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Interactive Comment

# *Interactive comment on* "Indications of nitrogen-limited methane uptake in tropical forest soils" *by* E. Veldkamp et al.

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Answers to referee #1:

'The paper "Indication of nitrogen-limited methane uptake in tropical forest soils" deals with impacts of nitrogen deposition on alteration of the sink strength of tropical forest soils for atmospheric CH4. Despite the finding that elevated nitrogen deposition can reduce CH4 uptake in temperate forest soils, so far data of tropical forest soils are quite scarce. Considering the potential increase of N deposition in tropical regions in the near future; the manuscript deals with an important topic worthwhile to be published in Biogeosciences. The datasets presented are of high scientific value since they are detailed (fluxes and environmental conditions) and long-term (> 4 years). The manuscript is mostly clear with regard to objectives and results presented. However,



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there are some methodological problems which might affect results, discussions and conclusions (see comments below).'

Answer: First we would like to thank referee #1 for sharing his/her insights with us which have been very helpful in improving the manuscript. We are glad that referee #1 recognized the importance of multiple year studies and we would like to take to opportunity to address concerns of referee #1.

General comments:

'The experimental setup for impacts of N deposition on soil CH4 exchange is not fully clear. Why 125 kg N urea, only rainy season fertilization only in the lowland but every quarter in the upland forest. It seems that this experiment is rather a nutrition experiment for forest growth than targeted to impacts of N-deposition on soil CH4 exchange.'

Answer: This experiment was not only set up to study the effects of N-enriched condition on soil trace gas fluxes (including CH4) but also as part of a long-term ongoing nutrient manipulation experiment in lowland tropical forest, which is the only one so far till present. This collaborative experimental set up is not unique only to our lowland forest site but also common to many nutrient manipulation experiments in tropical forests (e.g. Hawaii, Puerto Rico, Ecuador), which are aimed to address multi-disciplinary research questions. We don't think this is a weakness but as many researchers would agree we consider this a big advantage. Why 125 kg N ha-1 yr-1? Such rate is average, considering the rates of N fertilization (100 – 150 kg N ha-1 yr-1; (Hall and Matson, 2003; Cleveland and Townsend, 2006; Mo et al., 2008) that were used to investigate soil trace gas fluxes. For our lowland forest with a pronounced dry season, we avoided to apply the granular urea during the dry season when the fertilizer will stay as granules for a long time. During the rainy season, within six weeks from an N application, urea is hydrolyze and processed in the soil N cycling (trends of soil mineral N concentrations following intensive measurements after an N application from our sites were reported by (Koehler et al., 2009b). This is the main reason for the application of the urea during



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the rainy season in the lowland forest, whereas in the montane forest, there is no dry season and N was applied every quarter. We could not spread the application of the amount of N more often than 4 times a year because our lowland site has no road access such that we have to drive a boat and our montane forest site is 600 km away from our laboratory.

'For that reason your soil NH4+ concentrations are rather high (e.g. »10 mg kg compared to Zhang et al., 2008 < 10 mg kg-1). Surprisingly also soil NH4+ concentrations in the control treatment (Fig 2a) which sometimes even exceeds the concentration in the N-addition plots of the lowland forest. For the montane forest differences in soil NH4+ only appear in year 3 and 4. Under aerated conditions (September year 3) there are higher uptake rates in the control. Unfortunately in year 4 you have very high WFPS which may overwrites the impact of soil NH4+ to CH4 exchange. Independent from the correlation using all year data (majority when there is no difference in soil NH4+ between control and N-addition) I would put some emphasis on times when you observe differences in soil NH4+ across treatments. For that reason I would extend Table 1 and would not present data only at yearly basis since CH4 emissions seem to dominate the annual values.'

