Biogeosciences, 10, 5367–5379, 2013
www.biogeosciences.net/10/5367/2013/
doi:10.5194/bg-10-5367-2013

Biogeosciences Open Access

Indications of nitrogen-limited methane uptake in tropical forest soils

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Received: 14 March 2013 – Published in Biogeosciences Discuss.: 28 March 2013
Revised: 19 June 2013 – Accepted: 21 June 2013 – Published: 9 August 2013

Abstract. It is estimated that tropical forest soils contribute 6.2 Tg yr^{-1} (28 %) to global methane (CH_4) uptake, which is large enough to alter CH_4 accumulation in the atmosphere if significant changes would occur to this sink. Elevated deposition of inorganic nitrogen (N) to temperate forest ecosystems has been shown to reduce CH_4 uptake in forest soils, but almost no information exists from tropical forest soils even though projections show that N deposition will increase substantially in tropical regions. Here we report the results from two long-term, ecosystem-scale experiments in which we assessed the impact of chronic N addition on soil CH_4 fluxes from two old-growth forests in Panama: (1) a lowland, moist (2.7 m yr^{-1} rainfall) forest on clayey Cambisol and Nitisol soils with controls and N-addition plots for 9–12 yr, and (2) a montane, wet (5.5 m yr^{-1} rainfall) forest on a sandy loam Andosol soil with controls and N-addition plots for 1–4 yr. We measured soil CH_4 fluxes for 4 yr (2006–2009) in four replicate plots (40 m × 40 m each) per treatment using vented static chambers (four chambers per plot). CH_4 fluxes from the lowland control plots and the montane control plots did not differ from their respective N-addition plots. In the lowland forest, chronic N addition did not lead to inhibition of CH_4 uptake; instead, a negative correlation of CH_4 fluxes with nitrate (NO_3^-) concentrations in the mineral soil suggests that increased NO_3^- levels in N-addition plots had stimulated CH_4 consumption and/or reduced CH_4 production. In the montane forest, chronic N addition also showed negative correlation of CH_4 fluxes with ammonium concentrations in the organic layer, which suggests that CH_4 consumption was N limited. We propose the following reasons why such N-stimulated CH_4 consumption did not lead to statistically significant CH_4 uptake: (1) for the lowland forest, this was caused by limitation of CH_4 diffusion from the atmosphere into the clayey soils, particularly during the wet season, as indicated by the strong positive correlations between CH_4 fluxes and water-filled pore space (WFPS); (2) for the montane forest, this was caused by the high WFPS in the mineral soil throughout the year, which may not only limit CH_4 diffusion from the atmosphere into the soil but also favour CH_4 production; and (3) both forest soils showed large spatial and temporal variations of CH_4 fluxes. We conclude that in these extremely different tropical forest ecosystems there were indications of N limitation on CH_4 uptake. Based on these findings, it is unlikely that elevated N deposition on tropical forest soils will lead to a rapid reduction of CH_4 uptake.

1 Introduction

Methane (CH_4) is an important atmospheric trace gas because it influences both the energy and the oxidant balance of the earth's atmosphere. Presently, the atmospheric concentration of CH_4 is about 1800 ppbv, which accounts for about 0.48 W m^{-2} of the total anthropogenic radiative forcing (Denman et al., 2007). About 75 % of the global CH_4 source strength, which is about 600 Tg yr^{-1}, originates from biogenic sources wherein CH_4 is exclusively produced by methanogenic microorganisms (Conrad, 1989). Although CH_4 is primarily produced in wetland soils, CH_4 production can also occur in upland soils during high rainfall or wet season, for example in anaerobic microsites inside soil

aggregates (Keller and Reiners, 1994). In well-aerated soils, CH_4 is oxidized by methanotrophic microorganisms and CH_4 oxidation normally exceeds production, which results in a net CH_4 uptake. The largest biogenic sink of atmospheric CH_4 is through uptake by upland soils, which contributes about 5 % to the total removal of CH_4 from the atmosphere (Reeburgh, 2003).

Tropical ecosystems play an important role in the production and uptake of atmospheric CH_4 (Keller and Matson, 1994). In tropical forest areas, known wetland sources of CH_4 production do not suffice to explain the observed high CH_4 concentrations over Neotropical forests (Frankenberg et al., 2008), and some "canopy" wetlands may contribute significantly to the CH_4 production (Martinson et al., 2010). Most tropical forests grow on well-drained upland soils that are too dry to emit CH_4 but act instead as an important sink for atmospheric CH_4 (Kiese et al., 2003). In a review where measurements were stratified according to climatic zone, ecosystem and soil texture, the total global CH_4 uptake was estimated at 22.4 $Tg\,yr^{-1}$, of which 9.2 $Tg\,yr^{-1}$ (41 %) occurred in tropical ecosystems (Dutaur and Verchot, 2007). The contribution of tropical forest soils to global CH_4 uptake was estimated at 6.2 $Tg\,yr^{-1}$ (28 %), which is large enough to alter the CH_4 accumulation in the atmosphere if significant changes would occur to this sink.

CH_4 fluxes at the soil surface are the result of methanogenesis and CH_4 oxidation, which can occur simultaneously in aerated soils (Yavitt et al., 1995). The microorganisms involved in CH_4 oxidation are methanotrophic bacteria and ammonium-oxidizing bacteria. Most methanotrophic bacteria use CH_4 as their only source of carbon and energy and all use methane monooxygenase in the first step of CH_4 oxidation (Hanson and Hanson, 1996). Methanotrophic bacteria are separated into Type I and II according to their biochemical pathways of oxidizing CH_4. Type I methanotrophs are generally non-N-fixing organisms, while Type II methanotrophs can fix atmospheric N_2 but can also assimilate mineral N (Hanson and Hanson, 1996). Depending on the CH_4 concentration that they live on, two groups of methanotrophs can be distinguished: one group contains "low affinity" methanotrophs which are adapted for growth at high CH_4 concentrations (e.g. in rice fields), and the other group contains "high affinity" methanotrophs which are able to make use of the atmospheric CH_4 concentrations (around 1.8 ppm). Ammonium-oxidizing bacteria can also oxidize CH_4 through the enzyme ammonia monooxygenase, which can react with CH_4 instead of NH_4^+ (Bédard and Knowles, 1989).

The increased use of nitrogen (N) fertilizers, fossil fuel, and cultivation of N-fixing crops have more than doubled the amount of "reactive" nitrogen (N_r) cycling worldwide (Vitousek et al., 1997). In the past decades, this has led to enhanced N_r input in forest ecosystems, especially in economically developed regions of the temperate zone. Projections are that the input of N_r will increase substantially in tropical regions such as Southeast Asia and South and Central America due to increasing agricultural and industrial use of N (Galloway et al., 2008). A recent study suggested that elevated anthropogenic N_r deposition is probably already widespread in tropical forests (Hietz et al., 2011).

Elevated depositions of mineral N (ammonium (NH_4^+) and nitrate (NO_3^-)) and N fertilization to forest ecosystems have been shown to affect CH_4 fluxes from forest soils (Steudler et al., 1989; Brumme and Borken, 1999). Several mechanisms have been proposed to explain how mineral N affects CH_4 fluxes in upland soils. Most commonly, the inhibition of CH_4 oxidation in the soil by increased NH_4^+ levels is mentioned, not only in temperate soils (Steudler et al., 1989; Crill et al., 1994) but also in tropical soils (Veldkamp et al., 2001). The enzyme methane monooxygenase, which initiates the oxidation pathway of CH_4, is also able to oxidize NH_4^+. When NH_4^+ competes with CH_4 for reactive sites of methane monooxygenase, this can cause inhibition of CH_4 oxidation (Bédard and Knowles, 1989).

