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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 #3:

'Understanding effects of nitrogen deposition and fertilization of methane uptake by soils, especially tropical soils is of utmost importance to better understand sinks and sources of global methane. Long term studies, under relevant conditions are rare and in this respect the present study and dataset obtained is of high value. Long-term nitrogen fertilization effects on methane flux was assessed in two sub-tropical forest soils with very different moisture regimes. Although primarily controlled by soil physical factors, the authors propose that methane uptake by these soils was N-limited as indicated by correlative evidence with soil mineral ammonium and nitrate.'

Answer: We thank referee #3 for the comments and suggestions. We would like to point out that our sites are located in the tropics, not the subtropics. Our soils do not only differ in soil moisture regime but also in the degree of soil development (and thus soil characteristics) and N status (see section 2.2 Site description). Below we address the specific points mentioned by this referee:

'Comments: 1: The manuscript has to be checked by a native speaker. In terms of style and grammar there is a lot to be improved. Besides grammar and style, the manuscript is rather long for the actual data that is shown and discussed.'

Answer: The revised manuscript was checked by a native speaker. We think that our manuscript is not exceptionally long for the data presented: the manuscript includes four years of field data and a review of available data on CH4 fluxes from tropical forests. Overall, our manuscript is 30 pages long (in the PDF format of BGD). We have looked through a number of other experimental terrestrial studies presently under discussion in BGD: from the twelve examined manuscripts the total pages ranged from 28 to 54 pages. Only one manuscript was shorter than our manuscript.

'2: I think in the introduction the authors have to mention the microbiology behind methane consumption in soils. Mention high vs. low affinity methane oxidizers. Mention that methane oxidizers are not always strict obligatory methanotrophic. Also mention that not only methane but also ammonia oxidizers can oxidize methane. I think, some microbiology is necessary for the reader to understand the interpretations later on.'

Answer: We thank referee #3 for this good suggestion and we included all these points in the introduction as follows (lines 74-88):

'CH4 fluxes at the soil surface are the result of methanogenesis and CH4 oxidation, which may occur simultaneously in aerated soils (Yavitt et al., 1995). The microorganisms involved in CH4 oxidation are methanotrophic bacteria and ammonium-oxidizing bacteria. Most methanotrophic bacteria use CH4 as their only source of carbon and

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energy and all use methane monooxygenase in the first step of CH4 oxidation (Hanson and Hanson, 1996). Methanotrophic bacteria are separated into Type I and II according to their biochemical pathways of oxidizing CH4. Type I methanotrophs are generally non N-fixing organisms while Type II methanotrophs can fix atmospheric N2 but can also assimilate mineral N (Hanson and Hanson, 1996). Depending on the CH4 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 CH4 concentrations (e.g. in rice fields), and the other group contains 'high affinity' methanotrophs which are able to make use of the atmospheric CH4 concentrations (around 1.8 ppm). Ammonium-oxidizing bacteria can also oxidize CH4 through the enzyme ammonia monooxygenase which can also react with CH4 instead of NH4+ (Bédard and Knowles, 1989).

'3: A number of times reference is made to reviews on a specific topic mentioning a certain fact. I would rather see the reference cited that show actual data proving the fact mentioned. 1: page 6009 (line 18): Conrad 2007 for 5% methane uptake by upland soils. 2: page 6010 (line 23): Conrad 1996 for inhibition of methane oxidation by ammonium. 3: Page 6011 (line 2); Conrad 2007 for inhibition by NOx of methanogenesis.'

Answer: In principle we agree with referee 3 that it is preferable to cite the original publication instead of a review. However, in some cases we did not have the publication available especially when they are books or book chapters. In other cases it is not always clear who had made the first claim. We have replaced the following references as suggested by the referee: Page 6009 (line 18) Conrad, 2007 was replaced with Reeburgh, 2003 (line 57) Page 6010 (line 23) Conrad, 1996 was replaced with Bédard and Knowles, 1989 (line 116) Page 6011 (line 2) Conrad 2007 was replaced with Klüber and Conrad, 1998 (line 124)

'4: Materials and methods: I would suggest to first give site description and experimental design followed by description of the N-amendment and flux measurements.'

