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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.

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)?

The manuscript is aiming at unraveling 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.

2) Scientific quality: Are the scientific approach and applied methods valid? Are the

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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.

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 to account for.

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.

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 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.).

3) Presentation quality: Are the scientific results and conclusions presented in a clear,

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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.

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?

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 borders of the ordination.

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'.

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

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Specific comments

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

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

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

p 3, l 18: Where all samples besides the ones for molecular data stored at 4 °C? 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.

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.

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?

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.

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

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?

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