Ideas and perspectives: varying sources and sinks of CO_2, CH_4 and N_2O along an altitudinal gradient - a pilot study from an Ecuadorian Neotropical forest

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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 temporal (seasonality) and spatial (topographic positions) variability are needed 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, 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), while CH_4 is consumed by methanotrophic bacteria (Jang et al., 2006); however, forest soils prone to inundation (anaerobic) emit CH_4 by methanogenic microorganisms (*Archaea* domain). On the other hand, 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 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 of tropical forests is ~ 0.4 t CO_2-C $ha^{-1}y^{-1}$. 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).

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.

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 more severe in the tropics, and even though forests can be managed to mitigate climate change as well, it is important to understand and integrate the magnitude of their feedbacks in the Earth system for future climate projections (Bonan, 2008; Li et al., 2020).

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. 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). 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 confirm 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 budget 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_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}. 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 due to imbalances or limitations in resource (water and/or nutrients) availability at higher altitudes (Bauters et al., 2017; Leuschner et al., 2007). Consequently, the observed increase in CO_2 emissions at high altitude (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, only one chamber at one site (S_400) and a specific date (08/09/2018) showed a net source of CH_4 (45.8 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.

On the other hand, only S_400 (both months) and M_1100 (September) (i.e. plots located at the lower locations) acted as net sources of N_2O (Fig. 1c and Table 1). In contrast, the lowest N_2O flux was observed in the plot located at the highest stratum (P_3010) during August and September, which showed a general net consumption.

The high 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. 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). 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 presented the highest consumption (negative values, i.e. fluxes from the atmosphere to the soil), followed by C_2200 (Fig. 1c and Table 1). From 37 valid measurements only one resulted in net emission at P_3010 (range: -12.9 to 1.3 μg N_2O-N m^{-2} h^{-1}), whereas from 36 measurements, 20 resulted in net emissions at C_2200 (range: -11.1 to -0.3 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). 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. more 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).

Besides the soil isotope signatures, the bulk N_2O isotope signatures (Fig 2) confirm 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 (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 enriched $\delta^{15}N_s^{Bulk}$ values measured during N_2O consumption, while the opposite 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.

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, 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 trend of net N_2O 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_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. 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, the coupling of microbial analysis with N_2O fluxes, and the response of tropical forests to current and future changes in N content.

For instance, in terms of spatial variation, N_2O 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). 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 (e.g. bi-weekly) 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 collaboration with local communities (i.e. citizen science) could contribute in terms of data acquisition, 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 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.

Acknowledgments

This research has been supported by Ghent University, the VLIR-UOS South Initiative COFOREC (EC2018SIN223A103) and COFOREC II (EC2020SIN279A103). We also thank Mindo Cloud Forest Foundation, El Cedral Ecolodge and the Escuela Politécnica Nacional del Ecuador, for the logistic support in Ecuador. M. Barthel was supported through ETH Zurich core funding provided to Johan Six, and M. Bauters is funded as a postdoctoral fellow of the research Foundation – Flanders (FWO).

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Table 1. Average measurements±standard deviations (SD) of 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 (µg C m^{-2} h^{-1})	Average N_2O flux (µg N m^{-2} h^{-1})
August	S_400	68.4±18.2	-63.2±22.9	11.1±18.1
	M_1100	69.0±8.5	-55.6±22.3	-0.2±7.7
	C_2200	55.3±12.1	-57.5±22.9	-0.3±0.9
	P_3010	137.6±32.8	-55.5±17.6	-6.1±3.2
September	S_400	95.3±19.9	-63.4±31.7	3.7±6.1
	M_1100	74.6±24.0	-56.3±16.2	13.2±31.3
	C_2200	68.6±24.8	-74.4±25.0	-0.3±2.8
	P_3010	124.0±26.9	-46.7±14.7	-5.1±1.9

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 ($R^2 > 0.60$).

