



RESEARCH ARTICLE

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Nitrogen and Phosphorus Control Soil Methane Uptake in Tropical Montane Forests

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Key Points:

- Projected increases in nitrogen and phosphorus depositions in the tropics will stimulate soil methane uptake in tropical montane forests
- The direction, magnitude, and timing of nutrient deposition effects on soil methane uptake will depend on forests' nutrient status
- Nutrient limitations on ecosystem processes have to be investigated in actual field conditions

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract Tropical forests contribute about one third to global annual CH₄ uptake by soils. Understanding the factors that control the soil-atmosphere exchange of CH₄ at a large scale is a critical step to improve the CH₄ flux estimate for tropical soils, which is presently poorly constrained. Since tropical forest degradation often involves shifts in nutrient availabilities, it is critical to evaluate how this will affect soil CH₄ flux. Here, we report how nitrogen (N; 50 kg N ha⁻¹ yr⁻¹), phosphorus (P; 10 kg P ha⁻¹ yr⁻¹), and combined N + P additions affect soil CH₄ fluxes across an elevation gradient of tropical montane forests. We measured soil CH₄ fluxes in a nutrient application experiment at different elevations over a period of 5 years. Nutrient additions increased soil CH₄ uptake after 4–5 years of treatment but effects were not uniform across elevations. At 1,000 m, where total soil P was high, we detected mainly N limitation of soil CH₄ uptake. At 2,000 m, where total soil P was low, a strong P limitation of soil CH₄ uptake was observed. At 3,000 m, where total P was low in the organic layer but high in mineral soil, we found N limitation of soil CH₄ uptake. Our results show that projected increases of N and P depositions may increase soil CH₄ uptake in tropical montane forests but the direction, magnitude, and timing of the effects will depend on forests' nutrient status and plant-microbial competition for N and P.

Plain Language Summary CH₄ is a potent greenhouse gas that contributes to global warming. Tropical forests are a natural sink of CH₄ but increasing nutrient depositions due to industrialization may alter the sink strength of tropical forests. Our results show that projected increases of nitrogen and phosphorus depositions may increase soil CH₄ uptake in tropical montane forests but the direction, magnitude, and timing of the effects will depend on forests' nutrients and plant-microbial competition.

1. Introduction

Atmospheric concentrations of methane (CH₄), an important greenhouse gas, have more than doubled since pre-industrial times (Denman et al., 2007) and continue to increase at present (Turner et al., 2017, 2019). About 75% of global CH₄ production, which is in total about 600 Tg yr⁻¹, is produced by microbial methanogenic sources, primarily in wetland soils (Conrad, 1989). In contrast, the only global biogenic sink is CH₄ uptake in aerobic soils by CH₄-oxidizing bacteria, which perform an important ecosystem service by contributing about 5% to the total global CH₄ sink strength (Reeburgh, 2003). However, there are indications that this CH₄ sink is declining (Ni & Groffman, 2018).

Tropical ecosystems play an important role in the global CH₄ budget both in terms of production, with inundated wetlands contributing about 50% to global CH₄ production (Bloom et al., 2010; Z. Zhang et al., 2017), and uptake, with tropical forest soils contributing about one third to global annual CH₄ uptake by soils (Dutaur & Verchot, 2007; Zhao et al., 2019). However, studies that compare satellite observations of CH₄ concentrations with inventories of known CH₄ sources and sinks using bottom-up modeling reveal that there are unexplained large discrepancies between modeled and observed atmospheric CH₄ concentrations in the tropics (Riley et al., 2012). This illustrates that the location and strength of CH₄ sources and sinks in the tropics are poorly constrained (Bergamaschi et al., 2009; Bloom et al., 2010; Pangala et al., 2017). Improving our understanding of the factors that control the exchange of CH₄ in tropical soils is thus a critical step toward a better-constrained CH₄ budget for tropical ecosystems.

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In soils, CH₄ production by anaerobic methanogenic archaea, and CH₄ oxidation by CH₄-oxidizing bacteria, can occur simultaneously, and the relative magnitude of these two processes determines whether soils are net sources or sinks of CH₄ (Conrad, 1996; Yavitt et al., 1995). The microorganisms that are responsible for CH₄ oxidation are ammonium-oxidizing bacteria and methanotrophic bacteria. Both groups use the enzyme methane mono-oxygenase in the first step of CH₄ oxidation while methanotrophic bacteria use CH₄ as the sole carbon and energy source (Hanson & Hanson, 1996). Methanotrophic bacteria can be further grouped into Type I methanotrophs, which are generally non-N-fixing microorganisms, and Type II methanotrophs, which can fix N₂ but can also switch to mineral N assimilation as a more energetically favorable metabolism (Hanson & Hanson, 1996). In well-drained soils, CH₄ oxidation normally dominates because such soils typically support the so-called high affinity methanotrophic bacteria that oxidize CH₄ at concentrations that are near atmospheric (Conrad, 2007). CH₄ uptake in these soils is typically constrained by the rate of CH₄ diffusion from the atmosphere into the soil. This, in turn, is strongly influenced by the fraction of soil pores filled with water, since the rate of CH₄ diffusion in water is much lower than in air (Keller & Reiners, 1994; Veldkamp et al., 2013). In wet soils, the low rate of diffusional oxygen supply often leads to anaerobic microsites, where CH₄ production can occur. In these soils, high CH₄ concentrations are common and a different group of “low affinity” methanotrophic bacteria that typically settle at the interface of oxic and anoxic zones is responsible for CH₄ consumption (Conrad, 1996; Teh et al., 2005).

Since the diffusion rate of CH₄ and oxygen in soils strongly affects CH₄ production and consumption, water-filled pore space (WFPS) is often the dominant factor controlling seasonal variation in CH₄ fluxes at the soil surface (Keller & Reiners, 1994; Matson, Corre, Langs, & Veldkamp, 2017; Veldkamp et al., 2001). Soil texture, which indirectly influences WFPS, often explains variations across sites as was shown for tropical lowland and montane forest soils (Veldkamp et al., 2013). Apart from WFPS and soil texture, other soil-related factors, especially soil nutrient availability and enzyme substrate competition have been reported to control CH₄ exchange at the soil surface. For example, in many fertilized soils, methanotrophic activity is inhibited by high concentrations of inorganic nitrogen (N), especially ammonium (NH₄⁺). The proposed mechanism behind this observation is that NH₄⁺ and CH₄ compete for the same active sites of methane mono-oxygenase, the enzyme facilitating CH₄ oxidation (Bédard & Knowles, 1989; Steudler et al., 1989). Similarly, high contents of nitrate (NO₃⁻) in soils can inhibit methanogenesis, since NO₃⁻ is favored as an electron acceptor over bicarbonate (Conrad, 1989). Since methanotrophic bacteria need N for their metabolic activities, they can also be N-limited (Bender & Conrad, 1995), and accordingly N limitation of CH₄ uptake has been reported, mainly in unfertilized systems (Bodelier & Laanbroek, 2004). In response to increases in soil mineral N, Type II methanotrophs can reduce their N₂-fixing activities in favor of soil mineral N assimilation since this is an energetically beneficial metabolism (Bodelier & Laanbroek, 2004; Koehler et al., 2012). In tropical forest soils, indications of N limitation of CH₄ uptake have been reported for Panama (Matson, Corre, Langs, & Veldkamp, 2017; Veldkamp et al., 2013), Indonesia (Hassler et al., 2015), and Ecuador (Wolf et al., 2012) but neither for a Peruvian (Jones et al., 2016) nor a French Guiana site (Bréchet et al., 2019). N limitation of CH₄ uptake in soil following deforestation has also been reported for a deforested landscape in Indonesia (Hassler et al., 2015).

