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Interactive comment on "Soil properties impacting denitrifier community size, structure, and activity in New Zealand dairy-grazed pasture" *by* Neha Jha et al.

Neha Jha et al.

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3 April, 2017

Re: Final comments to the Associate Editor of Biogeosciences

Dear Sir/Madam

Thank you very much for the opportunity to submit our manuscript 'Soil properties impacting denitrifier community size, structure and activity in New Zealand dairy-grazed pasture' (bg-2016-390) to Biogeosciences. We are very grateful for the insightful comments you and the three reviewers have made, which made it clear to us that the manuscript required a major overhaul.

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We also wish to express our gratitude to you and your editorial team for the flexibility you have shown us with regard to the timeline of this review. An unusual combinations of events, which include two newborn babies and a heart attract, affected our team and so our energies were diverted from this manuscript longer than we would have wished. Now however, we are pleased to report that we have very carefully considered and responded to each of the reviewer's comments. These efforts have resulted in a vastly improved manuscript, including changes to all figures, a new figure, an overhaul of the introduction, methods and results sections and a complete rewrite of the discussion and conclusions. While the main results of the manuscript are unchanged, they are much more clearly communicated, and we feel confident that you and the reviewers will be satisfied with this progress.

Here we characterise the size, structure and diversity of nirS, nirK and nosZ genes in soils that varied widely in physicochemical characteristics to address the question of whether different denitrifier communities develop under these varied soil conditions, and if so, whether they are associated with different denitrification activities and likely to generate different N2O emissions. Overall, we found a strong correlation between MBC and DEA and that moderately high to highly fertile soils supported the largest populations of denitrifiers. Given that the more fertile soils were also likely to harbour significant populations of nitrifiers MBC may be an important coarse-scale indicator of total potential N2O emissions from such soils. However, our results for allophanic soils suggest that even relatively low rates of denitrification may lead to significant N2O emissions given their relatively low nos:nir. Consequently, we conclude that management strategies to limit N2O emissions through denitrification are likely to be most important for dairy farms on fertile or allophanic soils during wetter periods. Finally, our data suggest that new techniques that would selectively target nirS denitrifiers may be the most effective for limiting N2O emissions through denitrification across a wide range of soil types.

We eagerly look forward to the opportunity to submit our revised manuscript to Biogeo-

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sciences.

With very best wishes,

Dr. Neha Jha (On behalf of: Surinder Saggar, Donna Giltrap, Russ Tillman, and Julie Deslippe)

Response to reviewers' comments:

Soil properties impacting denitrifier community size, structure and activity in New Zealand dairy-grazed pasture.

Neha Jha, Surinder Saggar, Donna Giltrap, Russ Tillman, and Julie Deslippe

In this document we provide a comprehensive description of how we have responded to all the changes suggested by the associate editor.

Anonymous Referee #1 Received and published: 15 November 2016 Comments

Scientific significance: Does the manuscript represent a substantial contribution to scientific progress within the scope of Biogeosciences (substantial new concepts, ideas, methods, or data)?

The paper content falls within the scope of BG. The objective was to gain insight into relationship between denitrifier community size, structure and activity. This was performed by analyzing genes: nirS, nirK and nosZ. Also denitrifier enzyme activity was analysed. 10 soils each sampled at 6 locations with 25 samples at two depths respectively, and pooled. All analysis was performed later at the laboratory.

The study is motivated by N2O emissions, since a potent greenhouse gas, and that complete denitrification to N2 is better. The authors motivate the study by 'denitrifier community structure is not always strongly correlated to soil or environmental parameters (Dandie et al., 2011;Enwall et al., 15 2010;Philippot et al., 2009) indicating that our understanding of the factors controlling the diversity and function of denitrifying communities is still inadequate.' In contrast Graham et al. (2016 Frontiers in Microbiology)

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concludes environmental variables are the strongest predictors of process rates, however that microbial data was the next important explanation factors. So what is the hen and the egg?