Answer: We appreciate this thoughtful suggestion. However we need to point out two reasons why our measured NH4+ concentrations should not be directly compared with the values reported by (Zhang et al., 2008). First, our group found out and published that in tropical forest soils the processes of N mineralization and nitrification continue after soil samples are taken in the field, transported to the laboratory, and stored in a refrigerator (Arnold et al., 2008). If this is ignored, this will result in NH4+ values which are generally lower and NO3- values which are much higher than when extraction is done immediately in the field. Depending on how long the soils samples are stored, NO3- becomes more and more dominant while NH4+ becomes less and less as nitrification continues. Also soil N-cycling rates measured from stored samples do not reflect the soil N-cycling measured in situ (Arnold et al., 2008). Since this discovery, our group



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always conducts the extraction of soil mineral N immediately in the field and we do not first take the soil samples, store/transport and do the extraction in the laboratory, which we suspect happened only after 1 day or even more for many other studies. We have pointed this out in our earlier publications on soil N cycling (Corre et al., 2010) and soil mineral N (Koehler et al., 2009b) from our present study sites. When soils are not extracted for mineral N right in the field, the resulting values will be more a reflection of the transport and storage time and conditions rather than a reflection of the original mineral N concentrations. Using immediate extraction in the field, our group has published extractable NH4+ values of four tropical forest locations in Ecuador without significant N deposition which were all higher than 10 mg kg-1 (Arnold et al., 2009). The second reason why our measured NH4+ concentrations should not be directly compared with the values reported by Zhang et al. (2008) is the high N deposition in the site of Zhang et al. (2008). Zhang et al., (2008) reported N deposition of more than 30 kg N ha-1 yr-1 which has been going on at least since the 1990's. This has very likely led to changes in the soil N cycle as we also detected in the N addition plots: increases in gross N mineralization and gross nitriïňĄcation rates, and decreases in microbial biomass and NH4+ immobilization rates compared to the control (Corre et al., 2010). The result of these multiple changes to the N cycle in Panama were that the extractable NH4+ was low while extractable NO3- was high in the N addition plots. We expect that in the study site of Zhang et al., (2008) similar changes to the N cycle happened as a result of chronic N deposition, which makes it not a good reference to compare to our sites (of which the control plots still receive much lower amounts of N deposition (Corre et al., 2010). Finally, the treatment differences pointed by referee #1 (taken from above: i.e. 'control treatment (Fig 2a) which sometimes even exceeds the concentration in the N-addition plots of the lowland forest; for the montane forest differences in soil NH4+ only appear in year 3 and 4') are not based on statistical tests but only on visual guessing from Fig. 2. For the lowland forest, NH4+ concentrations did not differ (meaning statistically) between control and N-additions plots in the first 2 years (2006-2008; Koehler et al., 2009), in the last 2 years and in the whole 4 years (both statistically tested in our present study and mentioned in section 3.1). For the montane forest, it is also not true that the treatment differences in $NH_4^+$ concentrations only appeared in year 3 and 4. We reported that $NH_4^+$ concentrations differed between control and N-addition plots in the first 2 years of treatment (Koehler et al. 2009). We chose to give the statistical analysis results for the entire 4 years in section 3.1, because it is redundant to keep mentioning that in year 1, 2, 3 and 4 $NH_4^+$ concentrations differed between treatments. Also, what referee #1 mentioned (i.e. aerated conditions September year 3) as aerated condition, we disagree on where this is based from, when in fact there were no differences in WFPS between treatment or years (see 3.1, Fig. 1b). If what referee #1 meant for aerated condition was the high $NH_4^+$ and $NO_3^-$ in Sept of year 3, we tested $CH_4$ uptake rates for this period and there was no significant difference between treatments. We suggest not to focus only on the means but also on the SE bars or values, because these are indicators of whether the means are statistically significant or not. Thus, we did not follow this suggestion of referee #1, because the basis of his/her suggestions does not hold true for our data. Besides, Table 1 is for the purpose of giving the annual estimates in case readers or reviews in the future will be interested for the annual values. But we give the times-series measurements in the figures and averages $\pm$ SE of measurements in the Results' texts.

'Furthermore, the separation of fluxes in short term (first 6 weeks after fertilization) and long term (> 6 weeks) is somehow artficial. Why did you not cluster in dependence of $NH_4^+$ (and $NO_3^-$) soil concentrations? This would allow also a more detailed comparison on basis of soil DIN to other studies in temperate and tropical forests (Zhang et al., 2008, 2011). I would suggest also including soil DIN, and WFPS into Table 1.'