Answer: The order proposed by Referee #3 is almost the same as how we have organized the Materials and methods in the present manuscript. The only difference is that we have as a first section ‘approach’, which in our view has to be mentioned first because this is where we explain the rationale for adding N at relatively high doses. Following ‘approach’ we have exactly the order proposed by referee #3: ‘Site description and experimental design’ this includes at the end the description of the N-amendments. This is followed by the ‘Flux measurements’. We have chosen not to change the order because we do not see the advantage.

’5: In section 2.5 a comprehensive statistical analyses is described for analyzing fertilizer and site effects corrected for the sampling time. Where can I find the outcome of this analyses? Should this not be displayed somewhere in Table 1?’

Answer: In the original and revised manuscript, the outcome of the stat analysis are clearly presented throughout the ‘Results’ section e.g. when sites and treatments are compared (lines 322, 326, 328, 330-332, 337, 356, 367 and 374; previously we only give the P values that are significant but now we give both significant and non-significant P values to clearly support all our claims of whether differences are detectable or not). We present the results of the statistical analysis on actual measured values across four years and the means $\pm$ SE and P values are given in the text. Since the values in Table 1 are interpolated over time between actual measured fluxes, we did not conduct statistical analysis on these annual estimates. We employed the same statistical methods in our study on the soil respiration from the same sites which was 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 the annual values in Table 1. The seasonal pattern of CH4 fluxes is exactly the reason why the trapezoidal method is appropriate for estimating annual fluxes (Table 1) and not by just simply extrapolating the average fluxes within a year into an annual value. Statistical comparisons, however, should always be conducted on the actual measured fluxes.

’6: I find the conclusion that methane consumption in montane forest soil is N-limited not very strong, or at least only one perspective is taken. The authors base this conclusion on negative correlations between the fluxes and ammonium concentration. Looking at the data in the montane soils, organic as well as mineral layer, there is a positive correlation with nitrate and flux in the control soils. This can mean that with higher nitrification the flux increases due to inhibitory effects of nitrification on methane consumption (via nitrite or pH). In case of the negative correlations between ammonium and flux it may very well be that nitrifiers are stimulated that subsequently oxidize more methane. The consumption of methane by nitrifiers is not considered.’

