We would like to thank you for the time and effort taken to review our manuscript “Laboratory measurements of nitric oxide release from forest soil with a thick organic layer under different understory types”**.** We have carefully responded to each of the referees’ comments in the text below, and indicated where we have revised the manuscript. We hope that our revised version of the manuscript will be accepted for publication in BG

Referee #1:

1.1 The method for measuring gravimetric soil water content has not been described in the methods section. The water content is given in numbers between zero and 4, which is odd. I would have expected units of volume water per mass soil or per volume soil, but cannot figure out how these would amount to a value of 4. Please clarify!

>> We will insert a short description of the method for measuring soil water content in section 2.4. The corresponding paragraph (p. 210, l. 8-17) will read as follows:

“***Gravimetric soil water content was measured by tracking the evaporated water vapour throughout the measurement period and relating this temporal integral to the gravimetric soil moisture content observed at the start and end of the measurement period.*** *Soil samples are completely dry within 4 to 7 days. This procedure provides us the response of the net NO release rates over the entire range of gravimetric soil moisture. Gravimetric soil water contents in our study ranged between 0 and 4 kg kg^{-1}.”*

>> Values between zero and 4 gravimetric water content are not unexpected, because the fresh weight of organic soils is often a multiple of their dry weight. For example, Muhr et al. (2008) observed gravimetric water contents up to 2 kg kg^{-1} for organic layers from the Fichtelgebirge, Germany. Because gravimetric water content was directly measured, throughout the paper we used gravimetric water content as explicatory variable. For calculating volumetric water content or water filled pore space (WFPS) from gravimetric water content, bulk and particle density are necessary (both add additional uncertainty to the water content measure used for further analyses). However, for eventual conversion, bulk and particle density for our samples are given in Tab. 3”.

1.2 The soil was incubated in batches of 100 g inside Plexiglas cuvettes. This is a rather large amount. Was the rate of NO release under these conditions still proportional to the amount of soil; at all the different water contents? If not, then only part of the soil acted as the reactive body exchanging the NO with the gas phase. If the soil layer in the cuvette is too deep, then the NO produced in the lower layers will not be exchanged with the gas phase but be (partially) consumed during diffusion to the surface. This is analogous to the incubation of a soil core or to flux measured under field conditions. The reference of NO release, production or consumption to 100 g soil mass may then not be correct.

>> Former experiments showed that net NO release rates increase fairly proportionally with soil mass in the chambers up to 100 g, after which the slope declines. This indicates that from this soil mass onwards gas diffusion through the soil could be limiting. These results are similar to those of Remde et al. (1989) where the NO flux rate was shown to be proportional to the soil mass in the chamber up to 150 g. Above 150 g the relationship between NO flux and soil mass was no longer linear. As in Remde et al. (1989), we found a soil mass of less than 100 g in each chamber to be appropriate.

1.3 A negative relationship between NO production and pH has been observed before, as discussed. This negative correlation does not only hold true for NO production by nitrification but also for denitrification, see papers by Koskinen & Keene (1982) and Nagele and Conrad (1990). There is also an enhanced chemical NO production from nitrite at low pH (VanCleemput & Baert, 1984), which can happen even if nitrite does not accumulate to detectable amounts (it is nevetherless produced during both nitrification and denitrification).

>> Thank you for these helpful comments. We will add the references in the corresponding paragraph (p.227, l.11-21). The paragraph will read as follows in the revised ms:

"Nevertheless, net potential NO fluxes showed a weak relationship with soil pH values. During laboratory incubation measurements, there might have been microsites in the soil samples with a soil pH different from the measured mean pH, indicating that nitrification occurred in microsites having pH higher than the surrounding soil (Paavolainen and Smolander, 1998). That could also be a reason for the relatively high NO emission despite of the low pH values. A pH value between 7 and 8 is ideal for nitrification. However, Paavolainen and Smolander (1998) reported coniferous soils that exhibited acid-tolerant nitrification. In this respect, a series of studies reported relationships between NO exchange processes and soil pH ***(Gödde and Conrad, 2000; Venterea et al., 2004; Nägele and Conrad, 1990). There is also an enhanced chemical NO production from nitrite at low soil pH (Cleemput and Baert, 1984), which can happen even if nitrite does not accumulate to detectable amounts. In contrast, o****ther studies found no strong relationships between NO exchange and soil pH (Dunfield and Knowles, 1998)."*

1.4 Referring to P.228, L.15, the effect of tree species on N_2O turnover has been reported by Menyailo (2006) and Menyailo & Hungate (2006).