Answer: Earlier studies in the humid tropics have shown that the elevated mineral N concentrations occur within a month following N addition (Keller et al., 1988; Steudler et al., 2002). We were able to confirm this for our study. Clustering observations based on DIN values as suggested by referee #1 is actually what we did in using the 6 weeks as the cut-off period. During the first 2 years when we intensively measured trace gas



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fluxes and soil mineral N concentrations (e.g. after 1, 3, 7, 14, 30, 45 days following N addition), we observed maximum $NH_4^+$ and $NO_3^-$ concentrations within the ïňĄrst 2 weeks and concentrations went down to the background levels (i.e. levels prior to an N application in the N-addition plots) at most after 6 weeks of N application (also shown in Fig. 2). Based on this, we decided on using an equilibration period of 6 weeks and this is not artificial because this decision was based on actual measurements of mineral N. We believe we explained this sufficiently in section 2.1. As to the suggestion to use extractable N to cluster our measurements and for comparison of our soil mineral N values to other studies, we refer to our discussion above on the methodological issues of mineral N extraction. Soil DIN ($NH_4^+$ & $NO_3^-$) is displayed in Figure 2, while WFPS is displayed in Fig. 1a, b. The large seasonal variability of both DIN and WFPS illustrate that presenting these data in figures are more useful rather than averaging away these temporal patterns by using mean values for each year. Besides redundancy in presenting values already in Figures also in Tables, Table 1 is for the purpose of giving annual values and the figures are for the spatial (means/SE on each sampling day) and temporal patterns of the entire 4 years.

‘Due to the above I am not sure if the conclusions taken so far might change. Taking into account the wealth of data you have I wonder why you did not apply multiple regression (even though linear mixed effect models are mentioned in the statistics part) rather than showing Pearson correlation coefficients in Table 2.’

Answer: Referee #1 is confusing linear mixed effects models (that are used to assess treatment differences on variables measured repeatedly over time) with regression analysis (that is a parametric test of relationship between dependent and independent variables). However, we appreciate this suggestion; before we wrote the manuscript we discussed in our group whether to include multiple regressions in the results. We decided to only present the Pearson correlation coefficients since our goal was to explore which factors potentially affect $CH_4$ fluxes. Multiple regressions are typically used to make predictions, and given the methodological differences that exist (e.g. in the way

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mineral N is extracted, see above) a regression might lead to wrong predictions if mineral N values were not obtained by extraction in the field.

‘Under this aspect and keeping in mind that you studied only two tropical forest systems, I am not fully convinced at the moment that the very general statement of N limited soil $CH_4$ uptake in tropical forest soils can be exposed as presented in the title and conclusions. Even though uptake rates have a negative sign (due to the perspective of the atmosphere) Iwould recommend presenting correlations not as $CH_4$ fluxes (i.e. more negative values are smaller fluxes) but rather as $CH_4$ uptake rates (i.e. more negative values represent higher uptake rates). Thus, P6008 Ln16ff your correlation would be not negative but positive as mentioned in the text with increased $NO_3$ stimulated $CH_4$ uptake.’

Answer: We appreciate the concern of referee #1 and are aware that we only studied two forest ecosystems and that together with the study of Zhang et al. (2008) only three tropical forests have been studied worldwide. First, we want to point out that the correlations analyses were conducted on actual values (including uptakes and emissions and hence it is not correct to refer to the correlations as only $CH_4$ uptake), and to facilitate the meaning of negative or positive correlations, we specifically explained this in lines 336-340 because we know this is not easy to grasp. Furthermore, the two ecosystems that we studied were extremely different and both did not show any signs of inhibition of $CH_4$ uptake, but both ecosystems showed indications of N limitation on $CH_4$ uptake. Our attempt to explain the differences with the study by Zhang et al. (2008) and extrapolate to the tropics is of course speculative, and we believe that we are quite careful in formulating this by using phrases like: “If our explanation for the contrasting eïňĂects of N additions between our study sites and that of Zhang et al. (2008) holds up throughout the tropics. . .”. Furthermore we do not present our study as evidence that $CH_4$ uptake is N-limited, but we write both in the title and in the conclusions that our study only shows “indications” of N limitation on $CH_4$ uptake. In the concluding paragraph we even leave the consequences of our study open by phras-



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ing: “Whether N additions to tropical forests with N-limited methanotrophic activity can indeed stimulate soil CH4 uptake remains to be seen”.