Although phosphorus (P) is a critical nutrient for methanotrophic bacteria and methanogenic archaea and both can thus be P-limited, much less information is available on the effects of P availability on soil CH₄ fluxes. A recent review on the role of P on CH₄ oxidation in soils and sediments concluded that this is clearly a knowledge gap (Veraart et al., 2015). There is only one P-addition study with in situ CH₄-flux measurements from tropical forest soils (Bréchet et al., 2019). The few other studies that analyzed the role of P availability in CH₄ fluxes were mainly conducted on rice paddies, landfills, and fertilized agricultural soils, and almost all these studies were conducted under laboratory conditions, either in microcosms, flasks or cores (Veraart et al., 2015). While there was no link between P-availability and CH₄ fluxes in tropical forest soils of French Guiana (Bréchet et al., 2019), a study on natural unmanaged soils, conducted in the Arctic, showed that soil P availability was positively correlated with in situ Type I methanotroph gene abundance, whereas no correlation with soil N availability was detected (Gray et al., 2014). On one hand, in a heavily fertilized subtropical forest, it was reported that P buffered inhibitive effects of added N (T. Zhang et al., 2011). On the other hand, a stimulation of CH₄ oxidation was found for combined N and P additions to landfill soils (Jugnia et al., 2012). Tropical montane forest sites, close to our present study sites, show a strong positive correlation between soil CH₄ uptake and total soil P in the top 0.05-m depth (either mineral soil or organic

layer) (Wolf et al., 2012). Furthermore, canopy soils in the same sites as our present study show a tendency to increase CH₄ uptake after P applications to the forest floor (Matson, Corre, Langs, & Veldkamp, 2017). Some of the N and P effects reported in the above-mentioned studies may have been caused indirectly by changes in soil oxygen and nutrient status as a result of changes in water and nutrient uptake by plants.

Across the tropics, forest degradation processes, such as deforestation, biomass burning, and N deposition, have a strong impact on factors controlling CH₄ uptake in soil, highlighting the vulnerability of this important ecosystem service (Hassler et al., 2015; Veldkamp et al., 2008, 2013). Elevated N deposition is already affecting natural tropical forest regions (Hietz et al., 2011) and rates of N deposition could soon exceed 25 kg N ha⁻¹ yr⁻¹ (Phoenix et al., 2006). Tropical montane forests may be particularly affected by elevated N deposition since cloud water deposition is important in these ecosystems (Carrillo et al., 2002). In addition, P deposition is predicted to increase in tropical South America because of biomass burning, anthropogenic mineral aerosols, and biogenic dusts from the neighboring Amazon Basin (Mahowald et al., 2005).

Since degradation of tropical forest often involves shifts in nutrient availabilities, it is critical to evaluate how and when this will affect steady state dynamics of important ecosystem services. Unfortunately, there is only one nutrient-addition study from tropical montane forests that reported CH₄ cycling dynamics over more than 3 years (Veldkamp et al., 2013). This was one of the main reasons to establish a long-term (5 years) nutrient addition experiment across an elevation gradient of tropical montane forests in southern Ecuador (Homeier et al., 2012). Since higher rates are applied in most other nutrient fertilization experiments in tropical forests (e.g., Wright et al., 2011 [150 kg N ha⁻¹ yr⁻¹ and 50 kg P ha⁻¹ yr⁻¹]), we consider the application rates in our experiment (50 kg N ha⁻¹ yr⁻¹ and 10 kg P ha⁻¹ yr⁻¹) to be moderate. We chose these moderate amounts to avoid immediate inhibition effects that may develop due to high doses of fertilizers (T. Zhang et al., 2011) and to simulate realistic long-term increases of N and P depositions in the study area. From this experiment, we report how 5 years of moderate N, P, and N + P additions affected soil CH₄ fluxes. In contrast to other studies from tropical montane forests (Jones et al., 2016; Veldkamp et al., 2013), where seasonality and WFPS controlled soil CH₄ fluxes and may have masked nutrient controls, our study area displayed only slight seasonal variability (Emck, 2008) that may help to identify nutrient controls on soil CH₄ dynamics in tropical montane forests. Since earlier studies conducted in the same area showed indications of N-limited CH₄ uptake in soils (Wolf et al., 2012) and that soil N availability controls forest productivity (Wolf et al., 2011), we hypothesized that (a) moderate amounts of N and P applied will be insufficient in an unresponsive phase but sufficient in a responsive phase to overcome nutrient limitations and to stimulate methanotrophy and soil CH₄ uptake, (b) limitations on methanotrophy and soil CH₄ uptake will be related to the initial availability of N and P in soils, and N and P limitations of net primary production that have been earlier observed at these sites (Homeier et al., 2012, 2013), indicating that there is a strong competition for these nutrients between vegetation and microbial biomass. Our present study contributes to the much needed information on long-term measurements of soil CH₄ fluxes (an important ecosystem regulating service) from tropical montane forests exposed to moderate N and P inputs, which are nutrient elements presently influenced by anthropogenic activities.

2. Materials and Methods

2.1. Study Area

Our study was conducted in the south Ecuadorian provinces of Loja and Zamora Chinchipe on the east slope of the Andes mountain range. We selected three sites located along an elevation gradient—spanning from 1,000 to 3,000 m above sea level—of natural montane rainforests. The sites were located within the Podocarpus National Park and in the adjacent privately owned Biological Reserve San Francisco forest that showed no evidence of human activities. Elevation is the surrogate variable that encompasses the naturally occurring variations in forest types, soil and climatic characteristics along this 2,000-m elevation gradient. At 1,000 m, vegetation consisted of premontane rainforest (Homeier et al., 2008) on a Dystric Cambisol soil, developed from deeply weathered granitic rock. At 2,000 m, vegetation consisted of lower montane rainforest on a Stagnic Cambisol soil with a thick organic layer, and at 3,000 m, vegetation consisted of upper montane rainforest on a Stagnic Histosol soil that also has a thick organic layer. Both at the 2,000 and 3,000 m, soils were developed from metamorphic rocks, mainly schists. Detailed information on forest and soil characteristics is in Table S1 (Homeier et al., 2013; Martinson et al., 2013). Across the elevation

gradient, mean annual temperatures varied from 19.4°C at 1,000 m, 15.7°C at 2,000 m, to 9.4°C at 3,000 m. Mean annual precipitation at 1,000 m was 2,230 mm yr⁻¹, at 2,000 m was 1,950 mm yr⁻¹, and at 3,000 m was 4,500 mm yr⁻¹, with small seasonal variability at all sites (Moser et al., 2007). In the study region, annual nutrient deposition measured between 1998 and 2010 ranges from 14 to 45 kg N ha⁻¹ and from 0.4 to 4.9 kg P ha⁻¹ (Boy et al., 2008; Homeier et al., 2013).

