depth at all sites during both months (Fig. S3), except at C\_2200 in September. The lowest WFPS at 5 cm depth was obtained at C\_2200 ( $16.8\% \pm 2.5$ ) and P\_3010 ( $14.4\% \pm 0.3$ ) in August and September, respectively, whereas the highest one at M\_1100 at 20 cm in both months (August:  $75.9\% \pm 0.3$ ; September:  $71.9\% \pm 6.3$ ).

### 125 2.2 Greenhouse gas fluxes

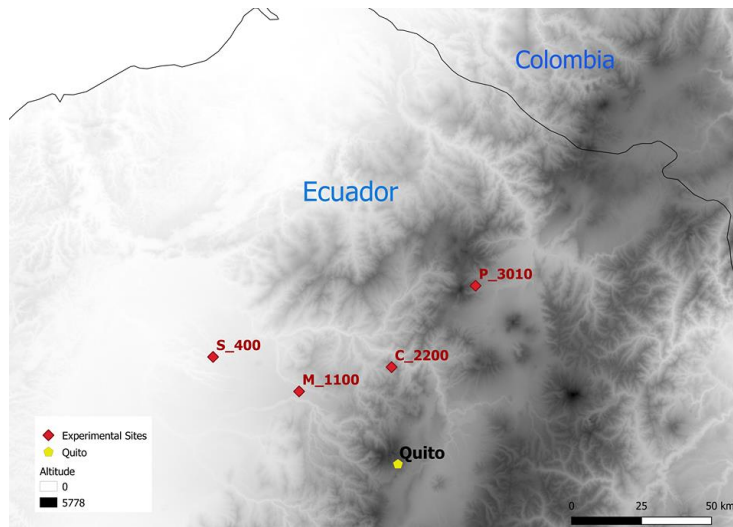
In general, all sites were sources of  $\text{CO}_2$  (Fig. 1a, Table 1). Except for P\_3010, mean  $\text{CO}_2$  emissions were higher in September compared to August, but due to the high variability in the measurements, there was no significant difference between months at M\_1100, C\_2200 and P\_3010 ( $P > 0.05$ ). The lowest and highest emissions were observed at C\_2200 (August and September) and P\_3010 (August), respectively. All mean  $\text{CH}_4$  fluxes were negative, indicating a net flux from the atmosphere to the soil (Fig. 1b, Table 1). Although the mean  $\text{CH}_4$  fluxes (except for P\_3010) were higher in September compared to August, there was no significant difference ( $P > 0.05$ ) between months at any site. Finally, the mean  $\text{N}_2\text{O}$  fluxes showed a general negative trend with increasing altitude (Fig. 1c). A marked net  $\text{N}_2\text{O}$  consumption was observed at the sites located at 2200 and 3010 m a.s.l.; however, there was no significant difference ( $P > 0.05$ ) in any plot between months. The highest consumption was observed in September at C\_2200, followed by P\_3010 in August, while the highest emission was in September at M\_1100 (Table 1).

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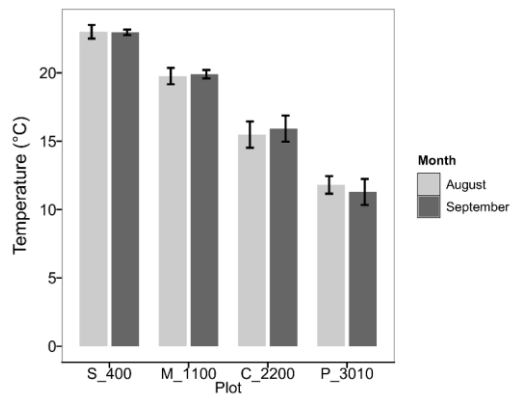
Although only monthly average fluxes were discussed, the large variability observed with most of the gas fluxes (Table 1 and Fig. 1) are the result of the spatial (i.e. differences in GHG fluxes between chambers) and temporal (i.e. differences in GHG fluxes per day) variability within each site.