An osmotic effect may also contribute to the inhibition of CH_4 oxidation (Nesbit and Breitenbeck, 1992; Veldkamp et al., 2001). There is a discrepancy in published literature about the duration over which NH_4^+ can inhibit CH_4 oxidation. An inhibition effect of NH_4 for 13 yr has been reported (Mosier et al., 1996), whereas in another study inhibition lasted only about four weeks (Veldkamp et al., 2001). On the other hand, increased NO_3^- levels can inhibit CH_4 production because NO_3^- is preferred as an electron acceptor over bicarbonate (Conrad, 1989), and some intermediates if NO_3^- is denitrified (NO_2^-, NO, N_2O) can be toxic for methanogenic microorganisms (Klüber and Conrad, 1998).

Methanotrophic microorganisms also need a N source and thus could be N limited (Bender and Conrad, 1995; Bodelier et al., 2000). However, Bodelier and Laanbroek (2004) showed through a literature review that many indications for N limitation of soil CH_4 consumption have been ignored in earlier studies. Apart from the effects N limitation has on the growth and activity of CH_4-oxidizing bacteria, they also proposed that switching from fixation of molecular N to assimilation of mineral N can cause almost instantaneous changes in CH_4-oxidizing activity.

To date, only one N-manipulation study has been published on N effects on soil CH_4 fluxes from (sub)tropical forests, and this was conducted in China (Zhang et al., 2008; Zhang et al., 2011). In this study, CH_4 uptake decreased with increasing N application rate, whereas in the disturbed and rehabilitated forest no N-addition effect was observed. The authors concluded that the response of soil CH_4 uptake to N addition in tropical forests varied depending on the soil N status; the lack of effect from the disturbed and rehabilitated forest was explained by intense competition for N by the vegetation (Zhang et al., 2008).

Here we report the impact of chronic N additions on soil CH_4 fluxes from two species-rich, old-growth forests in Panama: a lowland, moist forest on clayey Cambisol and

Nitisol soils, and a montane, wet forest on a sandy loam Andosol soil covered with an organic layer. We hypothesized that (1) in the lowland forest, with large soil N-cycling rates (Corre et al., 2010) and tree stem diameter growth (≥ 10 cm diameter trees), as well as fine litterfall that is not N limited (Wright et al., 2011), long-term N addition will inhibit CH_4 uptake; (2) in the montane forest, with comparatively small soil N-cycling rates (Corre et al., 2010) and tree stem diameter growth (10–50 cm diameter trees), as well as fine litterfall that is N limited (Adamek et al., 2009), long-term N addition will stimulate CH_4 uptake. We tested these hypotheses by comparing soil CH_4 fluxes over a period of four years (2006–2009) in the lowland forest between control and N-addition plots during 9 to 12 yr of N additions and in the montane forest between control and N-addition plots during 1 to 4 yr of N additions. Our objectives were to (1) assess changes in soil CH_4 fluxes as a result of long-term N addition, and (2) relate these changes to soil-extractable NO_3^-, NH_4^+ and soil water-filled pore space, which are factors that potentially control soil CH_4 fluxes. This is the first study to report how CH_4 fluxes change under chronic N addition in diverse, old-growth Neotropical forests.

2 Materials and methods

2.1 Approach

We applied N fertilizer to create N-enriched conditions, which ultimately will simulate future increased atmospheric N deposition. N deposition normally enters the ecosystem at the canopy level at low N concentrations with each rain shower whereas we applied N fertilizer to the soil at high N concentration in four doses per year (see below). One of the artefacts of N fertilization is the occurrence of pronounced peaks of soil mineral N concentrations, which can affect short-term CH_4 fluxes within the first weeks following N application (Veldkamp et al., 2001). We therefore did a separate statistical analysis for CH_4 fluxes that include all measurements conducted from 1 day to 3 months after an N application and for CH_4 fluxes that were measured ≥ 6 weeks after the last N application (hereafter referred as long-term CH_4 fluxes). The long-term CH_4 fluxes are unlikely to be affected by the artificially high mineral N concentrations directly following N application. Furthermore, the type of N fertilizer (in our case urea) will be less important for the long-term CH_4 fluxes because within six weeks following urea application in our study sites, urea-N was hydrolyzed and processed in the internal soil N cycle (Koehler et al., 2009).

2.2 Site description and experimental design

The lowland forest (25 to 61 m elevation) consists of an old-growth (> 200 yr), semi-deciduous forest and is located on the Gigante Peninsula (9°06′ N, 79°50′ W), which is part of the Barro Colorado Nature Monument, Republic of Panama. On the nearby Barro Colorado Island, annual rainfall averages 2715 ± 139 mm (1999–2010) with a dry season from January to April. Ambient N deposition from rainfall was $9\,kg\,N\,ha^{-1}\,yr^{-1}$, measured bi-weekly in 2006–2007 at the shore of Gigante Peninsula near the study site (Corre et al., 2010). The mean annual air temperature is 27.2 ± 0.1 °C. Stem diameter growth of trees with ≥ 10 cm diameter at breast height (dbh), fine litter production, and fine-root biomass within 0–10 cm depth were not affected by 11 yr of N addition (Wright et al., 2011). The soils are Endogleyic Cambisol in the lower parts of the landscape and Acric Nitisol in the upper parts of the landscape, both with heavy clay texture. Bulk density was $0.62\,g\,cm^{-3}$ in the top 5 cm depth of mineral soil (Koehler et al., 2009). After 8 yr of N addition, we measured significant decreases in soil pH (control = 5.1 ± 0.1, N addition = 4.8 ± 0.1) and base saturation (control = 67 ± 8%, N addition = 41 ± 7%), while exchangeable aluminium (Al) increased (control = $213 \pm 39\,g\,Al\,m^{-2}$, 8 yr N addition = $297 \pm 44\,g\,Al\,m^{-2}$) in the top 50 cm of mineral soil.

The montane forest (1200–1300 m elevation) consists of an old-growth lower montane forest and is located in the Fortuna Forest Reserve in the Cordillera Central (8°45′ N, 82°15′ W), Chiriquí Province, Republic of Panama. Mean annual rainfall is 5461 ± 250 mm (1997–2010) with no dry season. Ambient N deposition from rainfall was $5\,kg\,N\,ha^{-1}\,yr^{-1}$, measured biweekly in 2006–2007 at a forest clearing near the study site (Corre et al., 2010). The annual mean air temperature is 20.3 ± 0.2 °C. Stem diameter growth of trees with 10–50 cm dbh and fine litter production increased during the first two years of N addition compared with the control plots (Adamek et al., 2009), whereas fine-root biomass and production (from organic layer down to 20 cm depth of the mineral soil) were not affected by N addition (Adamek et al., 2011). The soil is Aluandic Andosol with sandy loam texture and has an organic layer thickness of 10 ± 1 cm. Bulk density of the organic layer was $0.07\,g\,cm^{-3}$ and the underlying mineral soil had a bulk density of $0.47\,g\,cm^{-3}$ in first 5 cm depth (Koehler et al., 2009). After 3 yr of N addition no significant changes in pH (control = 4.7 ± 0.1, N addition = 4.6 ± 0.2), base saturation (control = 8 ± 3 %, N addition = 11 ± 4 %), and exchangeable Al (control = $252 \pm 16\,g\,Al\,m^{-2}$, N addition = $280 \pm 24\,g\,Al\,m^{-2}$) were observed in the top 50 cm of mineral soil.