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 $\delta^{15}N$ 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}	$0.62\pm0.09^{a,b}$	0.86 ± 0.12^{a}	0.70 ± 0.11^{a}	0.92 ± 0.05^{a}	$0.62\pm0.09^{a,b}$	0.81 ± 0.06^{a}
Porosity (%)	83.8 ± 5.5^{a}	78.0 ± 2.5^{a}	$76.5\pm3.3^{a,b}$	67.7 ± 4.7^{b}	73.7 ± 4.1^{b}	65.4 ± 2.0^{b}	$76.7\pm3.4^{a,b}$	69.6 ± 2.1^{b}
pH_{water}	$4.6\pm0.7^{a,b}$	5.2 ± 0.5	4.6 ± 0.8^{b}	5.5 ± 0.4	$4.8\pm0.4^{a,b}$	4.8 ± 0.6	5.7 ± 0.5^{a}	5.6 ± 0.5
pH_{KCl}	4.4 ± 0.2^{b}	$4.9\pm0.3^{a,b}$	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}	$4.9\pm0.2^{a,b}$
NO_3-N (μg g^{-1})[2]	71.9 ± 39.5^{a}	35.7 ± 29.5^{a}	23.1 ± 15.9^{b}	$6.7\pm7.7^{a,b}$	$30.6\pm19.4^{a,b}$	$7.3\pm4.3^{a,b}$	0.8 ± 0.3^{b}	3.6 ± 7.1^{b}
NH_4-N (μg g^{-1})[2]	34.3 ± 14.8	$27.9\pm16.1^{a,b}$	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	$0.3\pm0.0^{a,b}$	0.6 ± 0.0	$0.4\pm0.2^{a,b}$
C (%)	8.9 ± 2.4	$4.0\pm1.0^{a,b}$	7.1 ± 1.8	2.4 ± 0.7^{b}	6.6 ± 1.7	$3.3\pm0.4^{a,b}$	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}
$\delta^{15}N$ (‰)[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.