>> Thanks for this helpful information! We will add the references in the corresponding paragraph (p. 228, l. 5-19). The paragraph will read as follows in the revised ms:

"One substantial difference between the four understory vegetations, where the soil samples were taken under, is, that spruce and blueberries are both higher plants with woody and larger roots in comparison to moss and grass. The root system affects the physical, chemical and biological properties of soil. Roots are vital sources of food and energy for microorganisms like nitrifiers and denitrifiers. Slemr and Seiler (1991) found, that the presence of roots may stimulate the NO emission rate. Also, Stöhr and Ullrich (2002), and Stöhr and Stremlau (2006) demonstrated that roots can generate NO. Vos et al. (1994) measured 2 to 12-fold higher NO emissions from plots covered with green manure than from fallow plots, probably caused by increased microbial activity in the rhizosphere of the green manure plots compared to the bare soil. Unfortunately, no field studies exist examining the influence of plant roots on NO emissions. ***However, Menyailo (2006) and Menyailo and Hungate (2006) have reported a effect of tree species on nitrous oxide exchange.*** *A few studies have also shown a strong influence on nitrous oxide emissions by roots (Mosier et al., 1990) and it is generally accepted that denitrification decreases with distance from plant roots (Smith and Tiedje, 1979a). The size and the density of the nitrifier and denitrifier communities are also influenced by plant roots (Philippot et al., 2009)."*

1.5 The ms frequently uses the term "fumigation". I found this term awkward. This term is normally used in soil science when treating the soil with toxic fumes in order to sterilize it. I think a term such as "gassing" or "flushing" would be more appropriate.

>> We will replace "fumigation" by "flushing" in the revised ms.

1.6 The paragraph 2.6 (calculation of Q_{10}) would be better placed after paragraph 2.8. Otherwise, the reader does not yet know what net potential NO flux is.

>> In the revised manuscript we will place paragraph 2.6 after paragraph 2.8.

1.7 P.211, L.14: J_{opt} is probably wrong and must be replaced by J(teta). Please check!

>> J_{opt} can also be expressed as $J_{\theta opt}$. We will clarify this in the revised ms.

1.8 Typo in P.206, L.6: centimeter instead of centimetre

>> We will correct this in the revised ms.

1.9 Typo in P.206, L.19: Deschampsia not Descampsia

>> We will correct this in the revised ms.

1.10 P.206, L.23: better replace "in this direction" with "in this respect".

>> We will correct this in the revised ms.

1.11 P.208, L.25: Using a spade normally does not allow taking soil cores. This requires a corer.

>> This is correct for taking samples from mineral soils. However, we took our samples from the organic horizon. The volume of a soil corer is too small for a correct measurement of the bulk density. Taking a soil sample with a spade and afterwards determine the dimensions of the sample allows us to take larger soil samples, resulting in a higher accuracy.

1.12 The abbreviations PTFE and PC (P.210) are not explained. I would simply use the full name.

>> We will include the full names (*polytetrafluorethylene and computer*) in the revised ms.

1.13 Typo in P.224, L.18: Therefore, not Therefor.

>> We will correct this in the revised ms.

1.14 P.226, L.13: The significance level for the correlation NH_4^+ with NO consumption coefficient is not shown in Table 4.

>> We will correct this in the revised ms (significance level = 0.10).

1.15 Table 3: Clarify whether it is NH_4^+ and NO_3^- or NH_4^+-N and NO_3^--N.

>> We will change the values in Tab. 3 to NH_4^+-N and NO_3-N, and clarify it in the revised ms.

Referee #2

2.1 p 204, l 20-25: The two last sentences of the abstract should be improved: It is insufficient to state that effects of:"are discussed", instead the most important effects should be highlighted. The circular reasoning in last sentence should be replaced by tighter message.

>> We will delete the last sentence.

>> The second sentence will read as follows in the revised ms:

"*Therefore, as an alternative explanation for the differences in soil biogenic NO emission we consider more biological factors like understory vegetation type, amount of roots, and degree of mycorrhization; they have the potential to explain the observed differences of net potential NO fluxes.*"

2.2 p 205, l 12-14: "Although:" Split this awkward sentence into several shorter ones.

>> We will change this sentence in the revised ms. It will be read as follows:

"*Soils have the potential for acting as a sink for atmospheric NO (Conrad, 1994). Only a few studies provide an indication of soils acting as a sink (Dunfield and Knowles, 1998; Skiba et al., 1994; Slemr and Seiler, 1991)*".