2.2. Experimental Design

At the 1,000, 2,000, and 3,000 m sites, we established a factorial nutrient addition experiment with control, N, P, and N + P treatments (Homeier et al., 2013; Martinson et al., 2013). At each site, we implemented a stratified complete block design with four replicate blocks, each of which consisted of the four treatment plots (20 × 20 m each) with at least 10 m distance between plots. The plots were almost flat. However, since bigger flat areas do not exist in these mountains, we established blocks along moderate slopes as the landscape position can affect biogeochemical processes (Wolf et al., 2012). Treatments were assigned within a block using a stratified random procedure with the constraint that within each block control treatments were located at the higher parts and the N + P treatments were located at the lower parts of the landscape, to avoid unintended transfer of nutrients through lateral water movement. Additionally, nutrients were only applied during sunny days to avoid surface runoff.

We started the manual application of nutrients in early 2008, with the following rates: 50 kg N ha⁻¹ yr⁻¹ which was applied as urea (CO(NH₂)₂) and 10 kg P ha⁻¹ yr⁻¹ which was applied as sodium hydrogen phosphate (NaH₂PO₄·H₂O and NaH₂PO₄·2H₂O, analytical grade quality). We chose analytical grade P chemicals to prevent addition of micronutrients such as molybdenum that may influence other processes not attributable to P (Barron et al., 2009). We split N and P additions into two equal applications per year, in February or March and in August or September to ensure a gradual ecosystem fertilizer uptake over the study period. In 2010, the second fertilizer application was delayed for 4 months due to logistical problems related to the shipping of P fertilizer. During fertilizer application, each chamber base area received the respective amount of fertilizer.

2.3. Soil CH₄ Flux, Temperature, Moisture, and Mineral N

We measured soil CH₄ fluxes using vented static chambers that consisted of permanently installed polyvinyl chloride chamber bases (area 0.04 m², height 0.15 m) that were inserted ~0.03 m into the soil, and vented polyethylene chamber hoods, equipped with a Luer-lock sampling port. The total enclosed air volume of the chambers was ~12 L. In each treatment plot at each site, we installed four chamber bases along two perpendicular random transects and all chamber bases were placed at a minimum distance of 2 m from the plot's borders to avoid any edge effects. We measured soil CH₄ fluxes monthly from January 2008 to September 2009 and from November 2010 to August 2012. During measurement, we collected four gas samples at 2, 14, 26, and 38 min after chamber closure. We selected this interval because (a) gas concentration in our chamber headspace changed linear over this time frame and (b) to measure all four chambers per plot within 1 h. All measurements were conducted after 3 weeks following nutrient application since we were not interested in short-term effects of nutrient applications, which are influenced by the pulse amount of applied nutrient, but focused instead on the long-term effects when the applied nutrients are already incorporated into the soil nutrient cycling processes (Koehler et al., 2009; Veldkamp et al., 2013). We were unable to measure in the period of June and July 2009 and from May–July 2011 because of malfunctioning of gas chromatograph.

Gas samples collected from January 2008 to April 2012 were analyzed using our gas chromatograph (GC; Shimadzu GC-14B, Duisburg, Germany) at the nearby laboratory in the Universidad Técnica Particular de Loja, Ecuador. During this time, gas samples were stored in pre-evacuated 60 ml glass containers that were equipped with airtight stop cocks. Starting in April 2012, we transported the gas samples to the University of Goettingen, Germany, for analysis. During this time, gas samples were stored in pre-evacuated 12 ml Labco Extainer[®] with septa (Labco Limited, Lampeter, UK), which we previously tested to be leak proof (Hassler et al., 2015). The gas samples were analyzed using a GC 6000 Vega Series 2 (Carlo Erba Instruments, Milan, Italy). Both GC were equipped with an auto-sampler and a flame ionization detector that measured carbon dioxide (CO₂, ppm) concurrently with CH₄ (ppb). The CO₂-concentration increase with chamber closure

served as our strong indicator that field sampling and gas storage in the glass vials did not have errors. Calibration of the GC was done daily by comparing integrated peak areas of gas samples with three or four standard gas mixtures with CH₄ concentrations ranging from 1,000 to 20,000 ppb (Deuste Steininger GmbH, Mühlhausen, Germany). In Ecuador, gas analyses were typically conducted within 1 day after sampling in the field, while gas samples transported to Germany were stored for up to 2 months before analyses. The CO₂ concentrations always linearly increased during the chamber closure duration (the coefficient of determination [R^2] of the linear increase was almost always above 0.95) and justified the concentration change of CH₄ from the same gas samples as true. Hence, we calculated CH₄ fluxes from the linear increase or decrease of measured CH₄ concentrations in the chamber headspace over time and adjusted with the actual air temperature (GTH 175/Pt-E; Greisinger electronics GmbH, Regenstauf, Germany) and pressure (GMH 3310; Greisinger electronics GmbH, Regenstauf, Germany) measured in the field at the time of sampling. We estimated annual soil CH₄ fluxes using linear interpolation between soil CH₄ fluxes and sampling day intervals (Veldkamp et al., 2013). In total, we calculated 5,616 soil CH₄ fluxes of which 540 fluxes were discarded due to leakage problems. There were 4,697 negative fluxes, 379 positive fluxes, and 83 fluxes between -1 and $+1 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$.

We also measured the following soil variables as potential controlling factors during soil CH₄ flux measurement in the field: soil temperature, gravimetric moisture, and mineral N concentrations (extracted in situ with 0.5 M K₂SO₄ solution, as described in details by Martinson et al., 2013) in the top 0.05-m depth. For the determination of soil mineral N concentrations and moisture content, we took composite soil samples within each plot during each measurement period. Gravimetric moisture content was converted to WFPS using the measured soil bulk densities in the top 5 cm of soil and the particle densities of 2.65 g cm⁻³ for mineral soil at 1,000 m and 1.4 g cm⁻³ for organic layers at 2,000 and 3,000 m (Müller et al., 2015). The data for these soil factors were already reported in our separate study (Müller et al., 2015) and placed in Table S2. Thus, we only report here their patterns wherever they were significantly correlated with soil CH₄ fluxes.

2.4. Statistical Analysis

All statistical analyses were conducted using the means of the four or five chambers that represent each replicate plot on a given sampling day. We first checked for each parameter distribution normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test), and when these assumptions were not fulfilled we applied either a square root or logarithmic transformation (after adding a constant value of 110 because of the negative values of soil CH₄ consumption). First, we tested if control forests across elevations differ in soil CH₄ fluxes and soil controlling factors (soil temperature, WFPS, soil NH₄⁺, and NO₃⁻ contents) during each year and across years using linear mixed effects (LME) models; elevation was a fixed effect and replicate plot and sampling day were random effects (Crawley, 2013; Piepho et al., 2004). Prior to determining nutrient addition effects, we checked for inherent differences among blocks at each site using the first soil CH₄ flux measurements prior to the start of nutrient applications. This test was conducted using one-way analysis of variance (ANOVA) with block as the fixed effect. Second, we tested nutrient addition effects on soil CH₄ fluxes at each elevation, using LME models for each year as well as across years; nutrient treatment was the fixed effect whereas the replicate plot and sampling day were random effects. All LME models were extended to include the following functions, if these improved the relative goodness of the model fit based on the Akaike information criterion: (a) a variance function that allows different variances of the fixed effect (Crawley, 2012), and/or (b) a first-order temporal autoregressive process that accounts for decreasing correlation of measurements with increasing time difference (Zuur et al., 2009). The significance of the fixed effects was determined by ANOVA and the differences among fixed effects (i.e., elevation in case of comparison among control forests and nutrient treatment at each elevation) were determined based on Tukey HSD test, all at $P \leq 0.05$. We further assessed the relationships between soil CH₄ fluxes and soil controlling factors with Pearson's correlation test, using the average of the replicate plots of each treatment on each sampling day during 2010–2012 measurement period; we conducted this correlation tests for each elevation and across elevations. Mean values are given with \pm standard error (SE). All statistical analyses were conducted using R 2.14.0 (R Development Core Team, 2013).