Answer: We thank Referee #3 for these considerations. Making deductions how mineral nitrogen affects CH4 oxidation has been done quite regularly before e.g. (Chan and Parkin, 2001). However, most studies do this in the laboratory while we chose to use mineral N values extracted in the field (please see our answer to referee #1 on this under general comment 2). We did not ignore the potential effects of nitrification on CH4 fluxes in the montane forest. From Figure 2 d and f, it can be seen that the measured extractable NO3- values were one to two orders of magnitude lower than the extractable NH4+ (please note the different scale on the Y-axis between Fig. 2 c and d and between Fig. 2 e and f). The opposing pattern of NH4+ and NO3- across four years was the cause of this opposing trends in correlations with CH4 fluxes (negative correlation with NH4+ and positive correlation with NO3-), as we mentioned in the original manuscript (Page 6018 Line15). The opposing patterns of NH4+ and NO3- across four years was probably related to the high water-filled pore space during the wet year of 2009 (last year of measurement) as shown in Fig. 1b (clearly shown by the mineral soil). However, differences in NO3- levels between years were all not statistically significant, because we are talking about only small values (barely above the detection limit). This is also the reason why the correlations of total extractable mineral N (NH4+ + NO3-) with CH4 fluxes follow the correlations of NH4+ as we reported in the original manuscript on Page 6018, lines 16-19. As can be expected, these low extractable NO3- values point at extremely low nitrification rates. In an earlier publication, the net nitrification rates in the control plots of the montane forests were reported as 15.17 ng N cm-2 h-1 in the organic layer and even negative or net NO3- immobilization (-3.17 ng N cm-2 h-1) in the mineral soil, while the corresponding net mineralization rates were 258.77 ng N cm-2 h-1 and 41.44 ng N cm-2 h-1, respectively (Koehler et al., 2009b). Gross nitrification rates were only 39 mg N m-2 day-1 in the organic layer and 2 mg N m-2 day-1 in the mineral soil while the corresponding gross N mineralization rates were an order of magnitude higher: 532 mg N m-2 day-1 in the organic layer and 570 mg N m-2 day-1 in the mineral soil (Corre et al., 2010). At such low nitrification rates it is very unlikely that nitrification played a significant role in CH4 uptake. This becomes even clearer when we look at the nitrification rates in the N amended plots. In the N addition plots in the montane forest both net and gross nitrification rates increased in the organic layer (Koehler et al. 2009a, Corre et al. 2010). Had nitrification played an important role, we would have found a positive correlation between NO3- and CH4 fluxes in the N addition plots, but this was not the case. To make clear that we did consider nitrification as a potential process affecting CH4 uptake we have added the following section in the discussion (line 436-447): "While the positive correlation of NO3- with CH4 fluxes may indicate inhibitory effects of nitrification on CH4 consumption, we think that this is very unlikely since the NO3- concentrations were one to two orders of magnitude smaller than the NH4+ concentrations (compare Fig. 2c, d and 2e,f and note the difference scales on the Y axis). Furthermore measurements of gross and net nitrification showed very low nitrification rates (Koehler et al., 2009b; Corre et al., 2010)."

'The other line of evidence the authors take is that higher methane concentrations (evidenced by periods of emission) can lead to growth of methanotrophs needing more nitrogen. However, the authors indicate methane concentrations of 2 ppm above soils surface, especially during heavy rainfall. This is barely above atmospheric concentrations. The concentrations in the soil indicated are even lower. Hence, I do not think that this is reason to belief that this would lead to N-limited growth of methanotrophs. I would argue that maybe facultative MOB feed on acetate during events of possible anoxia increasing population levels needing more nitrogen. Hence, I am not convinced of N-limitation purely based on correlations. The authors have very narrow way of explaining their results. I would suggest to take the microbiology of methane and ammonium oxidation more into account.'

Answer: Referee #3 correctly notices that we argue that higher methane concentrations in the soil can lead to growth of methanotrophs needing more nitrogen. However, the referee wrongly assumes that we base our argument on the periods of CH4 emission and the CH4 concentration of 2ppm above the soil surface. We have clearly presented the data summary of our measurements of soil air at various depths in these plots as basis of our arguments (Page 6021 line23 to Page 6022 line 6; Page 6022 lines17-24). At both sites auxiliary measurements of CH4 concentrations in the soil profile were conducted and those measurements showed concentrations above 2 ppm in 11% of the observations in the lowland forest and even in 34% of the observations in the montane forest. For the lowland forest, these soil-air CH4 concentrations were published earlier and we refer to this publication (Page 6022, lines 17-24): "…ancillary measurements of CH4 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-Jan 2009) showed that 11% of the observations had higher soil-air CH4 concentrations than the average soil-air CH4 concentrations at a specific depth. These high soil-air CH4 concentrations occurred in any depths of both N-addition and control plots regardless of seasons, indicating that inhibition by high NO3- levels in N addition plots on CH4 production was unlikely (Koehler et al., 2012)." For the montane forest we refer to these measurements on Page 6021, line 24 and further: "…we had ancillary measurements of the soil-air CH4 concentrations in our montane forest soil that showed CH4 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 works (Koehler et al., 2012). We found that 34 % of 421 observations had CH4 concentrations in the mineral soil higher than the concentration at 0.10 m above the soil surface of 2.0 ± 0.1 ppm CH4-C, particularly during periods of high rainfall and thus high soil water contents."

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