The N-addition experiment in the lowland forest was part of an on-going nutrient manipulation study established in 1998 (Wright et al., 2011). The experiment includes N-addition and control plots, among other treatments, laid out in four replicates across a 26.6 ha area in a stratified random design. In the montane forest, the experiment was set up in 2006 in a paired-plot design with four replicates of control and N-addition plots (Corre et al., 2010). At both sites, the size of the plots was $40\,m \times 40\,m$, separated by at least 40 m of buffer zone where no manipulation was

done. The N-addition plots received 125 kg urea-N ha^{-1} yr^{-1} split in four applications (i.e. during the rainy season (May–December) for the lowland forest, and every quarter of the year for the montane forest). Measurements were conducted in the central 20 m × 20 m area of the plot to prevent possible edge effects (e.g. roots from trees outside the plots growing into the N-fertilized plots).

2.3 CH_4 flux measurements

Soil CH_4 fluxes were measured using vented static chambers. Four permanent chamber bases (area 0.04 m^2, height 0.25 m, total volume with cover 11 L) were installed on each plot in a stratified random design along two perpendicular 20 m long transects that crossed in the plot's centre. During the first two years (2006–2007), sampling frequency was from once a month to four times a month when we intensively measured following an N application. During the third and fourth year (2008–2009), sampling was conducted at least once a month. Four gas samples (100 mL each) were removed at 2, 12, 22 and 32 min after chamber closure and stored in pre-evacuated glass containers with a teflon-coated stopcock. Gas samples were analyzed in the field station in Panama using a gas chromatograph (Shimadzu GC-14B, Columbia, MD, USA) equipped with a flame ionization detector and an autosampler (Loftfield et al., 1997). CH_4 concentrations were determined by comparison of integrated peak areas of samples with those of three to four standard gases (depending on concentrations: 250, 1499, 1996, 9900 and 20 010 ppb CH_4; Deuste Steininger GmbH, Mühlhausen, Germany). Gas fluxes were calculated from the concentration change in the chamber versus time and were adjusted for air temperature and atmospheric pressure measured at the time of sampling. To account for the decreasing diffusion gradient over time caused by the chamber feedback, we fitted both a linear and a quadratic regression model if CH_4 concentrations increased or decreased asymptotically (Wagner et al., 1997). We chose the statistically more adequate model based on the Akaike information criterion. The quadratic model was used in 14 % of the flux calculations in the montane forest and in 20 % of the gas flux calculations in the lowland forest. If CH_4 concentrations leveled out over time and the quadratic model was statistically inferior, we excluded the last data point and calculated the flux based on a linear model. These data screening and calculation procedures ensure that we minimized underestimations which may occur if a linear model was uncritically applied to static chamber flux data (Livingston et al., 2006). Positive fluxes indicate CH_4 emission from the soil; negative fluxes indicate CH_4 uptake by the soil. Zero fluxes were included. The annual CH_4 fluxes were approximated by applying the trapezoid rule (linear interpolation of time intervals between measured flux rates), assuming constant flux rates per day.

2.4 Soil mineral N and moisture

From earlier experience in tropical forests, we learned that short storage of disturbed soil samples can considerably alter mineral N concentrations (Arnold et al., 2008). We therefore conducted mineral N extractions in the field. Parallel to gas sampling, four samples of mineral soil (0–0.05 m depth) were collected within the central 10 m × 10 m of each plot. For the montane site, we sampled the organic layer and 0–5 cm depth mineral soil separately. While in the field, samples were pooled for each plot, leaves and roots were manually removed, and a subsample (50–60 g fresh weight) was added to a prepared extraction bottle containing 150 mL of 0.5 mol L^{-1} K_2SO_4. Shaking (1 h) and filtering continued upon arrival at the field station, which was at most 6 h after field extraction. Soil extracts were stored in a freezer and kept frozen during air transport to the University of Göttingen (Germany), where NH_4^+ and NO_3^- contents were analyzed using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands). NH_4^+ was determined using the Berthelot reaction method (Skalar Method 155-000) and NO_3^- was measured using the copper-cadmium reduction method (NH_4Cl buffer but without ethylenediamine tetraacetic acid; Skalar Method 461-000). The rest of the field-moist sample was stored in plastic bags for gravimetric moisture determination, conducted in the field station on the same sampling day. We dried 40–100 g of fresh-weight soil for 24 h at 105 °C. We expressed moisture content as WFPS using measured bulk density and particle densities of 2.65 g cm^{-3} for mineral soil (Linn and Doran, 1984) and 1.4 g cm^{-3} for organic layer (Breuer et al., 2002).

2.5 Statistical analyses

For CH_4 fluxes, statistical analysis was conducted on the plot means (average of 4 chambers) of each sampling day. Linear mixed effects models were used to test for the fixed effects of site (lowland vs. montane control plots) or treatment (control vs. N addition for each site) on the repeated measurements of soil CH_4 fluxes and soil factors (WFPS, soil temperature, NH_4^+ and NO_3^- concentrations). The spatial replication and time (sampling days) were included as random effect. A function which allows different variances of the response variable per level of the fixed effect and/or a first-order temporal autoregressive process was included if this improved the relative goodness of the model fit based on likelihood ratio tests. The significance of the fixed effect was evaluated using analysis of variance (Crawley, 2009). If residual plots revealed non-normal distribution or non-homogenous variance, square root or logarithmic transformation was used for right-skewed data and quadratic transformation for left-skewed data, and the analysis was repeated. Effects were considered significant if P value ≤ 0.05. Pearson correlation tests were conducted on treatment means (average of 4 plots) of each sampling day to investigate the linear influences

of WFPS, soil temperature, NH_4^+ and NO_3^- concentrations on soil CH_4 fluxes. A few CH_4 fluxes from the N-addition plots of the montane forest were exceptionally high (21 out of 196 plot means with emissions $> 60\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$), and correlation analyses were conducted both including (using logarithmic transformation) and excluding these high emissions. We also used Pearson correlation to test the influences of annual rainfall, soil clay and sand contents, organic layer thickness, and annual N deposition on annual soil CH_4-C fluxes of tropical forests published so far. Mean values in the text are given with ± 1 standard error. Analyses were conducted using R 2.15.2 (R Development Core Team, 2011).

3 Results

3.1 Soil water content, temperature and mineral N

In the lowland forest, the pronounced dry season from January to April caused a strong seasonality in WFPS, which ranged from approximately 55–70 % during rainy season to 35–45 % during dry season (Fig. 1a). Mean annual soil temperature was 25.5 °C and the seasonal variation was 2.5 °C (Fig. 1c). Neither WFPS nor soil temperature differed between the control and N-addition plots ($P = 0.37$ to 0.95). In the montane forest, where the dry season is absent, the WFPS in the mineral soil was high (70–80 %) throughout the year. The organic layer with its low bulk density had a much lower WFPS (20–35 %; Fig. 1b). Mean annual soil temperature was 18.1 °C and the seasonal variation was 3.8 °C (Fig. 1d). Also, WFPS and soil temperature were similar between the control and N-addition plots ($P = 0.31$ to 0.47).

In the lowland forest, NH_4^+ concentrations did not differ between the control and N-addition plots ($P = 0.82$) (Fig. 2a), but NO_3^- concentrations increased with N addition ($P < 0.01$) (Fig. 2b). In the montane forest, mineral N was dominated by NH_4^+ in both organic layer and mineral soil. N addition increased NH_4^+ concentrations in the mineral soil ($P < 0.01$) but did not show an effect on NH_4^+ concentrations in the organic layer ($P = 0.31$) (Fig. 2c and e). NO_3^- concentrations increased in both mineral soil ($P = 0.01$) and organic layer ($P = 0.03$) with very large increases in the fourth year of N addition (Fig. 2d and f).