Table 1

Mean Soil CH₄ Fluxes (±SE, n = 3 Plots) in Tropical Montane Forests Along a 1,000- to 3,000-m Elevation Gradient for 2008, 2009, 2010/2011, and 2012 and the Cumulative Years 2008–2009, 2008–2011 and the Whole Study Period From 2008 to 2012

Elevation (m)	Treatment	Soil CH ₄ flux (μg C m ⁻² h ⁻¹)						
		2008	2009	2010/2011	2012	2008–2009	2008–2011	2008–2012
1,000	Control	-22.17 ± 4.65 ^{A,a}	-32.85 ± 3.82 ^{A,a}	-28.13 ± 8.94 ^{A,b}	-24.96 ± 8.93 ^{B,b}	-26.78 ± 2.4 ^{A,a}	-27.25 ± 4.61 ^{A,c}	-26.67 ± 5.10 ^{A,b}
	N	-44.00 ± 22.28 ^a	-32.56 ± 8.83 ^a	-44.99 ± 14.72 ^a	-39.60 ± 20.51 ^a	-39.26 ± 16.59 ^a	-41.21 ± 15.60 ^a	-40.84 ± 16.63 ^a
	P	-26.85 ± 7.20 ^a	-31.41 ± 5.02 ^a	-33.31 ± 15.75 ^{ab}	-21.37 ± 12.90 ^b	-28.73 ± 6.23 ^a	-30.25 ± 9.63 ^{bc}	-28.29 ± 10.40 ^b
	N + P	-39.53 ± 11.60 ^a	-31.75 ± 6.25 ^a	-40.54 ± 16.26 ^a	-29.31 ± 14.97 ^{ab}	-36.13 ± 8.80 ^a	-37.75 ± 10.90 ^{ab}	-35.77 ± 11.88 ^a
2,000	Control	-28.12 ± 4.17 ^{A,a}	-31.57 ± 1.51 ^{A,a}	-30.19 ± 4.68 ^{A,b}	-34.45 ± 6.56 ^{A,b}	-29.63 ± 2.79 ^{A,a}	-29.79 ± 1.33 ^{A,b}	-30.91 ± 2.19 ^{A,c}
	N	-32.10 ± 7.84 ^a	-34.42 ± 4.95 ^a	-29.27 ± 1.98 ^b	-31.59 ± 1.56 ^b	-33.12 ± 6.14 ^a	-31.64 ± 4.49 ^b	-31.63 ± 3.76 ^{bc}
	P	-25.99 ± 4.89 ^a	-30.77 ± 3.31 ^a	-40.56 ± 5.73 ^a	-41.53 ± 7.66 ^a	-28.08 ± 2.39 ^a	-32.88 ± 0.95 ^{ab}	-34.92 ± 2.48 ^{ab}
	N + P	-35.67 ± 1.27 ^a	-30.62 ± 1.01 ^a	-43.96 ± 2.89 ^a	-44.15 ± 5.18 ^a	-33.46 ± 1.10 ^a	-37.42 ± 1.39 ^a	-39.00 ± 2.26 ^a
3,000	Control	-16.88 ± 5.62 ^{A,a}	-16.97 ± 8.01 ^{B,a}	-16.98 ± 5.80 ^{B,b}	-20.80 ± 6.49 ^{B,b}	-16.91 ± 6.41 ^{B,a}	-16.94 ± 6.00 ^{B,b}	-17.88 ± 6.12 ^{B,bc}
	N	-20.98 ± 6.72 ^a	-16.57 ± 7.03 ^a	-23.02 ± 6.02 ^a	-29.60 ± 7.40 ^a	-19.51 ± 6.80 ^a	-20.87 ± 6.49 ^a	-23.01 ± 6.66 ^a
	P	-17.60 ± 3.22 ^a	-15.73 ± 3.48 ^a	-17.99 ± 5.23 ^b	-21.27 ± 5.78 ^b	-16.97 ± 3.31 ^a	-17.38 ± 4.07 ^b	-18.32 ± 4.48 ^{ab}
	N + P	-14.70 ± 2.57 ^a	-14.65 ± 4.43 ^a	-14.77 ± 3.20 ^b	-15.59 ± 1.31 ^b	-14.68 ± 2.13 ^a	-14.73 ± 2.51 ^b	-14.92 ± 1.58 ^c

Note. Means followed by different capital letters indicate significant differences across the elevation gradient for the control plots, and means within each year or time period followed by different small letter indicate significant differences among treatments within each elevation (linear mixed effects model with Tukey HSD test at $P \leq 0.05$).

Abbreviations: N, nitrogen; P, phosphorus; SE, standard error.

3. Results

3.1. Control Plots: Soil CH₄ Fluxes and Soil Factors

While there were no differences in soil CH₄ fluxes among elevations in 2008, soil CH₄ uptake at 3,000 m was generally lower than the other elevations throughout the study period, and in 2012, soil CH₄ uptake at 2,000 m was higher than the other two elevations ($P < 0.01$; Table 1). Across years, annual soil CH₄ uptake in control plots at 1,000 m was 35% higher compared to 3,000 m, while at 2,000 m, CH₄ uptake was 48% higher than at 3,000 m (Table 2). Soil CH₄ uptake did not display a clear seasonal pattern at 2,000 and 3,000 m, but the temporal variability, as shown by the SEs on the sampling periods (Figure 1), was large at 1,000 m.

Over the entire measurement period, soil temperatures decreased with increasing elevation ($P < 0.01$; Table S2). The WFPS was highest at 2,000 m, intermediate at 3,000 m and lowest at 1,000 m ($P < 0.01$; Table S2). There was no clear seasonal pattern in soil temperature or WFPS at any of the sites. Soil mineral N content differed with elevation: NH₄⁺ content was highest at 2,000 m, followed by 1,000 m and lowest at 3,000 m ($P = 0.03$; Table S2); NO₃⁻ content was higher ($P < 0.01$) at 1,000 m and did not differ between 2,000 and 3,000 m (Table S2).

Across the elevation gradient, soil CH₄ fluxes correlated negatively with soil temperature and NH₄⁺ content (Figure 2; Table 3). Within each elevation, at 1,000 and 2,000 m, soil CH₄ fluxes did not correlate with any soil parameters while at 3,000 m soil CH₄ fluxes correlated with soil temperature (Figure 2; Table 3).