Specific comments: ‘P6011 Ln4: recent is almost 10 years ago, delete “Only recently,”’

Done

‘P6011 Ln10ff: this section needs to be shorted and put rather into discussion section’

Answer: We followed the suggestion to shorten this section. However, we did leave it in the introduction since this is the only other study under (sub-) tropical conditions which was comparable to our study.

‘P6013 Ln26ff: provide also bulk density measurements, since BD is one of the most important soil physical properties for diffusion processes.’

Answer: We followed this suggestion and included the information on bulk density.

‘P6014 Ln15ff: give info on sampling frequency.’

Answer: We included information on sampling frequency

‘P6015 Ln11ff: trapezoid rule: give more details, citation?’

Answer: The trapezoidal rule is a standard method for approximating integrals. It basically involves linear interpolation between the measured flux rates in time. Since this is a common mathematical method, a citation is not needed. We used the same trapezoidal method for estimating annual soil respiration and N-oxide fluxes (Koehler et al., 2009b; van Straaten et al., 2011). To clarify in the text we added in brackets: (linear interpolation of time intervals between measured ïňĆux rates).

‘P6018 Ln1ff: values are mean values, data presented in Tables are annual emissions. Should be harmonized or as suggested above make a new Table with seasonal CH4 uptake rates, wfps, and DIN.’

Answer: We chose on purpose to present the mean values in the text and annual



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values in the Tables. The reason is that if we compare sites or treatment effects, we should conduct the statistical analysis on the actual measurements across time, as represented by the mean/SE, and mentioned these with P values in the text. This is what we did on the soil respiration from the same sites which we also published in Biogeosciences (Koehler et al., 2009a). Since our study is the first tropical study that was conducted over a four-year period, readers or future reviews will also be interested in inter-annual variability, which is why we present these data in Table 1. Readers interested in temporal variability would otherwise have to guess the inter-annual variability from Figure 3. Giving the mean values for each year and separately for dry and wet season will also be misleading, because how would the readers extrapolate the seasonal average to an annual value when they know that there was seasonal pattern of $CH_4$ fluxes. This seasonal pattern is exactly the reason why the trapezoidal method is appropriate for estimating annual fluxes.

'P6021 Ln8ff: add already the finding of Ln14 to this sentence'

Answer: We did not follow this suggestion since the finding in Ln14 is a different topic which in our opinion deserves separate discussion. Besides, reporting the correlation with N deposition without pointing out the overall low N deposition values and the correlation with annual rainfall might be confusing.

'P6022 Ln6ff: This is a bit confusing, since population increase of methanotrophic bacteria would per se first increase uptake rates. However, high $CH_4$ concentrations at times of high rainfall should correlate with low oxygen concentration which anticorrelates with increase population increases. Also, your soil $NH_4$ concentrations are comparable high for tropical forest. This may indicate limited nitrification under high soil moisture conditions as shown in Figure 1.'

Answer: At the microsites where $CH_4$ is being produced, we would of course expect low oxygen concentrations. However, production of $CH_4$ in the soil profile does not mean that the entire soil is anaerobic, actually most of the soil profile is aerobic. In





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anaerobic microsites, $CH_4$ is produced and diffuses into the aerobic soil matrix where $CH_4$ concentrations above 2 ppm may stimulate growth of methanotrophic bacteria.

'P6022Ln26ff. Even though soil gas concentrations of $CH_4$ were published'

Answer: The $CH_4$ concentrations of the lowland forest were published and we refer to them (Koehler et al., 2012) in this part of the discussion section. We do not present the soil $CH_4$ concentration data from the lowland forest as new in our present manuscript. None of the data on $CH_4$ fluxes and soil $CH_4$ concentrations in the montane forest (included in the discussion as supporting evidence) presented in our manuscript was ever published before. Only the soil characteristics that we mentioned in the site description and the first 2 years of soil mineral N, WFPS and temperature, which were used as supporting data in our earlier study (Koehler et al. 2009). This is now clearly specified in the legends of Figs 1 and 2.

'Table 1 statistic is missing-'

Answer: This was done on purpose since Table 1 contains estimated annual fluxes by trapezoidal method, i.e. interpolation over time between the actual measured fluxes, similar to what we used for soil respiration and N-oxide emissions from the same study sites (Koehler et al. 2009a, b). In our manuscript we conducted the statistical analysis on actual measurements across time. On interpolated values we do not conduct statistical analysis.

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