2.3 p 205, l 19-20: "Nitrification…:"Delete this unnecessary sentence.

>> We will delete this sentence in the revised ms.

2.4 P 206, l 19: Deschampsia caespitosa is the correct spelling.

>> It should have said "Deschampsia flexuosa". We will correct this in the revised ms.

2.5 p 206, l 21-24:"Other…:" Delete this unnecessary sentence.

>> We will delete this sentence in the revised ms.

2.6 p 207, l 25 to p 208, l 1: I have qualms about naming soil samples according to ground vegetation following removal of biomass, sieving, and long time storage. Looking at Tab. 3, I get the impression that soil parameters within "vegetation types" differ more strongly than between them. You need to show that this variability is due to the (removed) ground vegetation rather than other factors. This will be difficult with two replicates.

>> (a) The reviewer is right: naming soil samples according to the understory cover is confusing. To avoid this confusion, we will rename blueberry 1 and 2 to "B1" and "B2" and so on throughout the revised manuscript. In addition we carefully reviewed the text of the entire ms and consistently used the introduced abbreviations of soil samples.

>> (b) Two replicates are not a lot. However, the laboratory procedure is very time-consuming. For the determination of a net potential NO flux four different measurements (incubations at two different NO concentrations and two different temperatures) are necessary. Due to the continuous drying cycle each measurement takes up to seven days. As we are able to analyze four soil samples simultaneously, analyzing our eight soil samples still took two months. Additionally we needed two weeks for calibrating the system and other service operations. To compromise scientific needs and laboratory constraints we decided for two replicates only.

2.7 p 208, l 11: “>3” The way soil moisture is presented in this contribution is uncommon outside the soil hydrology community. As you aim for an audience outside this community, you might consider employing another way of presenting soil moisture data.

>> Please, see our reply to comment 1.1 (referee #1).

2.8 p 209, l 20: “chemiluminescence” Shouldn’t it be “chemoluminesence?”

>> Both spellings are correct, but “chemiluminescence” is more common, so we kept “chemiluminescence” in the revised manuscript.

2.9 p 210, l 15-17; “This procedure…:” The presented procedure only gives data for a drying cycle, not wetting one. For CH_4 release, there are reports of hysteresis. Can this be expected for NO as well?

>> We are not aware of studies showing hysteresis effects on NO release. This may be investigated in future. In this study however, again due to the time restrictions (see reply to comment 2.6 b, referee #2), we investigated the drying cycle only.

2.10 p 212, l 1: obtain instead of “obtail”

>> We will correct this in the revised ms.

2.11 p 212, l 16-21: Constructing a Q_{10} based on just two data points is bold, if not impossible.

>> Instead of taking Q_{10} values of the literature we calculated Q_{10} values for each soil sample. Unfortunately, the measurement process is really time-consuming (see our reply on comment 2.6 b, referee #2). Therefore it was not possible to determine more data points for calculating Q_{10} values.

2.12 p 217, l 18-20: “Net NO release…:” You did not examine a soil under blueberry cover. Please find a more suitable expression.

>> Please, see our reply to comment 2.6 a (referee #2).

2.13 p 221, l 8-9: “not to mention…:” Again, I doubt that you really examined the influence of the understory type. In your setup, there were no live roots and the importance of live roots for gaseous N species is well known.

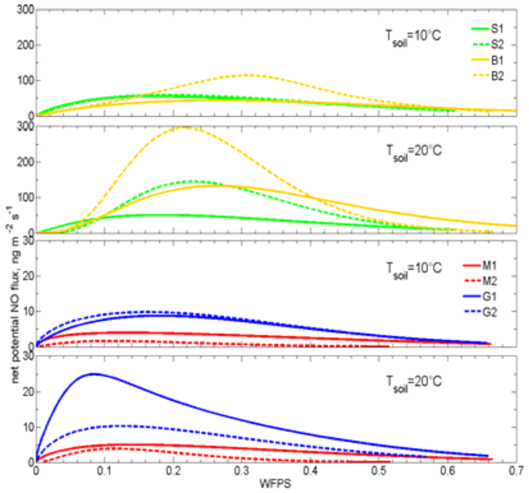
>> We will delete the last part (*"not to mention the influence of different understory types"*) in the revised ms.

2.14 p 223, l 16: "WFPS" Here, you switch to WFPS. Why don't you stick to WFPS throughout the manuscript?

>> We used gravimetric soil water content throughout the manuscript for the reasons outlined in comment 1.1 (referee #1). However, to compare fluxes between different soil types the WFPS is a useful term. WFPS makes different ecosystems comparable.