Author's Response: Thank you for drawing our attention to this important synthesis. Graham et al. 2016 address the question "When do we need to accurately predict microbial community structure to accurately predict function?" In this re-analysis of 82 existing datasets of bacterial community structure and a variety of ecosystem processes (both C and N cycling) the authors show that microbial community metrics had low power to explain ecosystem process rates but they improved models based on environmental variables alone by on average 8%, which while significant is admittedly not stellar.

In particular, they found that models based on all predictor sets (environmental variables only, microbial parameters only, or environmental + microbial parameters) had very low power to explain denitrification rates but that community diversity metrics added more explanatory power for denitrification rates than for any other process (which partly justifies our approach). The aim of our study was to achieve a better understanding of the relationships between the structure, abundance, and activity of denitrifiers over a range of dairy-pasture soils. As justification of this aim we suggest that this 'may enhance our ability to promote complete denitrification in order to reduce N2O emissions from pastoral agriculture'.

Given the results of Graham et al. 2016 we concede that this now seems overly optimistic and we have revised the introduction to reflect this, however, we point out that the former study did not directly analyse N2O:N2 ratios during denitrification. We have also made a large number of revisions to refocus the manuscript on our central question which is 'if the size and activity of bacterial denitrifying communities can be predicted on the basis of soil physicochemical characteristics'. We feel that this is clearly a separate question than that addressed by Graham et al. but one that could shed additional light on the environmental contexts wherein microbial community structure

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and diversity can inform ecosystem function.

Many new molecular methods have been developed over the last decennia, opening possibilities to study the microbial life in soils. The impression is that the availability of a method designed this study. Results and conclusions are vague.

Author's Response: This is unfortunate and points clearly to the need for a thorough revision of our manuscript in order to better frame its goal. In response to this comment we have completely revised the results and discussion.

Scientific quality: Are the scientific approach and applied methods valid? Are the results discussed in an appropriate and balanced way (consideration of related work, including appropriate references)?

The authors are familiar with molecular and microbial genetic and process studies, which were applied here. However one can ask what can the denitrifier community structure tell on the N2O emission size?

Author's Response: Here we present qPCR data for the number of gene copies for the functional genes nirS, nirK and nosZ, as well as for the ratio of nos: nir. The ratio of these genes has been interpreted previously as an index of the potential for complete denitrification (Phillipot et al. 2011, Braker et al. 2012, Jones et al. 2014). Generally, it is expected that soils with high nos: nir ratios are more likely to emit proportionally smaller N as N2O. We have now clarified this in the methods section.

A DEA assay gives a hint in combination with nosZ genes. But contrasting results were found, where soil of group had low DEA and low nosZ (Fig 4), so what to expect? And soil group 2 high in nosZ where DEA was the highest, does that hint low N2O in spite of high process rate?

Author's Response: Group 2 soils (based on soil physicochemical characteristics) varied widely with regard to both denitrification enzyme activity (DEA) and the number of nosZ gene copies (fig 2 and fig 4) but within a soil these parameters largely agreed.

This agreement drove the significant positive correlation among DEA and nosZ copy numbers which we report in supplementary table S3. Both high DEA and higher nosZ gene copy might indicate low N2O despite high denitrification rate under most favourable condition in these soils. The revised discussion is substantially clearer on this point.

It is not possible to guess that N2O may be emitted from a soil. This is not discussed in the paper. However N2O emission size was not the main aim of the study, but the study was motivated by it. The motivation of the study is vague (see above), and the objective told in the abstract 'to gain insight to relationships between structure and activity'.

Author's Response: As above, we have rewritten the introduction section to deemphasise a direct link between denitrifier community size/structure and N2O emissions from soils.

What was the insight gained? Ten soils were compared, but one soil (n=1?) is treated as a group of soils (group 2), however many samples at one site. This could be questioned?