3.2 CH_4 fluxes from control forest soils

CH_4 fluxes from the lowland forest control plots ($-21.47 \pm 1.57\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$) did not differ ($P = 0.82$) from the fluxes of the montane forest control plots ($-3.99 \pm 3.40\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$; Fig. 3, Table 1). This seemingly larger CH_4 uptake rates in this moist ($2.7\,m\,yr^{-1}$ rainfall) lowland forest soil than the wet ($5.5\,m\,yr^{-1}$ rainfall) montane forest soil was not statistically significant because of the large spatial and temporal variations (Fig. 3). Before elaborating on how soil factors influence CH_4 fluxes, we want to point out the implications of correlations: a positive correlation between CH_4 fluxes and a soil variable indicates a decrease in CH_4 uptake rates with an increase in the soil parameter values, whereas a negative correlation indicates an increase in CH_4 uptake rates with an increase in the soil parameter values. In the lowland forest, CH_4 fluxes were positively correlated with WFPS (Table 2). In the montane forest, CH_4 fluxes were negatively correlated with NH_4^+ concentrations and positively correlated with NO_3^- concentrations of the organic layer and mineral soil (Table 2). These opposing correlations of CH_4 fluxes with NH_4^+ and NO_3^- were because the temporal patterns of NH_4^+ and NO_3^- showed the opposite trend (Fig. 2c–f). The correlation between CH_4 fluxes and total soil mineral N ($NH_4^+ + NO_3^-$) concentrations (organic layer $R = -0.51$, $P = 0.01$, $n = 28$; mineral soil $R = -0.56$, $P = 0.00$, $n = 27$) followed that of NH_4^+, because NH_4^+ comprised the largest part of mineral N.

3.3 Effects of N addition on soil CH_4 fluxes

In the lowland forest, neither all CH_4 fluxes ($-24.22 \pm 1.64\,\mu g\,C\,m^{-2}\,h^{-1}$) nor the long-term CH_4 fluxes ($-26.14 \pm 2.00\,\mu g\,C\,m^{-2}\,h^{-1}$) from the N-addition plots differed ($P = 0.55$ to 0.57) from the CH_4 fluxes of the control plots (Fig. 3a and c; Table 1). The reason was the occasional CH_4 emissions from three of the four replicate plots of the control and N-addition treatment regardless of seasons (46 emission fluxes out of 373 plot-mean fluxes or 12 % of the observations, ranging from 0.4 to $210\,\mu g\,C\,m^{-2}\,h^{-1}$), resulting in the large spatial and temporal variations (i.e. large SE bars; Fig. 3a and c). For all CH_4 fluxes, we detected a positive correlation with WFPS and negative correlations with soil temperatures and NO_3^- concentrations (Table 2). The same soil factors showed similar trends of correlations with the long-term CH_4 fluxes (Table 2).

In the montane forest, despite the large mean CH_4 emissions from the N-addition plots (for all CH_4 fluxes $50.94 \pm 19.62\,\mu g\,C\,m^{-2}\,h^{-1}$; for long-term CH_4 fluxes $62.13 \pm 31.26\,\mu g\,C\,m^{-2}\,h^{-1}$), neither all CH_4 fluxes nor the long-term CH_4 fluxes differed ($P = 0.32$ to 0.71) from those of the control plots (Fig. 3b and d; Table 1). The reason was that frequent CH_4 emissions were observed from all eight plots (83 emission fluxes out of 351 plot-mean fluxes or 24 % of the observations, ranging from 0.2 to $2575\,\mu g\,C\,m^{-2}\,h^{-1}$). These CH_4 emissions were dominated by one pair of control and N-addition plots (49 emission fluxes out of 351 plot-mean fluxes), causing the large spatial and temporal variations (i.e. large SE bars; Fig. 3b and d). If we exclude this one pair of control and N-addition plots from the statistical analysis, there remained no difference between the N-addition and control plots ($P = 0.28$ to 0.82), but the mean CH_4 fluxes showed net uptake instead of net emission (Table 1). Also, a few CH_4 emissions from N-addition plots were exceptionally high (21 out of 196 plot means with emissions

Table 1. Annual soil CH_4-C fluxes (kg C ha^{-1} yr^{-1}, mean ± SE, $n = 4$) from the control and N-addition plots, separated into all and long-term fluxes, with the latter including only the fluxes measured at least six weeks after a N application. For the montane forest, values in parentheses are estimates that excluded one pair of plots (control and N addition) which dominated CH_4 emissions (49 emission fluxes out of 351 plot-mean fluxes).

Site	Treatment	2006	2007	2008	2009
Montane	Control	−1.69 ± 0.36	−1.18 ± 0.41	−0.53 ± 0.50	1.91 ± 2.49
		(−1.83 ± 0.48)	(−1.15 ± 0.58)	(−0.93 ± 0.42)	(−0.56 ± 0.57)
	1–4 yr N addition, all fluxes	−1.86 ± 0.57*	7.64 ± 9.40	4.42 ± 5.86	8.99 ± 10.41
		(−2.37 ± 0.35)	(−1.75 ± 0.30)	(−1.42 ± 0.59)	(−1.42 ± 0.38)
	1–4 yr N addition, long-term fluxes	−2.19 ± 0.76*	8.34 ± 9.97	5.36 ± 6.82	8.56 ± 9.89
		(−2.91 ± 0.37)	(−1.63 ± 0.30)	(−1.44 ± 0.63)	(−1.33 ± 0.43)
Lowland	Control	−1.93 ± 0.24	−1.82 ± 0.51	−2.38 ± 0.54	−1.60 ± 0.45
	9–12 yr N addition, all fluxes	−2.33 ± 0.85	−2.22 ± 0.60	−1.94 ± 0.98	−2.20 ± 0.50
	9–12 yr N addition, long-term fluxes	−2.09 ± 0.92	−2.42 ± 0.68	−2.15 ± 0.51	−2.16 ± 0.51

* The two pre-treatment measurements from January and February 2006 were not included in the calculation.