140

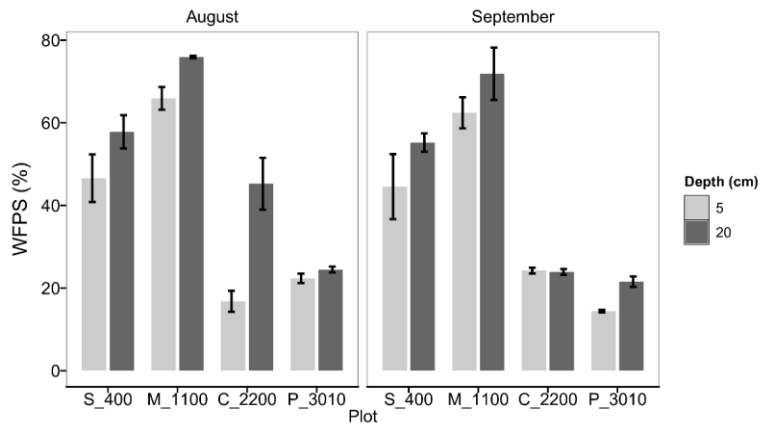
**Deleted: 2.3 Isotopic signature of  $\text{N}_2\text{O}$**  ¶  
ranged from  $-13.08$  to  $11.54\%$ , with the lowest and highest isotopic signature observed at S\_400 (September) (Fig. 2, Table S3.). During both months, values of M\_1100, C\_2200 and P\_3010 exhibited  $^{15}\text{N}$  enrichments relative to atmospheric  $\text{N}_2\text{O}$ , all of which reflecting chambers where negative fluxes were obtained, i.e. consumption of  $\text{N}_2\text{O}$  from the atmosphere to the soil (Table S3). ¶



**Fig. S1.** Overview map with the location 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). Study areas projected on a DEM based on SRTM data (Jarvis et al., 2008).



**Fig. S2.** Monthly average soil temperature (°C)±standard deviations (SD) 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 grey: average soil temperature in August; dark grey: average soil temperature in September.



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

165 Light grey: average WFPS at 5 cm, dark grey: average WFPS at 20 cm.

**Table S1.** Measured and estimated annual CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes from tropical forest soils of South America including elevation, mean annual precipitation (MAP), mean annual temperature (MAT) and period of measurement. N.R. stands for not reported.

Country	Elevation (m a.s.l.)	MAP (mm) MAT (°C)	Measured CO <sub>2</sub> flux (g CO <sub>2</sub> -C m <sup>-2</sup> h <sup>-1</sup> ) and period of measurement	Estimated annual CO <sub>2</sub> flux (Mg CO <sub>2</sub> - C ha <sup>-1</sup> y <sup>-1</sup> )	Measured CH <sub>4</sub> flux (µg CH <sub>4</sub> -C m <sup>-2</sup> h <sup>-1</sup> ) and period of measurement	Estimated annual CH <sub>4</sub> flux (kg CH <sub>4</sub> - C ha <sup>-1</sup> y <sup>-1</sup> )	Measured N <sub>2</sub> O flux (µg N <sub>2</sub> O-N m <sup>-2</sup> h <sup>-1</sup> ) and period of measurement	Estimated annual N <sub>2</sub> O flux (kg N <sub>2</sub> O-N ha <sup>-1</sup> y <sup>-1</sup> )	Reference
Brazil	50	2000 -	N.R.	N.R.	-11.5 April 1983	N.R.	43.6 April 1983	N.R.	(Keller et al., 1983)
Brazil	50	2000 -	N.R.	N.R.	0.7; -22.3 December 1983, and March 1984	N.R.	13.1; 31.0 December 1983, and March 1984	N.R.	(Keller et al., 1986)
Brazil	54	2200 -	N.R.	N.R.	N.R.	N.R.	15-35 April 1987-April 1988	1.9	(Luizão et al., 1989)
Brazil	130	1750 -	Dry season: 240±20 November 1992 <sup>3</sup> ; Wet season: 290±20 May 1992 <sup>3</sup>	N.R.	N.R.	N.R.	N.R.	N.R.	(Davidson and Trumbore, 1995)
Brazil	150	2200 25.5	N.R.	N.R.	-3.42 to -5.93 kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup> June 1992-December 1993 <sup>1</sup>	N.R.	N.R.	N.R.	(Steudler et al., 1996)
Brazil	130	1850 N.R.	N.R.	N.R.	N.R.	N.R.	<i>Fazenda Vitória – Primary forest</i> Dry season: 10.4±0.8 Wet season: 52.3±4 February 1995-May 1996	2.43	(Verchot et al., 1999)
							<i>Fazenda Vitória – Secondary forest</i> Dry season: 6.9±1.1 Wet season: 16.2±1.3 February 1995-May 1996	0.94	