Author's Response: The soils grouped into 3 distinct clusters based on their physicochemical characteristics. This is a result, not an aspect of our sampling design. We then ask whether microbial community diversity, structure and size varied according to these same major gradients in physicochemical characteristics. We find that they do not, but rather responded primarily to soil water content and Olsen P. This is much more clearly communicated in the revised manuscript.

References to papers describing methods are not appropriate, since the methods are not found there.

Author's Response: Thank you, we have replaced the erroneous reference with the correct one.

The Discussion section resembles a Result section however there are references after each paragraph.

Author's Response: As above, we have thoroughly revised the results and discussion sections.

Presentation quality: Are the scientific results and conclusions presented in a clear, concise, and well-structured way (number and quality of figures/tables, appropriate use of English language)?

The authors could have better worked the text through. Sometimes the text is difficult to follow. The overall structure is OK, however the content of the discussion could couple more to other work.

Author's Response: Thank you, we have thoroughly revised the results and discussion sections.

Specific comments

P2 L34 This hypothesis is not very visible through the paper. Management practices altering environment conditions at the different soils could not be found.

Author's Response: Given the centrality of soil water content in driving bacterial denitrifier community metrics in our study we have modified the discussion section to include a more thorough discussion of the ways in which pasture management can influence soil water content.

P3 L6 'Population therefore' something lacking, difficult to read.

Author's Response: Revised.

L17-20 This section describing soil sampling is messy, difficult to read, some things are lacking like only one soil depth here but two depths later on.

Author's Response: Additional information has been included to clarify the soil sam-

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pling.

L23 Standard protocols refers to Morales et al. (2015), but I could not find these methods referred to in this reference. L28 Refers to Morales also for DEA, not in that paper. I have to say I have not checked all references given in the manuscript.

Author's Response: As above, we have replaced this erroneous reference with the correct one.

P4 L25 Why was the 10 soils investigated described so sparsely?

Author's Response: Detailed description of the 10 soils investigated has been provided in the supplementary section.

P5 L32 Two soils (n=2) compose one group. Enough? P6 L2 More so for group 2 consisting only one soil.

Author's Response: As above, the soils grouped into 3 distinct clusters based on their physicochemical characteristics. This is a result, not an aspect of our sampling design.

P7 L12 two orders of magnitude? Only one as I can see.

Author's Response: Thank you, this was a typo that has been corrected.

Many vague and not very clear statements and conclusions, based on one or two soils follows.

Author's Response: We have thoroughly revised the manuscript to avoid any vague or unclear statement.

Anonymous Referee #2

Received and published: 23 November 2016 1)

Scientific significance: Does the manuscript represent a substantial contribution to scientific progress within the scope of Biogeosciences (substantial new concepts, ideas, methods, or data)?

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The manuscript is aiming at unravelling the relationships between denitrifier community structure and environmental parameters in pasture soils. It is well within the focus of the journal. The methods used are solid but not cutting edge and suited to answer some of the questions. However, the experimental design is not perfect for the big aim of understanding the connections between nitrous oxide emissions, denitrifier community structure composition and soil type and land management.

Author's Response: As in our responses to R1 above, we concede that our aim of understanding the link between the structure, abundance, and activity of denitrifiers based on soil physicochemical characteristics may not directly 'enhance our ability to promote complete denitrification in order to reduce N2O emissions from pastoral agriculture' and we have now revised the introduction to reflect this.

Scientific quality: Are the scientific approach and applied methods valid? Are the results discussed in an appropriate and balanced way (consideration of related work, including appropriate references)?

In principal I think the study has great potential but in present form suffers a little from too many variables between the different soils and not enough samples/replicates of similar soils to resolve their influences.

Author's Response: We present n=6 for all soil physicochemical datasets and n=3 for molecular microbial datasets. However molecular work was based on 6 separate DNA extractions followed by pooling 2 extractions/PCR amplification in attempt to better represent potential spatial variability among replicates.

