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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.

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

Interactive comment Discussion paper 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

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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) 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 char-

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acteristics'. 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 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 on nirS+K:nosZ. The ratio of nir:noz genes has been interpreted previously as an index of the potential for complete denitificiation (Phillipot et al 2011, Braker er al 2012, Jones et al 2014). Generally, it is expected that soils with low nir:nos 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 physiochemical characteristics) var-

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ied widely with regard to both 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

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correct one.

The Discussion section resembles a Result section however there are references after each paragraph. 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 - As above, 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.

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.

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.

Additional information has been included to clarify the soil sampling.

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.

As above, we have replaced this erroneous reference with the correct one.

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

Detailed description of the 10 soils investigated has been provided in the supplemen-

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tary section.

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

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.

Thank you, this was a typo that has been corrected.

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