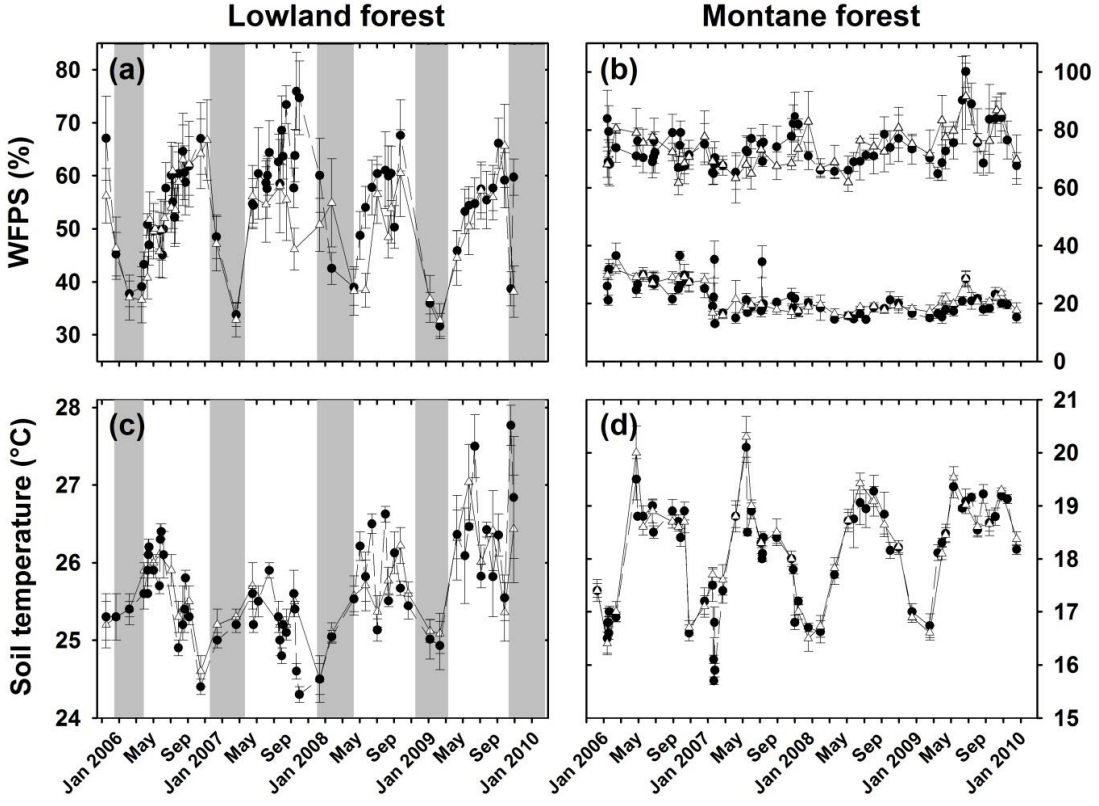

Fig. 1. Mean (±SE, $n = 4$) soil water-filled pore space (WFPS) and temperature at 0–0.05 m mineral soil in the control (△) and N-addition (•) plots of the lowland forest (**a** and **c**) with 9–12 yr of treatment and of the montane forest (**b** and **d**) with 1–4 yr of treatment. For WFPS in the montane forest, the upper and lower values are for the 0–0.05 m mineral soil and organic layer, respectively. Grey shadings in **(a)** and **(c)** mark the dry seasons. The first two years were previously reported by Koehler et al. (2009).

$> 60\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$). Thus, we looked critically at how these few high CH_4 emissions influence the relationships between CH_4 fluxes and soil factors. We first analyzed the correlations between CH_4 fluxes and soil factors that include all emission fluxes and that exclude the exceptionally high CH_4 emissions of $> 60\,\mu g\,CH_4\text{-}C\,m^{-2}\,h^{-1}$. Considering all CH_4 fluxes, we observed a positive correlation with WFPS of the mineral soil and a negative correlation with NH_4^+ concentrations of the organic layer when the large emissions were included. When the large emissions were excluded, CH_4 fluxes

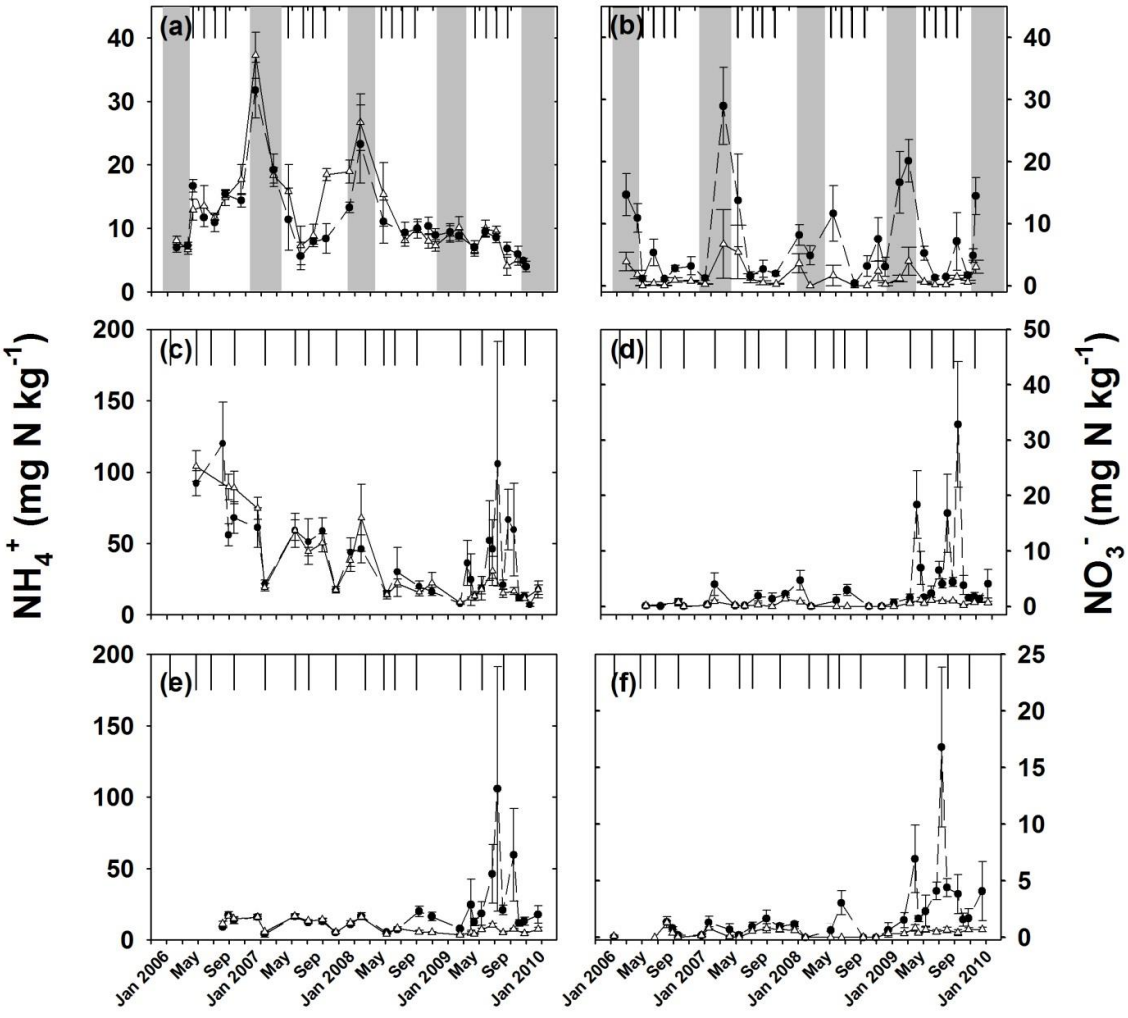

Fig. 2. Mean (±SE, $n = 4$) soil-extractable ammonium (NH_4^+, left panels) and nitrate (NO_3^-, right panels) at 0–0.05 m mineral soil in the control (△) and N-addition (•) plots of the lowland forest (**a** and **b**) and montane forest (**c** and **d** for organic layer, **e** and **f** for 0–0.05 m mineral soil). The black vertical lines indicate dates of N addition during 9–12 yr of treatment in the lowland forest and 1–4 yr of treatment in the montane forest. Grey shadings in **(a)** and **(b)** mark the dry seasons. The first two years were previously reported by Koehler et al. (2009).

remained negatively correlated with NH_4^+ concentrations of the organic layer (Table 2). Considering only the long-term CH_4 fluxes, we observed also a negative correlation with NH_4^+ concentrations of the organic layer both including and excluding the large emissions (Table 2).