			N.R.	N.R.	N.R.	N.R.	<i>Fazenda São José</i> 5.4±1.6 July 1996	N.R.	
			N.R.	N.R.	N.R.	N.R.	<i>Fazenda São Sebastião</i> 2.0±0.7 July 1996	N.R.	
<b>Brazil</b>	130	1800 N.R.	N.R.	20	N.R.	N.R.	N.R.	N.R.	(Davidson et al., 2000)
	130	1800 N.R.	N.R.	18	N.R.	N.R.	N.R.	N.R.	
<b>Brazil</b>	130	1850 N.R.	Dry season: 181±9 Wet season: 299±14 April 1995-May 1996	20	Dry season: -30.6±6.6 Wet season: 0.9±6.6 April 1995-May 1996	-1.6	N.R.	N.R.	(Verchot et al., 2000)
	130 (Secondary forest since 1976)	1850 N.R.	Dry season: 174±10 Wet season: 245±10 April 1995-May 1996	17.9	Dry season: -10.6±5.6 Wet season: -6.2±2.5 April 1995-May 1996	-0.7	N.R.	N.R.	
<b>Brazil</b>	145	2200 25.6	N.R.	N.R.	N.R.	N.R.	1.94±0.22 kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> June 1992-January 1996	N.R.	(Melillo et al., 2001)
<b>Brazil</b>	150	2270 18.8-25.6	N.R.	N.R.	N.R.	N.R.	Dry season: 1.3 August-September 1998 Wet season: 76.4, February-March 1998; 67.0 March 1999	N.R.	(Garcia-Montiel et al., 2001)
<b>Brazil</b>	150	2270 25.6	129.21± 31.93 November 2001	N.R.	N.R.	N.R.	13.13± 3.75 November 2001	N.R.	(Garcia-Montiel et al., 2003)
<b>Brazil</b>	200	2000 25	N.R. <sup>2</sup>	N.R.	N.R. <sup>2</sup>	N.R.	185 (clay soil), 15 (sandy soil) June-August 2000	N.R.	(Varner et al., 2003)
<b>Brazil</b>	150	2090-2270	<i>Nova Vida 1</i> 148.1±9.7	N.R.	N.R.	N.R.	<i>Nova Vida 1</i> 27.3±4.6	N.R.	



		18.8-25.6	1992-1999			1992-1999		
			<i>Nova Vida 2</i> 155.5±18.5 1992-1993	N.R.	N.R.	N.R.	<i>Nova Vida 2</i> 19.2±7.0 1992-1993	N.R.
			<i>Proto Velho</i> 163.92 October 1993 and March 1994	N.R.	N.R.	N.R.	<i>Proto Velho</i> 25.2 October 1993 and March 1994	N.R.
			<i>Jamari</i> 151.09 October 1993 and March 1994	N.R.	N.R.	N.R.	<i>Jamari</i> 34.5 October 1993 and March 1994	N.R.
			<i>Cacaulândia</i> 159.01 October 1993 and March 1994	N.R.	N.R.	N.R.	<i>Cacaulândia</i> 34.3 October 1993 and March 1994	N.R.
			<i>Ouro Preto</i> 144.7 October 1993 and March 1994	N.R.	N.R.	N.R.	<i>Ouro Preto</i> 30.5 October 1993 and March 1994	N.R.
			<i>Vilhena</i> 161.4 October 1993 and March 1994	N.R.	N.R.	N.R.	<i>Vilhena</i> 28.1 October 1993 and March 1994	N.R.
<b>Brazil</b>	200	2000 21 - 23	10.0±0.9 Mg CO <sub>2</sub> -C ha <sup>-1</sup> y <sup>-1</sup> September 1998-December 2002 <sup>1</sup>		-0.8±0.7 kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup> September 1998-December 2002 <sup>1</sup>		2.6±1.0 kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> September 1998-December 2002 <sup>1</sup>	(Davidson et al., 2004)
<b>Brazil</b>	200	2000 25	138.4±4.3 (clay soil), 160.0±8.6 (sandy soil) June 2000-May 2001	N.R.	-11.2±4.4 (clay soil), N.R. (sandy soil) June 2000-July 2011	N.R.	130±10 (clay soil), 14±2 (sandy soil) June 2000-July 2011	(Silver et al., 2005)
<b>Brazil</b>	200	2000 21 - 23	12.8±1.0 Mg CO <sub>2</sub> -C ha <sup>-1</sup> y <sup>-1</sup> September 1998-April 2005 <sup>1</sup>		-1.2±0.7 kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup> September 1998-April 2005 <sup>1</sup>		2.1±0.7 kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> September 1998-April 2005 <sup>1</sup>	(Davidson et al., 2008)
<b>Brazil</b>	100	3050	N.R.	13.3	-2.7±0.5 kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup>		3.4±0.4 kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup>	