I further have a slight problem with the determination of copy numbers for functional genes and using these numbers as 'abundances' of the organisms. The denitrifiers could be the same percentage of the total population in all soils and it would make sense to at least also determine the copy numbers of the bacterial 16S rRNA gene with a general primer set. Then there are still issues with gene copy number per genome, functional gene/16S rRNA gene ratio in a genome and such left, which would be harder

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to account for.

Author's Response: Yes, agreed. This problem is inherent in many qPCR studies of functional genes. We have revised the methods and results sections to reflect this limitation of our approach. In particular, we have moved figure 4a to the supplementary data so that our results and discussion focus on the nos: nir ratio only. Because these genes do not always (but can) co-occur within an organism their ratio may better reflect cell numbers of complete: incomplete denitrifiers. Of course, this assumes similar PCR bias among the different primer sets, but that assumption applies equally to amplification of a "housekeeper gene" like 16S rRNA or rpoB.

From an organismic point of view it has to be considered that the nirS/K and nosZ genes are not distributed completely independent. They are linked in organisms that can perform the full denitrification pathway. Therefore it is quite surprising that the NMS analysis of nosZ (Fig. 3c) doesn't show any clustering while nirS/K did. Would it be possible to identify the T-RFs of nirS/K that have similar distribution patterns over the samples than those from nosZ? That way only subsets of T-RFs could be analyzed in order to determine how the soil parameters influence their presence/abundance.

Author's Response: This is an interesting suggestion. It would certainly shed light on the how complete denitrifiers respond to varied soil conditions. However, this is really a separate question from the one we pose here because complete denitrifiers are typically only a small subset ($\sim$ 0.5%; Deslippe et al. 2014) of all denitrifiers in New Zealand pasture soils. Should we follow this suggestion, we would miss incomplete denitrifiers, which are equally likely to be affected by the soil physicochemical characteristics we study here, and they are especially of concern for GHG emissions.

The discussion is a bit lackluster and is missing a part in which the results are discussed in the frame of the bigger question, nitrous oxide emissions. Especially as the results of the study seem to suggest that all the soil parameters collected do not explain the distribution and abundance of the nosZ gene over the different soils. How does this

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fit with the question? I would have expected a more thorough discussion of this, also the potential pitfalls of the methods used that could have influenced this result (primer bias, etc.).

Author's Response: Yes agreed. We have thoroughly revised the discussion section and we now more fully address reasons that the distribution and abundance of nosZ genes respond primarily to SWC and Olsen P in our study.

Presentation quality: Are the scientific results and conclusions presented in a clear, concise, and well-structured way (number and quality of figures/tables, appropriate use of English language)?

The quality of the presentation is lacking a little with sentences that sometimes need re-reading before they make sense. Minor grammar mistakes here and there can be found too as well as layout issues.

Author's Response: We have given the manuscript a general overhaul and respond to specific issues in detail below.

The figures are not always as informative as they could be.

Figure 1 doesn't resolve the differences between the sites closely located next to each other well. It gives a general impression where the sites are located but why not move it to SOM and then add three zoomed in insert maps that resolve the three local areas where the samples were taken better?

Author's Response: Thanks we have revised this figure.

Figure 2 is really busy, especially with the legend for each dot. As the color code already defines which sampling site they are from, why not just put the numbers for the replicates on? And I don't think it adds anything to know which exact replicates are closer together as it is not mentioned elsewhere in the manuscript. So it might be an idea to leave the annotations in the figure off altogether and just rely on the color code explained in the legend. Further, the circles defining the clusters should not cross the

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borders of the ordination.

Author's Response: Done. Good suggestion, thanks.

Figure 3 is again pretty busy and would need some cleaning up. It would also make sense to stick to the same symbols/colors as in Fig. 2. Fig. 3 b is pretty meaningless as the majority of samples can't be resolved in the presented ordination. Here the question is if an outlier analysis could be used to remove the data points at the edges of the ordination. If not, I would suggest to at least show an ordination with only the data points that cluster tightly together in the SOM to resolve potential trends in this subset of samples that is not affected by the 'outliers'.