3.2. Nutrient Addition Effects on Soil CH₄ Fluxes

Soil CH₄ fluxes measured in January 2008, prior to the first nutrient application, showed no differences among blocks at each elevation. During the first 2 years of nutrient additions, we also did not detect any treatment effect on soil CH₄ flux at any elevation (Table 1). This changed following the 2010/2011 measurements when we detected significant effects of nutrient additions, which were different for each elevation (Figure 1; Table 1). When considering the data during the cumulative 4 and 5 years of nutrient additions, soil CH₄ uptake at 1,000 m increased with N and N + P treatments ($P \leq 0.02$), while application of P alone did not affect soil CH₄ fluxes within any single or cumulative set of years (Figure 1a; Table 1). In contrast

Table 2
Mean Annual Soil CH₄ Fluxes (\pm SE, $n = 3$ Plots) From Tropical Montane Forests During 5 Years of Nutrient Addition

Elevation (m)	Treatment	2008	2009	2010/2011 ^a	2012	2008–2012
		kg C ha ⁻¹ year ⁻¹				
1,000	Control	-2.02 \pm 0.41	-2.96 \pm 0.38	-2.57 \pm 0.71	-2.23 \pm 0.75	-2.23 \pm 0.52
	N	-2.12 \pm 0.81	-2.38 \pm 0.37	-4.11 \pm 1.24	-3.55 \pm 1.79	-2.94 \pm 0.89
	P	-4.07 \pm 1.73	-2.94 \pm 0.48	-2.96 \pm 1.30	-1.87 \pm 1.11	-3.50 \pm 0.96
	N + P	-3.29 \pm 0.96	-2.43 \pm 0.44	-3.77 \pm 1.42	-2.68 \pm 1.18	-3.27 \pm 1.04
2,000	Control	-2.42 \pm 0.30	-2.59 \pm 0.09	-2.49 \pm 0.38	-2.99 \pm 0.59	-2.77 \pm 0.15
	N	-2.77 \pm 0.73	-3.01 \pm 0.44	-2.52 \pm 0.19	-2.77 \pm 0.15	-2.82 \pm 0.39
	P	-2.23 \pm 0.41	-2.67 \pm 0.35	-3.39 \pm 0.44	-3.61 \pm 0.65	-3.16 \pm 0.25
	N + P	-3.08 \pm 0.15	-2.71 \pm 0.12	-3.73 \pm 0.24	-3.84 \pm 0.50	-3.28 \pm 0.20
3,000	Control	-1.52 \pm 0.50	-1.54 \pm 0.75	-1.52 \pm 0.52	-1.81 \pm 0.54	-1.45 \pm 0.63
	N	-1.81 \pm 0.56	-1.31 \pm 0.67	-2.03 \pm 0.55	-2.54 \pm 0.62	-1.79 \pm 0.55
	P	-1.52 \pm 0.29	-1.32 \pm 0.33	-1.64 \pm 0.49	-1.86 \pm 0.50	-1.36 \pm 0.39
	N + P	-1.29 \pm 0.23	-1.29 \pm 0.47	-1.28 \pm 0.24	-1.33 \pm 0.16	-1.25 \pm 0.13

Abbreviations: N, nitrogen; P, phosphorus; SE, standard error.

^aTrapezoidal interpolation for annual estimates in 2010/2011 included two measurements in 2010.

to 1,000 m, N addition at 2,000 m did not affect soil CH₄ fluxes within any year or cumulative set of years, whereas P addition and especially N + P addition increased soil CH₄ uptake during 2010/2011 and 2012 ($P \leq 0.01$) and over the cumulative 4–5 years of treatments ($P \leq 0.02$; Figure 1b; Table 1). At 3,000 m, N addition resulted in higher soil CH₄ uptake during 2010/2011 and 2012 ($P < 0.01$) and over the cumulative 4 and 5 years of treatment ($P < 0.01$; Figure 1c; Table 1) whereas P and N + P additions did not affect soil CH₄ fluxes within any year or cumulative set of years.

Across the elevation gradient and at 3,000 m, soil CH₄ fluxes correlated negatively with soil temperature in all nutrient addition treatments (Figure 2; Table 3). While N addition resulted in a negative correlation between soil CH₄ fluxes and NO₃⁻ content, P addition resulted in a negative correlation between soil CH₄ fluxes and NH₄⁺ content at 1,000 m and across the elevation gradient. The N + P addition resulted in a positive correlation of CH₄ fluxes with NO₃⁻ at 3,000 m and in negative correlations of soil CH₄ fluxes with NO₃⁻ and NH₄⁺ contents across the elevation gradient. At 1,000 and 2,000 m, soil CH₄ fluxes did not correlate with any soil parameters (Table 3).

4. Discussion

4.1. Soil CH₄ Flux and Its Soil Controlling Factors in Control Forests Along the Elevation Gradient

Annual soil CH₄ fluxes along the elevation gradient (Table 2) were within the range of values reported by studies with in situ year-round measurements in montane forests at comparable elevations in Indonesia (Purbopuspito et al., 2006), Brazil (Sousa Neto et al., 2011), Ecuador (Wolf et al., 2012), Panama (Veldkamp et al., 2013), Peru (Jones et al., 2016; Teh et al., 2014), and Africa (Gütlein et al., 2018; Wanyama et al., 2019). The negative correlations of soil CH₄ fluxes with NH₄⁺ content and temperature across the elevation gradient supported our earlier findings from other sites within a similar elevation gradient (Wolf et al., 2012). As was the case in this earlier study, we found no correlation between CH₄ uptake and WFPS either across the elevation gradient or at the individual elevation. In contrast, WFPS controlled soil CH₄ fluxes in montane forests of a Peruvian site (Jones et al., 2016). This discrepancy may be best explained by the low seasonality in rainfall at our site, which translates into minimal variation in soil moisture contents, resulted in statistically indistinguishable relationship of soil CH₄ fluxes with WFPS. In addition, WFPS was determined in the top 5 cm of the soil, which may not reflect the soil moisture condition at the transition between the organic