(Garcia-Montiel et al., 2004)

		19.1 - 25.5			September 2006-August 2007 <sup>1</sup>		September 2006-August 2007 <sup>1</sup>		
	400	3050 19.1 - 25.5	N.R.	13.6	-4.9±8.0 kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup> September 2006-August 2007 <sup>1</sup>		0.9±0.1 kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> September 2006-August 2007 <sup>1</sup>		(Sousa Neto et al., 2011)
	1000	2300 19.1 - 25.5	N.R.	12.9	-4.4±0.3 kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup> September 2006-August 2007 <sup>1</sup>		0.8±0.2 kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> September 2006-August 2007 <sup>1</sup>		
<b>Ecuador</b>	400	4500 -	171.6 August 1984	N.R.	19.4 August 1984	N.R.	7.5 August 1984	N.R.	(Keller et al., 1986)
	400 (Secondary forest of 5-10 years old)	4500 -	117.0 August 1984	N.R.	-25.1 August 1984	N.R.	7.4 August 1984	N.R.	
<b>Ecuador</b>	1000	2230 19.4	N.R.	N.R.	N.R.	N.R.	0.2±0.1 (lower slope), 0.3±0.1 (mid-slope), 0.4±0.1 (ridge) kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		
	2000	1950 15.7	N.R.	N.R.	N.R.	N.R.	1.3±0.2 (lower slope), 0.3±0.1 (mid-slope), 0.1±0.1 (ridge) kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		(Wolf et al., 2011)
	3000	4500 9.4	N.R.	N.R.	N.R.	N.R.	1.1±0.1 (lower slope), 0.1±0.1 (mid-slope), -0.05±0.1 (ridge) kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		
<b>Ecuador</b>	1000	2230 19.4	10.3±0.8 (lower slope), 10.3±0.1 (mid-slope), 9.8±0.9 (ridge) Mg CO <sub>2</sub> -C ha <sup>-1</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		-5.5±0.7 (lower slope), -5.4±0.9 (mid-slope), -5.9±1.0 (ridge) kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		N.R.	N.R.	
	2000	1950 15.7	8.8±0.4 (lower slope), 7.6±0.6 (mid-slope), 6.7±0.7 (ridge) Mg CO <sub>2</sub> -C ha <sup>-1</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		-2.3±0.3 (lower slope), -4.3±0.9 (mid-slope), -2.7±0.3 (ridge) kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		N.R.	N.R.	(Wolf et al., 2012)
	3000	4500 9.4	6.4±0.4 (lower slope), 5.7±0.7 (mid-slope), 3.7±0.5 (ridge) Mg CO <sub>2</sub> -C ha <sup>-1</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		-0.6±1.2 (lower slope), -1.6±0.4 (mid-slope), -1.0±0.1 (ridge) kg CH <sub>4</sub> -C ha <sup>-1</sup> y <sup>-1</sup> May 2008-May 2009 <sup>1</sup>		N.R.	N.R.	
<b>Ecuador</b>	1000	2230 19.4	N.R.	N.R.	N.R.	N.R.	0.2±0.1 (2008), 0.5±0.1 (2009) kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> January 2008-September 2009 <sup>1</sup>		(Martinson et al., 2013)