Author's Response: In this version of the manuscript we have recreated figure 3 (now figure 4). It now retains the same symbol colours as in figure 2, but has different symbol shapes to communicate the soil groups (based on the PCA result). We disagree that nirS ordination (formerly fig3b) is meaningless because it illustrates that nirK community structure responded to the same physicochemical characteristics (SWC and Olsen P) as nirS communities did, which is a major point of the manuscript. However we acknowledge that the importance of this result was not sufficiently described in the previous results section nor was it adequately discussed. Consequently, in this version of the manuscript we have corrected those issues as well. While we disagree that outlier analysis is appropriate in this case (removal of HR and PL soils constitutes a 20% data reduction), as requested we have, added an ordination of the nirK data without PL and HR soils to the supplementary materials, which shows that Olsen P and soil water variables remain the primary driver of nirK community structure, even for this reduced dataset. Likewise, we have added this information to the results and discussion.

The data presented in table 2 would also make a nice figure, maybe even in combination with Fig. 4.

Author's Response: Agreed. Since the patterns of significance were similar for gene richness, evenness and diversity we chose, (for the sake of simplicity) to make a figure

illustrating only gene richness by soil group. We have moved table 2 to the supplementary section.

Specific comments

Multiple pages: gene names are normally all italicized, also e.g. the 'K' from 'nirK'

Author's Response: Thanks, we have now thoroughly checked the manuscript for italicized gene name.

p 3, l 16: Sampling was conducted between August and December. Where there any kind of controls to test for seasonality effects?

Author's Response: Our aim was to sample from the range of soil conditions that occur on NZ pasture farms. It was therefore important to sample in both wet and dry seasons. However it was not our intention to characterise the amplitude of seasonal variation within any given soil, and so we did not design controls that would allow us to assess seasonal variation. However, to ensure that our sampling spanned the range of soil moistures that are typical for pasture soils in NZ we sampled the soils that were expected to be wettest (OH and TeK) in winter and the soils that were expected to be driest in summer (PS, LM MF) the other soils were sampled in between these. We have clarified this in the methods section

p 3, l 18: Were the 25 soil cores per replicate homogenized and mixed during the process of sieving?

Author's Response: Yes. Thanks for pointing out that this was unclear. This information has been added to the methods section.

p 3, l 18: Were all samples besides the ones for molecular data stored at 4 âÛęC? If some of the analyses were done 6 months later I would be worried about changes in the soils as microbial activity will continue, although much slower.

Author's Response: The soils used in this study were collected over a nearly 6-month

period. After each soil was sampled it was immediately sieved and pH, nitrate (NO_3^-) & ammonium (NH_4^+) –N (mineral-N), total nitrogen (TN), total carbon (TC), Olsen phosphorus (P), microbial biomass carbon (MBC), soluble C, and denitrification enzyme activity (DEA) were measured within 1-2 weeks. Measurements of DEA and MBC were prioritized so that they typically occurred within the first few days after sieving. However, after the first two sets of soils were sampled, a technical problem with our analytical set-up caused delay in measurements of MBC for nearly 3 months. Given that it was not possible to go back to farms and resample all soils (as their physico-chemical properties were likely to have changed in this time, we remeasured MBC on the initially sampled and stored soils after 3, 4 and 7 months, we determined that no significant changes in MBC occurred between the time period of 3 and 7 months. We therefore report MBC data for all soils that were stored between 4 and 6 months. We understand that this issue can be confusing to readers so we have clarified this in the methods section, as simply as possible.

p 6, l 8 ff/table 2/figure 3: The number of T-RFs used for the NMS analysis seems to be quite low and in the case of nirK also pretty different between the samples. This could result in problems with the ordination that is hard to evaluate. It would be nice to report stress values and also provide the data matrices used for the NMS analysis in the SOM so the reader can evaluate them.