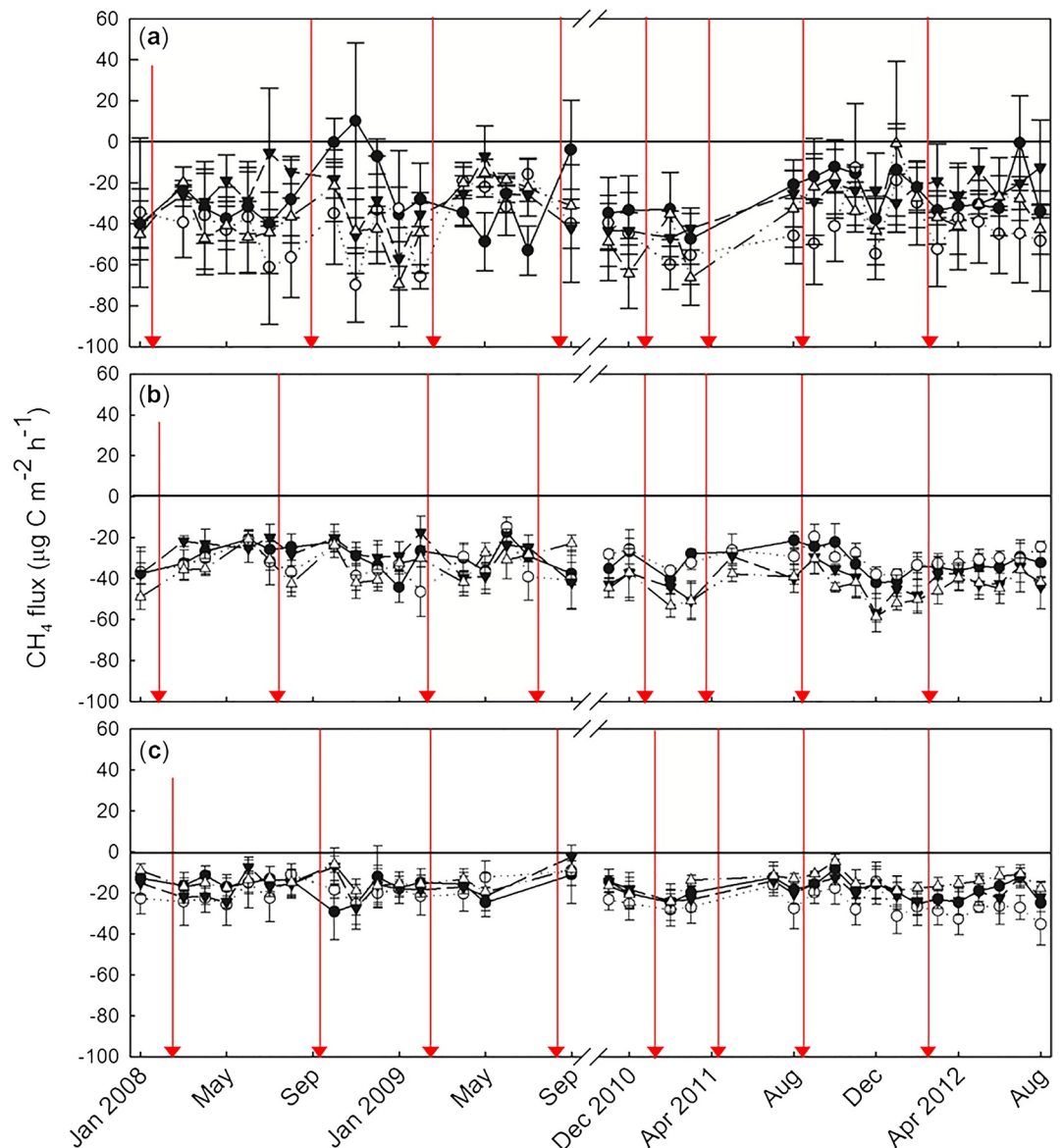


Figure 1. Mean (\pm standard error, $n = 3$) soil CH_4 fluxes from montane forests at (a) 1,000 m, (b) 2,000 m, and (c) 3,000 m elevations during 5 years of nutrient additions: control (filled circle), nitrogen (N) (open circle), phosphorus (P) (filled triangle), and N + P (open triangle). Red vertical lines indicate times of nutrient applications.

layer and the mineral soil, where most of the soil's CH_4 oxidation activity occurs (Wolf et al., 2012). Finally, the very low bulk density of the organic layer (Table S1) possibly renders CH_4 diffusion into the soil unresponsive to soil moisture. Instead, soil NH_4^+ and temperature largely influenced CH_4 uptake, of which the former implies N limitation (Wolf et al., 2012) and the latter suggests stimulation of CH_4 monooxygenase enzyme with increase in temperature (Hanson & Hanson, 1996).

4.2. N-, P-, and N + P-Addition Effects on Soil CH_4 Fluxes—Unresponsive Phase

The lack of effect of N and P additions on soil CH_4 fluxes during the first 2 years of nutrient addition (Table 1; Figure 1) was probably caused by combinations of the moderate amounts of N and P applied, strong microbial and plant competition for the applied N and P, vertical distribution of CH_4 oxidation activity in the soil (Wolf et al., 2012), and the spatial variability of soil CH_4 fluxes. The cumulative amounts of N and P applied during the first 2 years were 100 kg N ha^{-1} and 20 kg P ha^{-1} ; these were considerably lower than

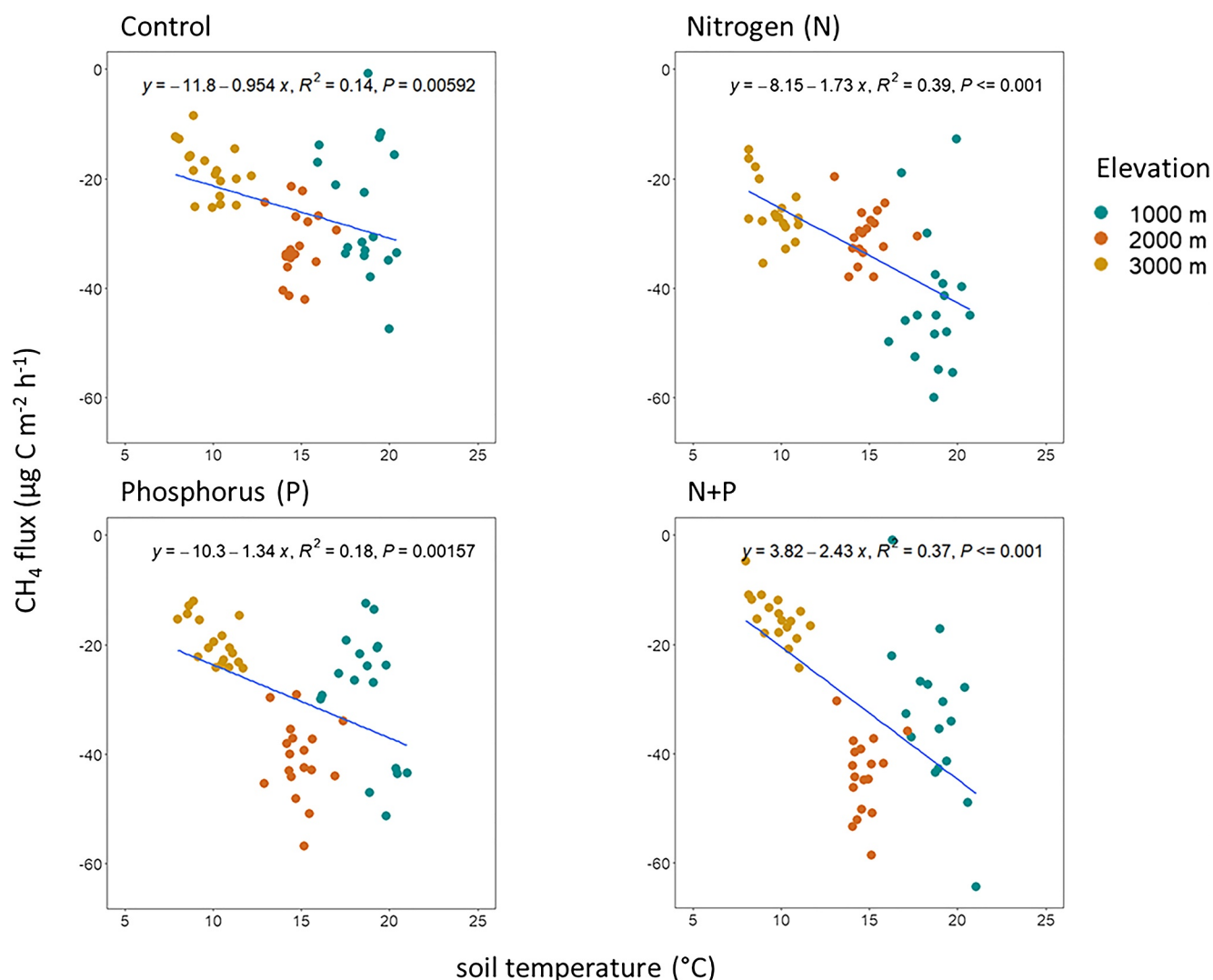


Figure 2. Linear regression of plot means of soil CH₄ fluxes against soil temperature ($n = 18$) along a 1,000- to 3,000-m elevation gradient, measured from 2010 to 2012 in the control, nitrogen (N), phosphorus (P), and N + P plots.