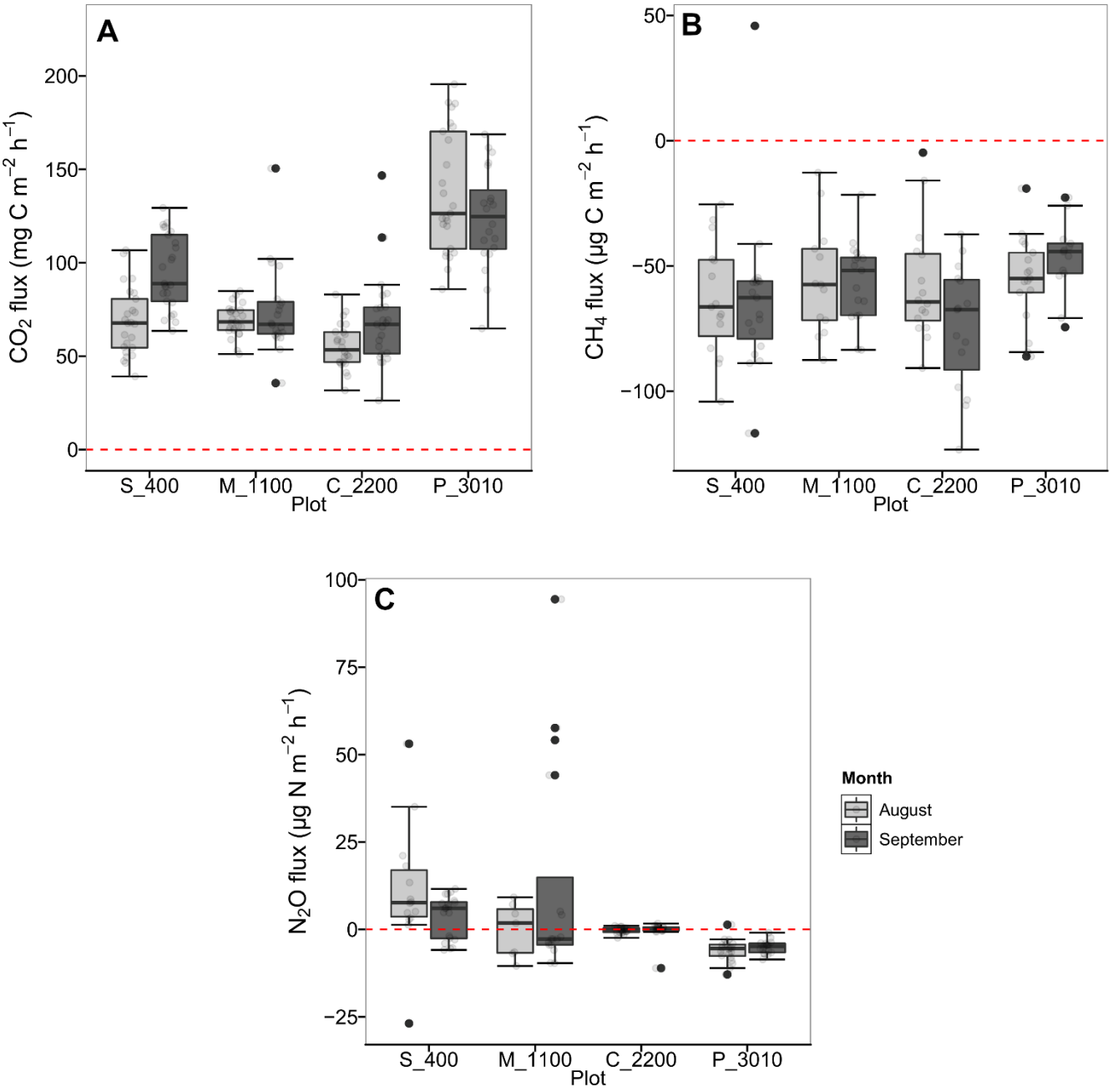

Fig. 1. A) CO_2 (mg C m^{-2} h^{-1}), B) CH_4 (µg C m^{-2} h^{-1}) and C) N_2O (µg N m^{-2} h^{-1}) fluxes per month at Río Silanche (400 m a.s.l.; S_400), Milpe (1100 m a.s.l.; M_1100), El Cedral (2200 m a.s.l.; C_2200) and Peribuela (3010 m a.s.l.; P_3010). Light gray boxplots indicate the fluxes in 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.

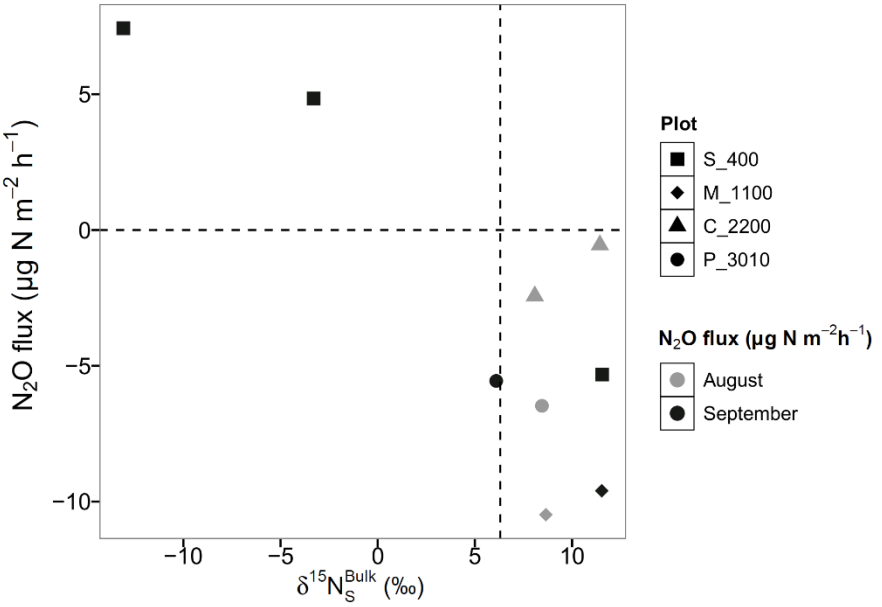

Fig. 2. N_2O fluxes plotted against the bulk isotopic signature of soil N_2O ($\delta^{15}N_S^{Bulk}$) for point samples taken at Río Silanche - squares (400 m a.s.l.; S_400), Milpe - diamonds (1100 m a.s.l.; M_1100), El Cedral - triangles (2200 m a.s.l.; C_2200) and Peribuela - circles (3010 m a.s.l.; P_3010) during August (grey) and September (black). Note: the dotted x axis 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.