4 Discussion

4.1 CH_4 fluxes from control forests in comparison with published values

The mean annual CH_4 uptake rate in the control plots of the lowland forest was within the range of published values from (sub)tropical forests below 800 m elevation (Table 3). The few published CH_4 uptake rates that were lower than our lowland forest soil were mainly from Amazon forest soils with low sand or high clay contents, while those with larger CH_4 uptake rates were mostly at sites with low clay content (Steudler et al., 1996; Sousa Neto et al., 2011). Indeed, from studies compiled (Table 3), the only significant correlation between annual CH_4 fluxes and site factors for the tropical forests below 800 m elevation was a positive correlation between annual soil CH_4 fluxes and clay contents ($R = 0.58$, $P = 0.02$, $n = 16$). A high content of clay decreases the contribution of coarse pores to the total porosity (Hillel, 1998). As coarse pores are especially important for gas diffusive transport, soil texture may be a good proxy variable for gas diffusion control on CH_4 uptake. Consistent with this correlation pattern, earlier studies have shown that CH_4 uptake is often limited by gas diffusion in the soil (Keller and Reiners, 1994). Also, the seasonal changes in CH_4 uptake of our lowland forest soil (Fig. 3a, c) were best explained by gas

Table 2. Pearson correlation coefficients between soil CH_4-C fluxes ($\mu g\,C\,m^{-2}\,h^{-1}$) and soil variables, using the mean values of each treatment on each sampling day, measured from 2006 to 2009. For the montane forest N-addition plots, coefficients in parentheses are from analyses that include the few events of large CH_4 emissions (please see Sect. 2.5).

Site and treatment	$n \geq$	Water-filled pore space (%)	NH_4^+ ($mg\,N\,kg^{-1}$)	NO_3^- ($mg\,N\,kg^{-1}$)	Soil temperature (°C)
Montane	Organic layer				
Control	28	−0.31	$-0.58^{b,c}$	$0.62^{b,c}$	–
1–4 yr N addition, including all fluxes	27	−0.05	$-0.43^{a,c}$	-0.12^{c}	–
		(−0.18)	($-0.38^{a,c}$)	(0.34^{c})	
1–4 yr N addition, long-term fluxes	24	−0.13	$-0.48^{a,c}$	0.11^{c}	–
(i.e. measured ≥ 6 weeks after N addition)		(0.06)	($-0.44^{a,c}$)	$(0.29)^{c}$	
Montane	0–0.05 m mineral soil				
Control	27	0.26	-0.56^{b}	$0.54^{b,c}$	−0.17
1–4 yr N addition, including all fluxes	26	0.14	−0.25	-0.35^{c}	−0.16
		(0.37^{b})	(−0.31)	(0.16^{c})	(0.09)
1–4 yr N addition, long-term fluxes	23	−0.09	−0.33	-0.01^{c}	0.02
		(0.30)	(−0.36)	$(0.08)^{c}$	(0.14)
Lowland	0–0.05 m mineral soil				
Control	32	0.57^{b}	-0.03^{c}	-0.14^{c}	−0.02
9–12 yr N addition, including all fluxes	33	0.49^{b}	0.16^{c}	$-0.40^{a,c}$	-0.34^{a}
9–12 yr N addition, long-term fluxes	28	0.54^{b}	0.27^{c}	$-0.37^{a,c}$	-0.39^{a}

[a], [b]: $P \leq 0.05$ and $P \leq 0.01$, respectively.
[c] Data were logarithmically transformed before analysis (please see Sect. 2.5).

diffusion, as was illustrated by the correlation of CH_4 fluxes with WFPS (Table 2); during the wet season when WFPS was high, CH_4 uptake was low because CH_4 diffusion from the atmosphere to this site's clayey soils was probably slowed down by the high soil water contents.

The mean annual CH_4 uptake rate in the control plots of the montane forest was the lowest published so far for tropical forests above 800 m elevation (Table 3). This was caused by the frequent CH_4 emissions from our wet, montane forest soil (Fig. 3b and d). From tropical forests above 800 m elevation (Table 3), we detected a positive correlation between annual CH_4 fluxes and rainfall ($R = 0.78$, $P = 0.04$, $n = 7$), which is in line with the gas diffusion control on soil CH_4 uptake as discussed above. Rainfall influences gas diffusion through its effects on soil moisture content. However, in contrast to the forests below 800 m elevation, we detected a negative correlation with clay contents ($R = -0.68$, $P = 0.04$, $n = 9$). This can probably be explained by the occurrence of thick organic layers (Table 3) at the surface of some of these soils, which may interfere with gas exchange between soil and atmosphere. From an earlier study we conducted in montane forests of Ecuador, we found that, contrary to common belief, the deeper part of such organic layers can contribute to the CH_4-oxidation capacity of soils (Wolf et al., 2012). The thickness, bulk density and CH_4-oxidation capacity of these organic layers may influence CH_4 uptake stronger than the soil texture of the underlying mineral soil. We also detected a positive correlation between annual CH_4 fluxes and annual N deposition rates ($R = 0.96$, $P < 0.00$, $n = 6$) of tropical forests above 800 m elevation. This may suggest that CH_4 uptake is lower at sites with higher N deposition. However, this correlation is based on six sites that had N deposition rates of only $\leq 5.0\,kg\,N\,ha^{-1}\,yr^{-1}$. At such low rates of N deposition, we think that inhibition of CH_4 oxidation by NH_4^+ is unlikely. Instead, we think that such correlation is only circumstantial because in these six sites annual N deposition was positively correlated with annual rainfall ($R = 0.89$, $P = 0.02$, $n = 6$), signifying that low CH_4 uptake was reported for sites with high rainfall and high N deposition. Thus, we think it is more likely that soil water content (which controls gas diffusion) as influenced by rainfall was the reason behind the observed correlation between annual CH_4 fluxes and annual N deposition rates.

For the control plots of the montane forest, we interpret the negative correlations of CH_4 fluxes with NH_4^+ and total mineral N concentrations as evidence that CH_4 consumption was N limited. We had similar findings of negative correlation between CH_4 fluxes and total mineral N concentrations in montane forest soils in Ecuador, suggesting N limitation on methanotrophic activity (Wolf et al., 2012). While the positive correlation of NO_3^- with CH_4 fluxes may indicate inhibitory effects of nitrification on CH_4 consumption,