the yearly doses applied in other nutrient manipulation studies in (sub)tropical forests (e.g., 150 kg N and 150 kg P ha⁻¹ yr⁻¹ in China [T. Zhang et al., 2011]; 150 kg N and 50 kg P ha⁻¹ yr⁻¹ in French Guiana [Bréchet et al., 2019]; 125 kg N ha⁻¹ yr⁻¹ in Panama [Veldkamp et al., 2013]). In laboratory incubation studies, nutrient additions are either mixed with the soil in slurries or added directly to the soil samples where CH₄ is produced or oxidized (Bodelier & Laanbroek, 2004). This results in direct short-term responses of CH₄ fluxes as the microbial community is immediately exposed to the added nutrients, but a major disadvantage is that the conditions in the laboratory are not comparable with those in the field. Contrary to laboratory studies, nutrients applied to ecosystems in actual field conditions have a much slower response time, as nutrients are spread at the soil surface whereas the majority of CH₄ oxidation activity is located at the interface between the organic layer and the mineral soil (Wolf et al., 2012). In our study, this means that at 2,000 and 3,000 m the nutrients had to first be transported through a 10–40-cm-thick organic layer (Table S1) before reaching this interface. If the net primary production of the forests at these elevations is co-limited by N and P, as was shown for the forest at 2,000 m (Báez & Homeier 2018; Homeier et al., 2012), there would be strong competition for the applied N and P, particularly in the organic layer where N immobilization by the microbial biomass is high (Baldos et al., 2015). Indeed, at 2,000 m, after 16 months of nutrient application, less than 10% of the applied N and less than 1% of the applied P had leached below the organic layer (Wullaert et al., 2010). At 1,000 m, the mineral soil was not covered with an organic layer (Table S1). However, at this

Table 3

Pearson Correlation Coefficients of Soil CH₄ Fluxes ($\mu\text{g C m}^{-2} \text{h}^{-1}$) With Soil Temperature, WFPS, and Soil Mineral N in Tropical Montane Forests Along a 1,000- to 3,000-m Elevation Gradient, Measured From 2010 to 2012 in the Top 0.05-m Depth of Soil

Treatment/elevation (m)	Soil temperature ($^{\circ}\text{C}$)	WFPS (%)	NH ₄ ⁺ (mg N m ⁻²)	NO ₃ ⁻ (mg N m ⁻²)
Control				
1,000	-0.23 (<i>n</i> = 18)	0.35 (<i>n</i> = 18)	-0.12 (<i>n</i> = 18)	-0.08 (<i>n</i> = 18)
2,000	0.13 (<i>n</i> = 18)	0.08 (<i>n</i> = 18)	-0.15 (<i>n</i> = 18)	0.13 (<i>n</i> = 18)
3,000	-0.49* (<i>n</i> = 18)	-0.33 (<i>n</i> = 18)	-0.17 (<i>n</i> = 17)	-0.19 (<i>n</i> = 17)
N				
1,000	0.04 (<i>n</i> = 18)	-0.05 (<i>n</i> = 18)	-0.08 (<i>n</i> = 18)	-0.28 (<i>n</i> = 18)
2,000	-0.02 (<i>n</i> = 18)	-0.02 (<i>n</i> = 18)	-0.18 (<i>n</i> = 18)	0.07 (<i>n</i> = 18)
3,000	-0.54* (<i>n</i> = 18)	-0.12 (<i>n</i> = 18)	0.05 (<i>n</i> = 17)	-0.03 (<i>n</i> = 17)
P				
1,000	-0.38 (<i>n</i> = 18)	0.76* (<i>n</i> = 18)	0.12 (<i>n</i> = 18)	-0.49* (<i>n</i> = 18)
2,000	-0.09 (<i>n</i> = 18)	0.20 (<i>n</i> = 18)	-0.11 (<i>n</i> = 17)	0.01 (<i>n</i> = 17)
3,000	-0.64** (<i>n</i> = 18)	-0.01 (<i>n</i> = 18)	0.07 (<i>n</i> = 16)	-0.03 (<i>n</i> = 16)
N + P				
1,000	-0.70** (<i>n</i> = 18)	0.72** (<i>n</i> = 18)	-0.09 (<i>n</i> = 18)	0.08 (<i>n</i> = 18)
2,000	0.00 (<i>n</i> = 18)	0.21 (<i>n</i> = 18)	-0.01 (<i>n</i> = 18)	-0.41 (<i>n</i> = 18)
3,000	-0.68** (<i>n</i> = 18)	0.04 (<i>n</i> = 18)	0.38 (<i>n</i> = 17)	0.58* (<i>n</i> = 17)

Abbreviations: N, nitrogen; P, phosphorus; WFPS, water-filled pore space.

* $P \leq 0.05$, ** $P \leq 0.01$.

elevation the spatial variability in soil CH₄ fluxes among replicate plots was larger, as indicated by the larger SEs on each measurement period, compared to the other elevations (Figure 1), and this large spatial variation resulted in statistically undetectable treatment effects.

4.3. N-Addition Effects on Soil CH₄ Fluxes—Responsive Phase

The increased CH₄ uptake with N addition at 1,000 and 3,000 m (observed in the fourth to fifth year; Table 1; Figures 1a and 1c) and a positive correlation between soil CH₄ uptake and NO₃⁻ content across the elevation gradient in the N-addition plots (Table 3) was in line with the previously observed indications of N-limited CH₄ oxidation in forest soils in Panama (Matson, Corre, Langs, & Veldkamp, 2017; Veldkamp et al., 2013) and in Indonesia (Hassler et al., 2015). However, in montane forests of a Peruvian site, there was a negative correlation between soil CH₄ uptake and NO₃⁻ content (Jones et al., 2016). The discrepancy between the Ecuadorian and the Peruvian sites was best explained by a stronger seasonality and higher precipitation leading to soil diffusional constraints and inhibition of soil CH₄ uptake at the Peruvian site (Jones et al., 2016). In a lowland forest of French Guiana soil, CH₄ uptake did not respond to N-additions after 1 year of nitrogen addition (Bréchet et al., 2019) indicating that soil CH₄ dynamics were still in an unresponsive phase. Furthermore, in a N-rich subtropical forest in China with high atmospheric N deposition, further N applications caused inhibition of CH₄ uptake (Zhang et al., 2011), which was probably related to the very high N fertilizer application (150 kg N ha⁻¹ year⁻¹ in form of NH₄NO₃) in an already N-rich ecosystem. In our study, mineral N availability in the soil of the control plots was low (Martinson et al., 2013; Müller et al., 2015) and the soil N cycle is conservative, with soil mineral N production rates closely coupled to microbial N immobilization rates (Baldos et al., 2015). Four years after the start of the experiment, N application decreased microbial N immobilization, decoupling the soil N cycle (Baldos et al., 2015) and increasing the mineral N contents in the soil (Table S2; Müller et al., 2015). These changes in soil-N cycling after 4 years of N addition were probably sufficient to alleviate N limitation of methanotrophic activity and consequently increased CH₄ uptake. Since many methanotrophic bacteria have the ability to fix N₂ but

can also switch to mineral N assimilation (Hanson & Hanson, 1996), the increased soil mineral N content may have allowed methanotrophs to switch from energy-demanding N_2 fixation to low-energy mineral N acquisition, which can increase CH_4 -oxidizing activity (Bodelier & Laanbroek, 2004). Despite a similar decoupling of soil N cycling in the top 5 cm at 2,000 m (Baldos et al., 2015), we did not observe any changes in CH_4 uptake, which may be related to the strong P limitation of methanotrophs such that a response to N addition was only elicited when applied together with P (see below).