The following figure presents the net potential NO fluxes versus the WFPS. Comparing this figure with figure 8 of the ms only small differences in the curves of net potential NO flux rates are noticeable. For our study a gravimetric soil water content of 4 corresponds to a WFPS of 0.51 -0.7 (depending on bulk and particle density of the soil sample).

Gravimetric water content is our directly measured variable. Conversion to WFPS would introduce additional uncertainty which we wanted to avoid.



2.15 p 224, l 10: vary instead of "are varying"

>> We will correct this in the revised ms.

2.16 p 224, l 18: Therefore instead of "Therfor"

>> We will correct this in the revised ms.

2.17 p 225, l 26-27: "We obtained…: " I am not surprised about your wide range of Q_{10} values: Two replicates per "plot" and two data points on the Q_{10} curve are not sufficient for drawing well founded conclusions.

>> Please, see our reply to comment 2.6b (referee #2).

2.18 p 226, 1st paragraph: Please condense the number of citations to 3 per sentence.

>> We feel that the total of the citations is necessary. All publications cited here substantially support our argumentation.

2.19 p 226, l 8-11: “no very significant” Statistical significance is well defined. So the word “very” is not needed here. Are you sure that a probability level of 0.1 is defined as “significant”?

>> We will delete “very” in the revised ms.

2.20 p 227, l 11: “small relationship” This sounds strange. Please rephrase.

>> We will correct this. P. 227, l. 11 will read as follows in the revised ms:

“*Nevertheless, net potential NO fluxes showed a **weak** relationship with soil pH values*.”

2.21 p 28, l 6: delete “biologically”

>> We will delete “biologically” in the revised ms.

2.22 p 28, l 20: “those species, which can exhibit…:” Please rephrase.

>> We will correct the sentence. The sentence will read as follows in the revised ms:

“Spruce and blueberry roots are maybe associated with ectomycorrhiza.”

2.23 Entire subchapter 4.3: The importance of understory vegetation on NO fluxes is very nicely described. Now you need to point out what this means to the fluxes you determined in the laboratory (presence of roots vs. no roots and other issues). Please extend this chapter. In the present state, you are not doing your data justice.

>> We are finally very grateful to referee#2 for this comment. Indeed, the importance of understory vegetation on NO fluxes deserves more than a nicely description. We have spent considerable time and effort to search for more soil microbiological background and stronger arguments. By doing this we got acquainted to the working group of Karin Pritsch (GSF-IBOE Neuherberg, Germany), who (by sharing with us her knowledge and some of her most recent results) finally contributed substantially to subchapter 4.3 and hence to the scientific content of our manuscript. Consequently, we included her into the authors’ list of this manuscript. Section 4.3 was considerably be extended and will now read as follows:

“A number of studies have detected effects of vegetation on NO emissions (Meixner et al., 1997; Feig et al., 2008; Davidson, 1991; Martin and Asner, 2005; Pilegaard et al., 1999). Our study suggests a strong relationship between understory type and the amount of net potential NO flux. As this relationship can hardly be explained by the measured physical and chemical soil parameters alone, it may originate from the complex biological interactions between plants and their soil environment. Because plant species differ in quantity and quality of resources that they return to soil, individual plant species may have important effects on components of the soil biota and the processes that they regulate (Wardle et al., 2004). Carbon derived from plant litter mainly influences the decomposer communities. In addition, providing carbon to the rhizosphere creates a hot spot for microbial activity in the soil. For example the size and the density of the nitrifier and denitrifier communities are strongly influenced by plant roots (Philippot et al., 2009).

In our experiments, soils were sieved through a 16 mm sieve and kept at 4°C for up to 2 months. Sieving may have removed the majority of roots but it cannot be excluded that fine roots passed the meshes resulting in a soil sample containing litter, roots, rhizosphere and root free soil. Stöhr and Ullrich (2002), and Stöhr and Stremlau (2006) demonstrated that roots can generate NO. The contribution of living roots to the observed net potential NO fluxes in our experiments should be rather low because most fine roots were removed by sieving. However, biochemical reactions of intact fine roots of spruce when stored in soil at 4°C are unchanged for up to 4 weeks and then slowly decline (Pritsch, unpublished results). Thus it cannot be excluded that a minor part of the observed NO emissions came directly from those fine roots that were not removed by sieving.