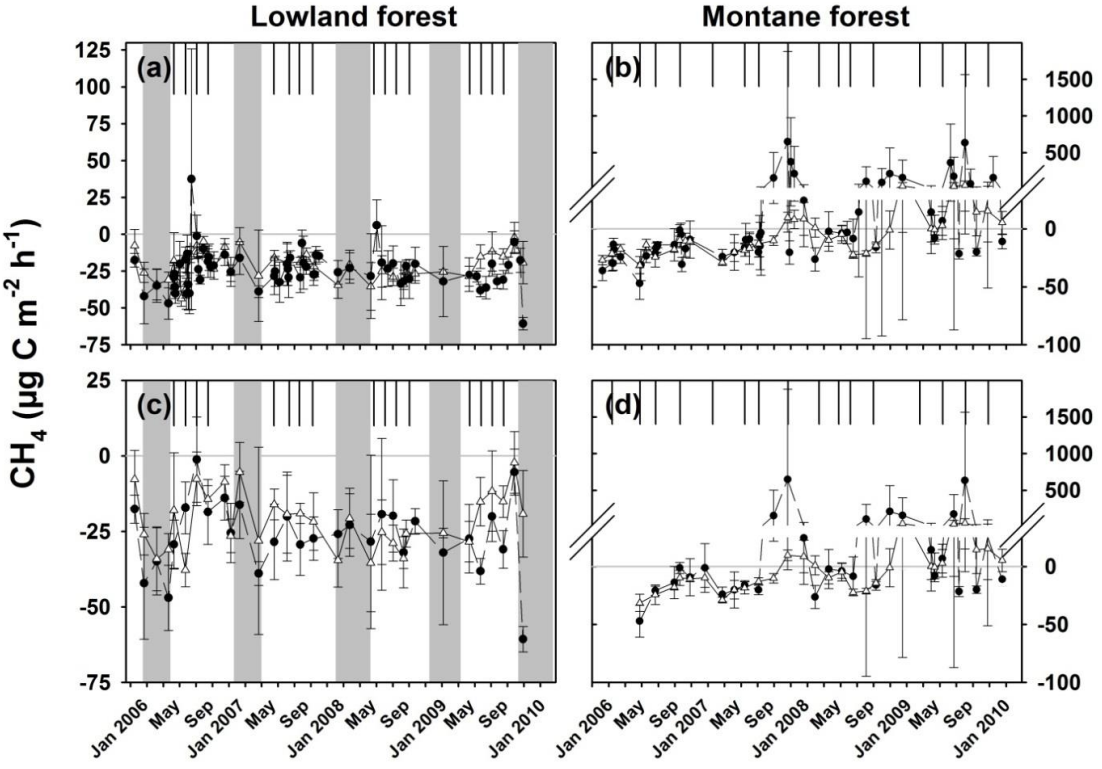

Fig. 3. Mean (±SE, $n = 4$) soil CH_4-C fluxes from the control (△) and N-addition (•) plots of the lowland forest (**a** and **c**) and montane forest (**b** and **d**). The black vertical lines indicate dates of N addition during 9 to 12 yr of treatment in the lowland forest and 1 to 4 yr of treatment in the montane forest; the grey horizontal lines mark the zero flux. The upper panels include all fluxes whereas the lower panels show only the long-term fluxes, which were measured at least six weeks after a N addition. Grey shadings in **(a)** and **(c)** mark the dry seasons.

we think that this is very unlikely since the NO_3^- concentrations were one to two orders of magnitude smaller than the NH_4^+ concentrations (compare Fig. 2c and d and 2e and f, and note the different scales on the *y* axis). Furthermore, measurements of gross and net nitrification rates showed very low nitrification activity (Koehler et al., 2009; Corre et al., 2010). Although Bodelier and Laanbroek (2004) suggest that N limitation of methanotrophic bacteria is less likely at (sub-)atmospheric CH_4 concentrations in the soil, we had ancillary measurements of the soil-air CH_4 concentrations in our montane forest soil that showed CH_4 concentrations in this forest soil were occasionally high. These measurements were conducted monthly from October 2008 to January 2010 in three control plots and three N-addition plots for various layers: 0.10 m above the soil surface, at the interface of the organic layer and mineral soil, at 0.05, 0.20, 0.40, 0.75 and 1.25 m depths in the mineral soil; we employed the same gas sampling methods described in our earlier study (Koehler et al., 2012). We found that 34 % of 421 observations had CH_4 concentrations in the mineral soil higher than the concentration at 0.10 m above the soil surface of 2.0 ± 0.1 ppm CH_4-C, particularly during periods of high rainfall and thus high soil water contents. Such high CH_4 concentrations in our montane forest soil air may allow for population increases of methanotrophic bacteria which, in turn, may lead to N limitation on their activity (Bodelier and Laanbroek, 2004).

4.2 Response of soil CH_4 fluxes to N addition in the lowland and montane forests

In contrast to the findings from temperate forest soils (Steudler et al., 1989; Brumme and Borken, 1999), tropical pasture soil (Veldkamp et al., 2001) and subtropical forest soil (Zhang et al., 2008), CH_4 uptake in our lowland forest soil was not inhibited by chronic N addition. Instead, the negative correlation of CH_4 fluxes with NO_3^- concentrations in the N-addition plots suggests that increased NO_3^- levels in these plots had stimulated CH_4 consumption (Bodelier and Laanbroek, 2004) and/or had inhibited CH_4 production (Conrad, 1989). The latter is however unlikely because our ancillary measurements of CH_4 concentrations at various depths of the mineral soil (0.05, 0.20, 0.40, 0.75, 1.25 and 2 m depth) in this lowland forest during the same study years (May 2006–January 2009) showed that 11 % of the observations had higher soil-air CH_4 concentrations than the average soil-air CH_4 concentrations at a specific depth. These high soil-air CH_4 concentrations occurred in all depths of both N-addition and control plots regardless of season, indicating

Table 3. Compilation of CH_4-C fluxes (kg CH_4-C ha^{-1} yr^{-1}) from soils of old-growth (sub)tropical forests, sorted from smallest to largest uptake rates within each elevation category.

Country	Elevation (m)	Annual CH_4-C flux	Annual rainfall (mm)	Clay content (%)	Sand content (%)	Organic layer thickness* (cm)	N deposition (kg N ha^{-1} yr^{-1})	Reference
Sites < 800 m elevation								
Brazil	120	−0.55	2000	80	18	0	not reported	Keller et al. (2005)
Brazil	120	−0.83	2000	75	20	0	not reported	Davidson et al. (2004)
China	720	−0.93	1557	54	17	0	18.0	Fang et al. (2010)
Brazil	100	−1.57	1850	80*	15*	0	not reported	Verchot et al. (2000)
Panama	43	−1.93	2715	69	7	0	9.0	Present study
China	300	−1.93	1564	22	20	0	38.0	Fang et al. (2010)
Australia	50	−2.35	4395	30*	60*	0	not reported	Kiese et al. (2008)
Australia	800	−2.41	1594	30*	60*	0	not reported	Kiese et al. (2008)
China	770	−2.58	1493	18	59	0	18.0	Werner et al. (2006)
Brazil	120	−2.60	2000	38	60	0	not reported	Keller et al. (2005)
Brazil	100	−2.74	3050	32	60	0	8.0	Sousa Neto et al. (2011)
Australia	50	−2.94	3609	60*	20*	0	not reported	Kiese et al. (2008)
Costa Rica	60	−3.45	4200	76	20	0	9.6	Keller and Reiners (1994)
Brazil	124	−3.50	2200	20*	75*	0	not reported	Steudler et al. (1996)
China	300	−3.60	1927	29	38	0	36.0	Zhang et al. (2008)
Brazil	400	−4.90	3050	16	67	0	8.0	Sousa Neto et al. (2011)
Sites > 800 m elevation								
Panama	1200	−0.37	5461	13	61	10	5.0	Present study
Ecuador	3000	−1.06	4500	17	30	14	4.4	Wolf et al. (2012)
Indonesia	2470	−1.45	not measured	17	59	15	not measured	Purbopuspito et al. (2006)
Indonesia	1190	−2.45	1590	12	64	0	2.6	Purbopuspito et al. (2006)
Ecuador	2000	−3.10	1950	18	25	13	2.9	Wolf et al. (2012)
Indonesia	1800	−3.32	not measured	32	51	20	not measured	Purbopuspito et al. (2006)
Brazil	1000	−4.40	2300	20	57	0	2.1	Sousa Neto et al. (2011)
Kenya	1600	−4.94	1662	34	43	0	not reported	Werner et al. (2007)
Ecuador	1000	−5.60	2230	25	41	4	1.5	Wolf et al. (2012)

* Percentages of clay and sand were estimated from the reported soil texture class. If no organic layer was mentioned, we assumed that it was absent (i.e. thickness of 0 cm).