4.4. P-Addition Effects on Soil CH_4 Fluxes—Responsive Phase

At 1,000 m, the lack of P treatment effects on CH_4 fluxes (Table 1; Figure 1a) suggests that the methanotrophic community was not limited by P. Since the soil at this elevation does not have an organic layer, litter decomposition is rapid, and nutrients in litterfall are quickly recycled. Low phosphatase activities (Dietrich et al., 2016) combined with the highest total P levels of the mineral soil (Table S1; Baldos et al., 2015), wherein methanotrophic activity is most favored (Wolf et al., 2012), both indicated that the methanotrophic bacteria at this elevation were possibly not P-limited.

At 2,000 m, competition between microbial biomass and plants for P was probably the strongest along the elevation gradient. This was suggested by the lowest total P concentrations both in the organic layer and underlying mineral soil across the elevation gradient (Martinson et al., 2013) and the very low P leaching losses (less than 1% of applied P; Wullaert et al., 2010). After 4–5 years of P addition, total P content in the organic layer increases (Baldos et al., 2015). Such an increase suggests that sufficient P may have reached the mineral soil, where methanotrophic activity is most active (Wolf et al., 2012), alleviating P limitation on methanotrophs and resulting in increased CH_4 uptake (Table 1; Figure 1b). In a study of canopy soil CH_4 fluxes conducted at the same plots, P addition to the forest floor leads to increase in CH_4 uptake in canopy soils, suggesting P-limited CH_4 oxidation (Matson, Corre, & Veldkamp, 2017). Enhanced soil CH_4 uptake by addition of P was also observed in a subtropical forest soil; however, the mechanism used to explain their observations was increased plant water uptake, which, in turn, reduced soil moisture and increased CH_4 diffusivity from the atmosphere into the soil (T. Zhang et al., 2011).

At 3,000 m, the lack of response to P addition (Table 1; Figure 1c) appeared to be for different reasons. On one hand, the organic layer at this elevation is the thickest (Table S1; Martinson et al., 2013) and it had the highest phosphatase activities (Dietrich et al., 2016), which may suggest strong microbial-plant competition for P. On the other hand, the underlying mineral soil, where methanotrophic activity is favored (Wolf et al., 2012), had higher total P concentrations than the other elevations (Martinson et al., 2013). This, combined with less acidic mineral soil pH than at 2,000 m (Martinson et al., 2013), suggests conditions of sufficient P availability in the mineral soil. These results may imply a decoupling of P availability between the organic and mineral soil at this elevation. While there was probably strong microbial-plant competition for P in the thick organic layer, in the mineral soil this may not be the case. Even if some of the added P may have reached the mineral soil after 3–5 years of P application, there was no detectable response because of the relatively sufficient P level in the mineral soil.

4.5. N + P-Addition Effects on Soil CH_4 Fluxes—Responsive Phase

At 1,000 m, there were comparable increases in CH_4 uptake in combined N + P and N treatments and a lack of response in the P treatment alone over 5 years (Table 1; Figure 1a), suggesting that the treatment effects were due to the applied N. As combined N + P application did not show a much larger CH_4 uptake than N application alone, this also supported our interpretation that P was not limiting CH_4 oxidation at this site. At 2,000 m, the larger increase in CH_4 uptake in combined N + P application than in the P treatment alone and the lack of response in N application alone over 5 years (Table 1; Figure 1b) suggested a serial nutrient limitation of methanotrophs with synergistic responses. This is akin to what has been described for nutrient co-limitation of primary producer communities (Harpole et al., 2011). In a serial nutrient limitation, not only P (the primary limiting nutrient), but also N, limits CH_4 uptake, whereby synergistic response to N application can only occur when it is applied together with P. Our observation that combined N + P application resulted in positive correlations of soil CH_4 fluxes with both NH_4^+ and NO_3^- contents over the whole

elevation gradient while N- and P-addition alone resulted in positive correlations of soil CH₄ fluxes only with NO₃⁻ and NH₄⁺, respectively (Table 3), supports this interpretation.

At 3,000 m, the increase in CH₄ uptake in N treatment alone and the lack of responses to P and combined N + P during 4 and 5 years of treatment (Table 1; Figure 1c) can be explained by the role of vegetation at this elevation. At this elevation, a strong microbial-plant competition for P may have occurred in the thick organic layer, while P level was sufficient in the mineral soil (see above), and there was N and P co-limitation of forest productivity. The latter was implied by a trend toward higher basal area increment in the N + P treatment compared to the control plots (Homeier et al., 2013). Thus, combined N + P application may have increased both N and P uptake by vegetation, such that added N may have not enriched down to the mineral soil where methanotrophic activity is most active (Wolf et al., 2012), and thus CH₄ uptake was not affected. This illustrates that laboratory incubations (in which vegetation is typically excluded) can lead to results that cannot be repeated in the field since vegetation can affect the magnitude and even direction of the results.

4.6. Implications for Elevated Nutrient Deposition in Tropical Montane Forests

In summary, we detected N and/or P limitations on soil CH₄ uptake across the elevation gradient in a delayed responsive phase, which supported our hypotheses. The complex pattern of nutrient limitations on soil CH₄ uptake across the elevation gradient can be related to the initial availability of N and P in soils and their changes with chronic additions. Although elevated deposition of N and/or P has the potential to increase soil CH₄ uptake, these nutrient-poor ecosystems have strong microbial-plant competition for nutrients, such that their stimulating effects on methanotrophic activity were only detected after several years of treatment. These delayed responses also reflected the time needed for the applied nutrients to enrich down to the depth at which methanotrophic activity is most active, at the interface of the mineral soil and organic layer. Together, these results strongly emphasize the need to investigate nutrient limitations on ecosystem processes, such as soil CH₄ fluxes, in actual field conditions. At present, elevated N and P depositions are a relatively recent phenomenon at our study sites, so stimulation of CH₄ uptake in these forest soils by N and/or P addition was the dominant feature. However, it is likely that a new steady state has not been reached and that chronic N and P deposition may ultimately lead to inhibition of CH₄ uptake as has been shown in other ecosystems that have been exposed to much larger nutrient additions and much prolonged periods of high N atmospheric deposition.

Data Availability Statement

The data for this manuscript are deposited in the GRO.data research data repository of the Göttingen Campus of the University of Göttingen (<https://doi.org/10.25625/XLKNKN>).

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