Author's Response: Thank you for this useful comment. Total T-RF richness was nirS=52, nirK=53, nosZ=47, which is quite typical for T-RFLP studies of functional genes. However, you are correct that the minimum and maximum number of T-RF varied among samples, which could possibly have contributed to instability in the NMDS ordinations we present. Final stress for the three ordinations in fig 3 were as follows: nirS=12.5, nirK=5.5, nosZ=9.4. So this was clearly not a major problem in our datasets. Nevertheless this is a good point and we have added this information to the discussion and SOM sections. We would also point out that the new T-RF richness figure (and specifically the size of the error bars on the histograms), which we have produced in

response to your earlier comment, will also help our readers to evaluate variability in gene richness among samples in our study.

p 8, l 14: Wouldn't it have been possible to avoid uneven grazing and excretal deposition by fencing off an area a couple of weeks prior to sampling? Or at least try to avoid these spots by a careful screening of the area to find representative spots?

Author's Response: All of the pastures sampled in this study were fenced from livestock and none had been grazed within 8 weeks of sampling. Thank you for pointing out this omission; this has now been added to the methods section. As explained in the methods section we also avoided any (old) dung patches when sampling, as bovine gut bacteria could have contaminated the soil sample if we had pushed the soil corer through a dung pile, and so we did not do this.

p 9, l 10 ff: I am not sure why the authors are so surprised by this. The sampling procedure (25 cores combined) should diminish the signals from different microniches and create an integrated signal.

Author's Response: True, but as we say we would then expect nirS and nirK to be negatively correlated overall. No significant negative correlation between nirS and nirK suggests independent environmental or stochastic controls on the size of these populations. This section has been expanded in the revised discussion.

p 10, l 21: 'saturated': I assume with water?

Author's Response: Yes, clarified.

p 10, l 24 ff: If the adsorption of copper is the reason that there is less nitrous oxide reduction, then why are there active nirKs, which also have copper as co-factor? There must be another explanation for this observation or could a reduction in the copy numbers of nirK be observed in these soils as well?

Author's Response: Yes, thanks for pointing out that our argument was confusing. We have revised the conclusions to make the point clearer. We did not intend to suggest

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that adsorption of copper is the reason that there is less nitrous oxide reduction in allophanic soils, but rather less nitrite reduction. We agree that because allophanic soils adsorb copper, they are likely to select against nirK denitrifiers. We expected this to reduce the overall number of genes encoding nitrite reductase in group 1 soils, but we didn't observe this (Fig 4). We have revised the discussion section of the manuscript to include this point. The point of interest in the conclusion section is that, the nos:nir gene ratio data we show agrees with previous work by our group showing that allophanic soils emit greater N2O: (N2O + N2O) relative to other soil types.

Anonymous Referee #3

Received and published: 24 November 2016

Summary:

They sampled soils from 10 different geographical locations in New Zealand. They did an ordination of soil characteristics and found that the 10 sample locations could be grouped into 3 groups based on soil characteristics. These groupings were used in the further analysis of T-RFLP, qPCR and DEA data.

General comments:

The study attempts to find how various pasture management (soil water, carbon and fertility) will affect the denitrifier community, which increase our knowledge on denitrification in different soil types, and maybe improve our ability to promote complete denitrification and avoid N2O emission. This is a relevant question within the scope of BG. They find that fertile soil with high microbial biomass promote complete denitrification, whereas allophanic saturated soil is a source of N2O production.

I found it hard to get a good overview of the results and discussion, maybe because of poor flow and clarity in writing. I agree with RC1 that the discussion resembles a result section. In general every section sums up observations and have some explanation with a reference. I don't think it reaches a high enough level of discussion. I'm also not

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confident that the data is strong enough to answer the question sufficiently. qPCR on RNA would be more reliable. To my knowledge the nir genes are very ubiquitous and not necessarily expressed.