that inhibition by high NO_3^- levels in N addition plots on CH_4 production was unlikely (Koehler et al., 2012). Instead, there were other supporting indications that methanotrophic activity was N limited aside from the negative correlation of soil CH_4 fluxes with NO_3^- concentrations: soil-air CH_4 concentrations and contents (or the total amount of CH_4 in a soil-air volume) down to 0.4 m depth were 30 % lower in N-addition than in control plots, and the minimum CH_4 concentration of 552 ± 42 ppb was reached at shallower depth (already at 0.40 m) in N-addition than in control plots (only at 1.25 m depth) (Koehler et al., 2012). It should be noted that these patterns were not influenced by WFPS because there were no differences in WFPS between control and N-addition plots at all depths. The reason why we did not detect significant differences in soil CH_4 fluxes despite stimulated CH_4 uptake by chronic N addition is first due to the large spatial and temporal variations of CH_4 fluxes (Fig. 3a and c). Similar large variability was reported for tropical lowland forest soils and was attributed to production of CH_4 by termites or in microsites of anaerobic conditions, and to temporal patterns of rainfall and soil moisture contents (Verchot et al., 2000; Davidson et al., 2004; Koehler et al., 2012). Second, CH_4 consumption was also largely limited by gas diffusion as shown by the positive correlation of CH_4 fluxes with WFPS (Table 2). Even if N addition stimulated methanotrophic activity, the supply of CH_4 as substrate from the atmosphere to the soil through diffusion did not change, and thus chronic N addition did not necessarily result in a larger CH_4 uptake rate. Stimulation of methanotrophic activity may be explained by a shift in N nutrition of type II methanotrophic bacteria from energy-demanding N_2 fixation to assimilation of soil mineral N (Bodelier and Laanbroek, 2004; Koehler et al., 2012), of which the NO_3^- concentrations had increased under chronic N addition (Fig. 2b).

In the montane forest soil, there was also an indication that methanotrophic activity was stimulated by chronic

N addition as shown by the negative correlations between CH_4 fluxes and NH_4^+ concentrations of the organic layer. However, this N-stimulated methanotrophic activity was masked by the frequent CH_4 emissions. The frequent CH_4 emissions in this wet montane forest soil indicated the regulation of WFPS of the mineral soil on CH_4 fluxes, as was shown by their positive correlation when all CH_4 fluxes are included in the statistical analysis (Table 2). WFPS did not only regulate CH_4 fluxes through the diffusive limitation of CH_4 as substrate for methanotrophs but also through the occurrence of anaerobic conditions for CH_4 production. Indeed, the WFPS of this montane forest was high throughout the year (Fig. 1b), and our ancillary measurements of WFPS at various depths in the mineral soil of these plots, conducted monthly during October 2008 to January 2010, showed WFPS between 96 ± 1 % and 88 ± 1 % from 0.20 m down to 1.25 m depth. Such high WFPS may have favoured CH_4 production and thus the frequent CH_4 emissions from all eight plots. This was probably the principal reason why we were not able to detect potential differences in CH_4 uptake rates between control and N-addition plots despite an indication of N limitation on CH_4 consumption. Exclusion of one pair of control and N-addition plots that strongly dominated the CH_4 emissions during our four-year measurements did not change the statistical trend even though the mean CH_4 uptake rates in the N-addition plots were seemingly larger than the control plots in all years (Table 1).

4.3 Consequences of chronic N deposition on soil CH_4 fluxes from tropical forests

Nine to twelve years of N addition to a lowland forest and one to four years of N addition to a montane forest did not affect soil CH_4 fluxes, although we found indications that CH_4 consumption may have been N limited at both sites. We proposed the following reasons why such N-stimulated CH_4 consumption did not lead to statistically larger CH_4 uptake: (1) for the moist, lowland forest soil, this was caused by limitation of CH_4 diffusion from the atmosphere into the clayey soils, particularly during the wet season when WFPS was high; (2) for the wet, montane forest soil, this was due to the high WFPS in the mineral soil throughout the year, which may not only limit CH_4 diffusion from the atmosphere into the soil but also favours CH_4 production; and (3) both forest soils showed large spatial and temporal variations of CH_4 fluxes. The lowland forest soil showed occasional but low CH_4 emissions whereas the montane forest soil showed more frequent CH_4 emissions with a few exceptionally large emissions (Fig. 3). Accordingly, such high CH_4 concentrations in the soil provide large amounts of substrate for methanotrophy and favour N limitation on methanotrophic bacteria (Bodelier and Laanbroek, 2004).

Our results contrast with the only published study about N-addition effects on soil CH_4 fluxes from (sub)tropical forests, which was conducted in China, where increasing N addition rates resulted in decreasing CH_4 uptake rates. These results were attributed to several possible causes: high N status, low pH values and Al toxicity (Zhang et al., 2008; Zhang et al., 2011). Although our lowland forest soil also had a high N status (Corre et al., 2010) and our montane forest soil also had low pH and high exchangeable Al (see Sect. 2.2), the differences in site conditions between our sites and this forest in China are that the Chinese site had suffered decades of high N deposition (Table 3) leading to soil pH values below 4.0, exchangeable Al of $> 400\,\mathrm{mg\,Al\,kg^{-1}}$ even in the control plots, and never emitted CH_4 during the first year of measurements. Sub-atmospheric soil CH_4 concentrations are possibly prevalent in this Chinese site, and in such conditions methanotrophic activity is less likely to be N limited (Bodelier and Laanbroek, 2004).

If our explanation for the contrasting effects of N additions between our study sites and that of Zhang et al. (2008) holds up throughout the tropics, it is unlikely that elevated N deposition on tropical forests will lead to a rapid reduction in CH_4 uptake. We expect that in tropical montane forests, which typically have low N availability, N deposition may stimulate CH_4 oxidation at sites where occasional CH_4 emissions occur or will cause no change in CH_4 uptake at sites where no CH_4 emissions occur. In tropical lowland forests, which often have a high N availability, N deposition only appears to inhibit CH_4 uptake if soil pH values have become so low that considerable Al toxicity occurs. In other situations, it seems more likely that N deposition will not affect CH_4 fluxes or may even stimulate CH_4 uptake. Whether N additions to tropical forests with N-limited methanotrophic activity can indeed stimulate soil CH_4 uptake remains to be seen. The most likely time when CH_4 uptake may be stimulated by N additions is during dry periods/seasons when CH_4 supply from the atmosphere is not or less limited by gas diffusion. The most likely place where CH_4 uptake may be stimulated by N addition is in forests with a strong seasonal rainfall where occasional CH_4 emissions occur during the rainy season and strong uptake occurs during the dry season.

Acknowledgements. We thank S. Joseph Wright, one of the PIs of the Gigante nutrient manipulation experiment, for hosting our study, valuable discussions and support; Oliver van Straaten for discussions and reading an earlier version of this manuscript; James Dalling for his help during the selection of the montane forest site; Rodolfo Rojas, Carlos Sanchez, Erick Diaz, Ignacio Del Cid, Olivier Gonzalez, Omar Hernandez and Rufino Gonzalez for their dedicated assistance during field measurements; Norman Loftfield, Milton Garcia and Jaime Florez for their help with the equipment; the Smithsonian Tropical Research Institute and ANAM, Panama, for extending excellent logistical and technical support; the laboratory staff of Soil Science of Tropical and Subtropical Ecosystems, for their assistance with laboratory analyses, and the three referees

for their thorough and helpful reviews. M. D. Corre acknowledges funding from the Robert Bosch Foundation (Germany) for her independent research group NITROF, and from the Deutsche Forschungsgemeinschaft (Co 749/1-1). B. Koehler acknowledges further funding by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning and from Erwin Zehe at the Karlsruhe Institute of Technology, Germany.

This Open Access Publication is funded
by the University of Göttingen.

Edited by: Z. Jia

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