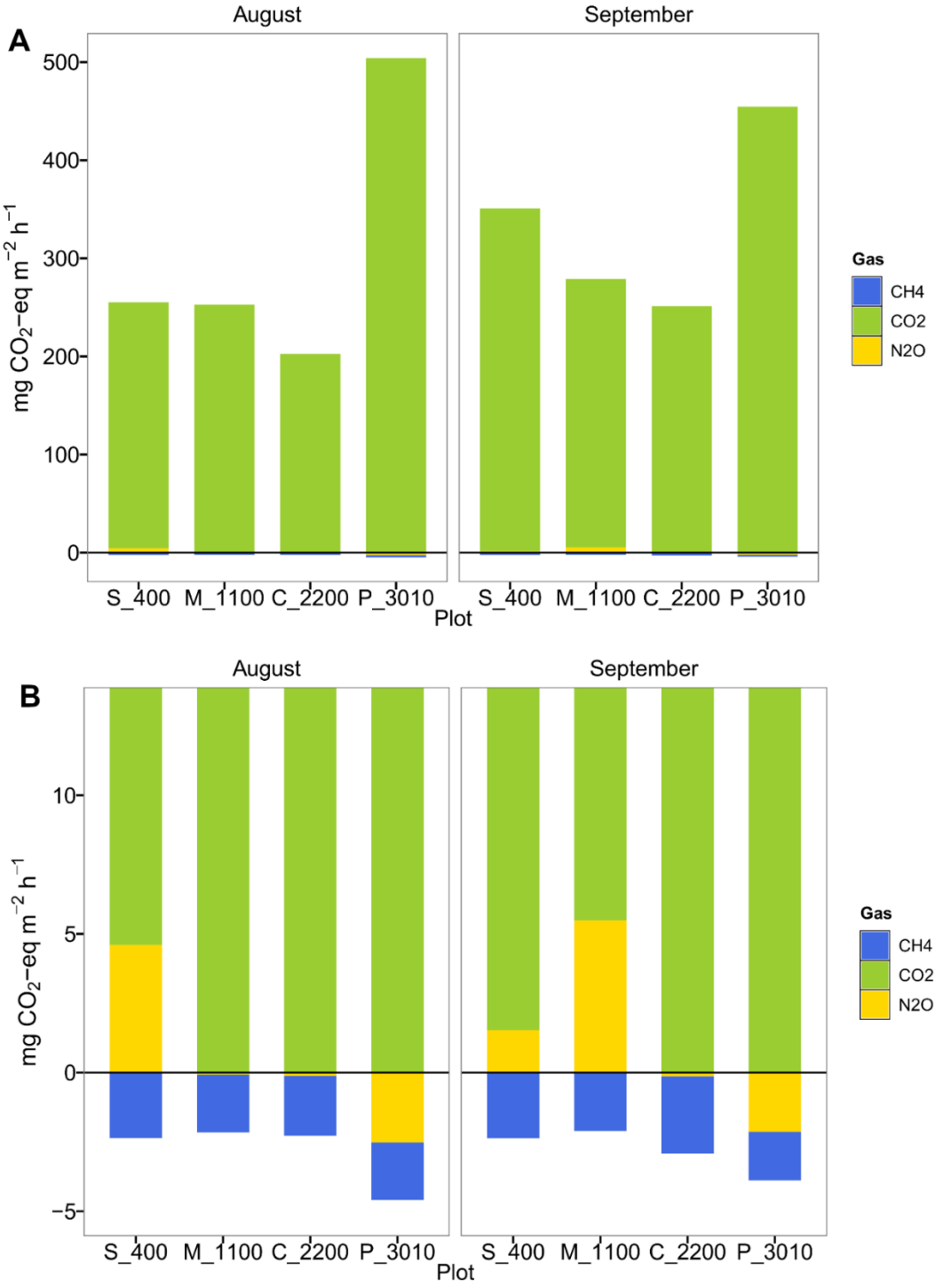

Fig. 3. CO_2 equivalent (CO_2-eq) emissions at Río Silanche (400 m a.s.l.; S_400), Milpe (1100 m a.s.l.; M_1100), El Cedral (2200 m a.s.l.; C_2200) and Peribuela (3010 m a.s.l.; P_3010) during August and September 2018, and expressed as mg CO_2-eq m^{-2} h^{-1}. Positive values indicate emission of GHGs whereas negative, consumption. Blue bars show the CO_2-eq emissions (-) of CH_4, green (+) of CO_2 and yellow (+ and -) of N_2O, using a global warming potential (GWP) of 1, 28 and 265, in each case and over a 100-year time horizon (Myhre et al., 2013). Figure B) is a zoom-in view of figure A).