A more likely explanation for the different net potential NO fluxes is that litter type and the influence of root exudates influenced functions of the soil microbial communities under the respective understory plants. Rhizosphere effects i.e. the influence of roots on NO emission rates was found by Slemr and Seiler (1991). Vos et al. (1994) measured 2 to 12-fold higher NO emissions from plots covered with green manure than from fallow plots, probably caused by increased microbial activity in the rhizosphere of the green manure plots compared to the bare soil. Unfortunately, no field studies exist examining the influence of plant roots on NO emissions. A few studies have shown a strong influence of roots on nitrous oxide emissions (Mosier et al., 1990) and it is generally accepted that denitrification is highest in the rhizosphere and decreases with distance from plant roots (Smith and Tiedje, 1979b).

According to our study, net potential NO fluxes as well as NO production rates, NO consumption coefficients, and net NO release rates displayed the highest values for soil samples taken under spruce and blueberry covered soils and the lowest values for soil samples taken under moss and grass covered soils. Our results on small net potential NO fluxes from soils taken under moss cover are in accordance with findings of Pilegaard et al. (1999). They suspected that mosses retain nutrients from throughfall but also hypothesized that moss cover simply reflects other factors such as canopy density *and water availability. Similarly small net potential NO fluxes were found for soil samples collected under grass cover in our study. Deschampsia flexuosa has a high potential to take up nitrogen in various forms and in competition to microbes (Harrison et al., 2008). This may explain a possibly reduced potential of its microbial communities in nitrogen cycling. The role of its arbuscular mycorrhizal (AM) associates has not been studied at the field site but colonisation by AM seems to be low on acidic soils (Göransson et al., 2008). Inferior competition of microbial communities under moss and grass cover therefore could explain low NO emissions.*

Soils taken under blueberry and spruce cover, in contrast, produced high net potential NO fluxes. Both plant species are associated with asco- and basidiomycetes forming ericoid mycorrhizae (blueberry), respectively ectomycorrhizae (spruce). NO accumulation can occur in mycorrhizal symbioses (Stöhr and Stremlau, 2006). Wallenda et al. (2000) also demonstrated that intact mycorrhizal roots of Norway spruce took up substantial amounts of NH_4^+*. This* NH_4^+ *may act as precursor of nitrification. During nitrification NO can be released as an intermediate. However, due to the fact that only very few roots may have been present and in an active state NO released from mycorrhizae may be of minor relevance. The 10 fold higher NO fluxes from the soils beneath spruce and blueberry are difficult to explain from our data. One factor may be that both plants produce litter types rich in lignin and phenolics (Adamczyk et al., 2008). Tannins formed in degradation of these litter types can form complexes with proteins. Protein phenol complexes can be degraded by ericoid mycorrhizal fungi and saprotrophic fungi but not by ectomycorrhizal fungi (Wu et al., 2003). It has been suggested that relatively more dissolved organic nitrogen (DON) compared to inorganic nitrogen is released upon degradation of these phenol rich litters (cf. from (Hofland-Zijlstra and Berendse, 2010). Since DON as a possible substrate for nitrification and N-mineralisation has not been measured in our study it can only be speculated if nitrogen sources other than* NH_4^+ *could explain the high NO net release or which part of the soil microflora may have contributed to the results. It could be speculated that fungi as decomposers may have played a role in this process. In a beech forest, measurements of* N_2O *emission from forest floor samples indicated that net* N_2O *production was the result of predominantly fungal* N_2O *production and predominantly bacterial* N_2O *consumption (Blagodatskaya et al., 2010).*

Altogether our results indicate a challenging field for unravelling the underlying processes of different understory plants on NO net release from forest soils."

2.24 p 229, 1st paragraph: It should say: "In this study, we investigated the net potential NO fluxes from the organic layers of soils…"

>> We will change this sentence in the revised ms. It will read as follows:

"*In this study, we investigated the net potential NO fluxes from the organic layers of a spruce forest soil covered with four different understory types (moss, grass, spruce and blueberry)."*

2.25 p 229, l 13: “of this study…:” Delete “is” following “study”. Please write “vegetation” instead of “types”.

>> We will correct this in the revised ms.

2.26 p 229. L 16: the comma following “indicated” in not needed.

>> We will correct this in the revised ms.

2.27 Tab. 4, caption: Please give an explanation of the abbreviation PD.

>> We will replace “PD” in Tab. 4 by “*particle density*”.

2.28 Fig. 8, caption: Sorry, but the caption suggests that the measurements were done on patches covered by different kinds of vegetation. Instead, they were done on sieved organic topsoil

>> Please, see our reply on comment 2.6a (referee #2)

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