	2000	1950 15.7	N.R.	N.R.	N.R.	N.R.	0.2±0.03 (2008), 0.1±0.2 (2009) kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> January 2008-September 2009		
	3000	4500 9.4	N.R.	N.R.	N.R.	N.R.	-0.03±0.1 (2008), -0.3±0.3 (2009) kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> January 2008-September 2009		
<b>Ecuador</b>	1000	2230 19.4	N.R.	N.R.	N.R.	N.R.	0.57±0.26 kg N <sub>2</sub> O-N ha <sup>-1</sup> y <sup>-1</sup> November 2010-August 2012 <sup>1</sup>		
	2000	1950 15.4	N.R.	N.R.	N.R.	N.R.	0.17±0.06 kg N <sub>2</sub> O-N ha <sup>-1</sup> y <sup>-1</sup> November 2010-August 2012 <sup>1</sup>	(Müller et al., 2015)	
	3000	4500 9.4	N.R.	N.R.	N.R.	N.R.	0.05±0.04 kg N <sub>2</sub> O-N ha <sup>-2</sup> y <sup>-1</sup> November 2010-August 2012 <sup>1</sup>		
<b>French Guiana</b>	49	2200 26	99.4 July-September 1994	N.R.	N.R.	N.R.	N.R.	N.R.	(Janssens et al., 1998)
<b>French Guiana</b>	30	2771.2±62 8.8 27.3±0.5	N.R.	N.R.	N.R.	N.R.	15.83±2.1 April 2010-April 2011	1.32 <sup>5</sup>	(Petitjean et al., 2015)
<b>French Guiana</b>	147-194	2990-3041 25.7	<i>Nouragues forest</i> Dry season: 92.6±34.3 (top hill), 89.9±37.8 (middle slope), 131.0±64.2 (bottom slope) October 2015 Wet season: 159±36.5 (top hill), 191.7±66.5 (middle slope), 188.8±50.2 (bottom slope) May 2016	N.R.	<i>Nouragues forest</i> Dry season: -64.0±69.7 (top hill), 6.6±237.9 (middle slope), -49.9±50.1 (bottom slope) October 2015 Wet season: -19.9±70.3 (top hill), 43.2±274.0 (middle slope), 9.4±64.9 (bottom slope) May 2016	N.R.	<i>Nouragues forest</i> Dry season: -20.4±15.0 (top hill), -20.1±17.4 (middle slope), -32.5±21.6 (bottom slope) October 2015 Wet season: -30.7±30.9 (top hill), -31.9±15.5 (middle slope), -55.4±47.6 (bottom slope) May 2016	N.R.	(Courtois et al., 2018)

			<i>Paracou forest</i> Dry season: 165.2±50.2 (top hill), 131.7±34.3 (middle slope), 189.6±96.8 (bottom slope) October 2015 Wet season: 161.6±62.9 (top hill), 138.3±88.7 (middle slope), 94.8±57.4 (bottom slope) May 2016	N.R.	<i>Paracou forest</i> Dry season: -44.0±139.7 (top hill), - 19.9±79.5 (middle slope), 6.6±103.7 (bottom slope) October 2015 Wet season: 3.7±40.1 (top hill), - 1.9±41.2 (middle slope), 23.9±34.6 (bottom slope) May 2016	N.R.	<i>Paracou forest</i> Dry season: -31.2±27.5 (top hill), -41.4±54.5 (middle slope), -35.7±21.8 (bottom slope) October 2015 Wet season: -49.7±49.8 (top hill), - 19.3±45.1 (middle slope), -18.0±70.9 (bottom slope) May 2016	N.R.	
<b>French Guiana</b>	40.	2929 <sup>4</sup> 27	Dry season: 99.6±7.9 Wet season: 111.3±5 May 2011-November 2014	N.R.	Dry season: -12.9±10.8 Wet season: -12.1±9.2 May 2011-November 2014	N.R.	Dry season: 8.3±2.1 Wet season: 12.9±1.7 May 2011-November 2014	N.R.	(Petitjean et al., 2019)
<b>Peru</b>	180	2200 26	N.R.	N.R.	-29.0 to -32.1 October 1997-October 1999	-2.6	8.1 to 18.8 October 1997-October 1999	0.80	(Palm et al., 2002)
	200	2700 26.4	Dry season: 0.19±0.01 <sup>6</sup> 2007	N.R.	N.R.	N.R.	N.R.	N.R.	
<b>Peru</b>	1000	3100 21.3	Dry season: 0.18±0.007 <sup>6</sup> 2007	N.R.	N.R.	N.R.	N.R.	N.R.	(Zimmermann et al., 2009)
	1500	2600 18.3	Dry season: 1.17±0.007 <sup>6</sup> 2007	N.R.	N.R.	N.R.	N.R.	N.R.	
	3030	1700 12.5	Dry season: 0.18±0.008 <sup>6</sup> 2007	N.R.	N.R.	N.R.	N.R.	N.R.	
<b>Peru</b>	600 - 1200	5318	N.R.	N.R.	Dry season: -13.3±4.6	-0.14±0.12	Dry season: 40.0±19.6	0.54±0.32	