Author's Response: We agree with R3's assessment that these doubts stem from poor flow of the manuscript and a lack of clarity in writing. These comments align with those of the other reviewers and made it clear to us that the manuscript required a major overhaul. To this end we have revised all parts of our manuscript as detailed elsewhere. Now that this is done we feel that our aim of achieving a better understanding of how soil physicochemical characteristics' affect the size, structure and activity of bacterial denitrifying communities is clear, and we think that R3 would agree that qPCR of RNA would not be an appropriate tool with which to address it.

Both title and abstract are descriptive and clear, reflecting the study well.

Specific comments:

The whole introduction argumentation for this study (P2, L11 – P3, L2) makes a good background, but somehow it's a bit vague. The idea of the study is very good and this framework can make it more visual with clearer and stronger formulations.

Author's Response: To this end we have added to the introduction one sentence, immediately after the statement of aim: "In particular, we asked if the size and activity of bacterial denitrifiers could be predicted on the basis of soil physicochemical characteristics."

P3, L22-23 I would mention which physicochemical characteristics were used in this study here, otherwise you only see it when reading the statistical analysis.

Author's Response: Added, thank you.

Regarding methods for physicochemical characteristics, DEA and qPCR, they refer to Morales et al. (2015). This seems to be another study of the very same soil sampling, and this manuscript is reusing data from Morales et al. (2015), right? It should appear

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more clearly that this study is an extension of Morales et al. (2015) with reuse of data. It would also seem natural to refer more to the earlier study since it's the same topic. There should be references to this in the introduction and/or discussion, not only for methods description.

Author's Response: Yes that's right, some of the physicochemical and molecular data presented here also appears in Morales et al. (2015), although the data analysis and objective of both the studies is entirely different. We revised the methods to more clearly convey that point, we also now refer to the Morales et al. (2015) in introduction of our paper.

P10, L25-29 Suddenly in the end of the conclusion this new stuff about allophanic soils comes up, this should have been included earlier on. The conclusion should instead round and wrap up. New stuff should not be introduced like this.

Author's Response: Yes, we agree and include the point about N2O emissions from allophanic soils in the discussion too.

Technical corrections:

Inconsistent use of water content terms and abbreviations: "Moisture"/"soil water"/"soil water content"/"SWC" and also "% SWC at field capacity"/"% FC SWC"/"high moisture at FC". Also "Field fresh" (P3, L20) and "field-moist" (P3, L22). This was all quite confusing to me.

Author's Response: Thank you, we have revised all parts of the manuscript with an eye for consistency.

Figure 2 have too many abbreviations in caption, the figure itself should be more descriptive.

Author's Response: This same comment was made by R2 and so we have changed Fig 2 accordingly.

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In caption for Figure 4, SEM should first be defined and then used. Not the other way around.

Author's Response: Agreed, done that.

P1, L3 There should not be a dot in the end of the manuscript title. This also occurs in the titles in the references.

Author's Response: We have removed dot from the end of the manuscript title and also from the titles in the references.

P2, L34 With enhanced structure, do you then mean diversity?

Author's Response: This comment has been rephrased for clarity.

P3, L19 "2 depths" not "2 depth". I can't find which depths you chose (mm/cm?), should be stated in the methods.

Author's Response: Yes thanks, we have fixed this and also added the unit of measurement.

P4, L7-8 "2.5 ul of 10xPCR buffer (1 mM MgCl2), 0.5 mM MgCl2". Final concentrations in reaction mix should be stated, this looks weird to me.

Author's Response: Okay, we have rewritten as final molarity. P4, L24 I would specify that the qPCR was performed on DNA

Author's Response: The title of the section "Quantitative polymerase chain reaction (qPCR) of total bacterial and denitrifier genes" makes this point clear.

P5, L19 Isn't the right abbreviation NMDS? Not NMS

Author's Response: Both abbreviations are in common use, with variation stemming from the term used by the particular stats package. PCOrd software refers to NMS ordinations (McCune and Grace, 2002), thus our use of that abbreviation here.

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