		23.4				Wet season: 21.3±17.1 July 2011 – December 2011		Wet season: -6.3±17.9 July 2011 – December 2011			
	1200 - 2200	2631 18.8	N.R.	N.R.		Dry season: -35.0±2.9 Wet season: -20.4±5.4 December 2010 - December 2011	-0.69±0.09	Dry season: 40.8±9.6 Wet season: 6.7±5.4 December 2010 - December 2011	0.50±0.13	(Teh et al., 2014)	
	2200 - 3200	1706 12.5	N.R.	N.R.		Dry season: -50.8±1.7 Wet season: -22.5±4.6 December 2010 - December 2011	0.80±0.08	Dry season: 7.9±7.1 Wet season: 0.8±9.2 December 2010 - December 2011	0.12±0.13		
	1070 - 1088	5300 23.4		N.R.		Dry season: 216.7±12.5 Wet season: 212.5±12.5 July 2011-June 2013		Dry season: -8.3±4.2 Wet season: -4.2±4.2 July 2011-June 2013	N.R.	N.R.	
<b>Peru</b>	1532 - 1769	2600 18.8		N.R.		Dry season: 179.2±12.5 Wet season: 170.8±12.5 February 2011-June 2013		Dry season: -45.8±4.2 Wet season: -1.34±4.2 February 2011-June 2013	N.R.	N.R.	(Jones et al., 2016)
	2811 - 2962	1700 12.5		N.R.		Dry season: 210.8±12.5 Wet season: 166.7±12.5 February 2011-June 2013		Dry season: -66.7±4.2 Wet season: -45.8±4.2 February 2011-June 2013	N.R.	N.R.	

<sup>1</sup>Measured mean annual fluxes.

170 <sup>2</sup>Soil-atmosphere fluxes of CO<sub>2</sub> and CH<sub>4</sub> were measured weekly during June-August 2000, but average values are not reported.

<sup>3</sup>Measured CO<sub>2</sub> fluxes from the soil surface.

<sup>4</sup>Average of the annual rainfall measured at the experimental site during 4 years.

<sup>5</sup>Cumulative N<sub>2</sub>O fluxes from the 11/05/2010 to the 09/05/2011

<sup>6</sup>Values for undisturbed “native” soil. This study evaluated the climate dependence of heterotrophic soil respiration from a soil-translocation experiment. As a matter of comparison

175 with the others studies, only control cores are depicted, i.e. soil cores re-installed at the same site.

**Table S2.** Characteristics 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), including mean annual precipitations (MAP) and mean annual temperatures (MAT) extracted from the Worldclim data set, using average monthly data from 1970-2000 with a spatial resolution of ~1 km<sup>2</sup> (Fick and R.J. Hijmans, 2017). Forest classification has been done based on the system used by the country (FAO, 2017; Ministerio del Ambiente, 2015).

Study area	Forest type	Coordinates		Altitude (m a.s.l.)	MAP (mm)	MAT (°C)
		Latitude	Longitude			
<b>S_400</b>	Lowland evergreen forest of Choco	00°08'45.58'' N	79°08'34.22'' W	400	3633	23.0
<b>M_1100</b>	Andean foothill evergreen	00°02'07.17'' N	78°51'59.72'' W	1100	2856	21.1
<b>C_2200</b>	Andean montane evergreen	00°06'47.87'' N	78°34'10.88'' W	2200	1464	16.8
<b>P_3010</b>	Upper montane evergreen	00°22'27.35'' N	78°18'0.36'' W	3010	956	12.8

Note: the coordinates were taken at the center of the plots.

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**Deleted: Table S3.** values for the point samples taken 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) during August and September 2018. The last column indicates the N<sub>2</sub>O flux measured for the respective chamber ID. Note: 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.¶

**Chamber ID**

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