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

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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 N_2O emissions, since a potent greenhouse gas, and that complete denitrification to N_2 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 N_2O 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 N_2O:N_2 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 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 N_2O emission size?

Author's Response: Here we present qPCR data for the number of gene copies for the functional genes *nir*S, *nir*K and *nos*Z, 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 N_2O. We have now clarified this in the methods section.

A DEA assay gives a hint in combination with *nos*Z genes. But contrasting results were found, where soil of group had low DEA and low nosZ (Fig 3), so what to expect? And soil group 2 high in nosZ where DEA was the highest, does that hint low N_2O 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 *nos*Z gene copies (Fig 1 and Fig 3) but within a soil these parameters largely agreed. This agreement drove the significant positive correlation among DEA and *nos*Z copy numbers which we report in supplementary table S5. Both high DEA and higher *nos*Z gene copy might indicate low N_2O 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 N_2O may be emitted from a soil. This is not discussed in the paper. However N_2O 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 N_2O 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 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.

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)?

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 N_2O 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 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 *rpo*B.

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. 3) 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 (~ 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 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 *nos*Z 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 and moved it to supplementary section (Fig S1).

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.

Author's Response: Done. Good suggestion, thanks. We have made the changes to this figure as suggested (Fig.1).

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. 1. Fig. 3 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. 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 *nir*S ordination is meaningless because it illustrates that *nir*K community structure responded to the same physicochemical characteristics (SWC and Olsen P) as *nir*S 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 *nir*K data without PL and HR soils to the supplementary materials, which shows that Olsen P and soil water variables remain the primary driver of *nir*K 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 (fig. 2) 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 ◦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.

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 physicochemical 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 *nir*S=52, *nir*K=53, *nos*Z=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: *nir*S=12.5, *nir*K=5.5, *nos*Z=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 *nir*S and *nir*K to be negatively correlated overall. No significant negative correlation between *nir*S and *nir*K 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 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 *nir*K 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 N_2O: (N_2O + N_2O) relative to other soil types.

Anonymous Referee #3

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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 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 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 N_2O 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.

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.

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

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Abstract. Denitrification is an anaerobic respiration process that is the primary contributor of the nitrous oxide (N_2O) production from grassland soils. Our objective was to gain insight to the relationships between denitrifier community size, structure, and activity for a range of pasture soils. We collected 10 dairy pasture soils with contrasting soil textures, drainage classes, management strategies (effluent irrigated or non-irrigated), and geographic locations in New Zealand, and measured their physicochemical characteristics. We measured denitrifier abundance by quantitative polymerase chain reaction (qPCR) and assessed denitrifier diversity and community structure by terminal restriction fragment length polymorphism (T-RFLP) of the nitrite reductase (*nir*S, *nir*K) and N_2O reductase (*nos*Z) genes. We quantified denitrifier enzyme activity (DEA) using acetylene inhibition technique. We asked whether varied soil conditions lead to different denitrifier communities in soils ~~develop under these varied soil conditions~~, and if so, whether they are associated with different denitrification activities and likely to generate different N_2O emissions.

Differences in the physicochemical characteristics of the soils were driven mainly by soil mineralogy and the management practices of the farms. We found that *nir*S and *nir*K communities were strongly structured along ~~the~~ gradients of soil water and phosphorus (P) contents. By contrast, the size and structure of the *nos*Z community was unrelated to any of the measured soil characteristics. In soils with high ~~soil~~ water content the richnesses and abundances of *nir*S, *nir*K and *nos*Z genes were all significantly positively correlated with DEA. Our data suggest that management strategies to limit N_2O emissions through denitrification are likely to be most important for dairy farms on fertile or allophanic soils during wetter

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periods. Finally, our data suggest that new techniques that would selectively target *nir*S denitrifiers may be the most effective for limiting N_2O emissions through denitrification across a wide range of soil types.

1 Introduction

Nitrous oxide (N_2O), is a potent greenhouse gas that is produced as an intermediate product of biological nitrogen conversions in soils (Stevens et al., 1997). Denitrification is the stepwise anaerobic reduction of aqueous nitrate (NO_3^-) to nitrite (NO_2^-) and into the gaseous forms N_2O and benign dinitrogen (N_2). It is the major global contributor to N_2O production in grassland soils (Saggar et al., 2013) and is responsible for a significant fraction of agricultural greenhouse gas emissions (IPCC, 2014). Denitrification is mediated by the action of four enzymes: NO_3^- reductase (NAR), NO_2^- reductase (NIR), nitric oxide (NO) reductase (NOR), and N_2O reductase (N_2OR) (Zumft, 1997), which are encoded by the *nar*/*nap*, *nir*, *nor*, and *nos* genes, respectively. Taxonomically diverse bacteria, archaea (Philippot et al., 2007;Tiedje, 1994;Ishii et al., 2010) and eukaryotes (Zumft, 1997) are known to harbour two or more denitrification enzymes. Denitrifying bacteria are particularly widely distributed in pasture soils (Graham et al., 2014) and more than 60 genera have ~~so far~~ been identified (Chen et al., 2012). Denitrifiers with all four reductases are capable of emitting N_2 and are said to be 'complete' denitrifiers. Those denitrifiers that lacks N_2OR emit N_2O, as the final product of denitrification are called 'incomplete' denitrifiers.

*Nir*S, *nir*K, and *nos*Z genes have been targeted as functional markers of both complete and incomplete denitrifiers in soils (Stres et al., 2008; Throbäck et al., 2004; Morales et al., 2010; Enwall et al., 2010). The balance of complete and incomplete denitrifiers in soils can determine the ratio of N_2O: N_2 produced during denitrification (Philippot et al., 2011; Bakken et al., 2012)~~, and thus the environmental impact of biological denitrification.~~ . Thus, denitrifier community structure and abundance can be important factors in determining nitrogen (N)-loss and agricultural greenhouse gas emissions from soils. Indeed, a recent synthesis of 82 datasets relating bacterial community structure and environmental characteristics to a variety of carbon (C) and nitrogen (N) cycling processes found that microbial community structure data improved the power of models to explain denitrification process rates better than for any other ecosystem process (Graham et al., 2016). ~~However~~Still, strong relationships are not always observed between denitrification rates, and denitrifier community structure and abundance (Cavigelli and Robertson, 2000,

2001; Chèneby et al., 1998; Mergel et al., 2001). In particular, the structure of ~~Moreover,~~ d~~d~~enitrifier communities~~ty~~ in environmental samples is often poorly ~~structure is not always strongly~~ correlated ~~to soil or environmental parameters~~ with soil or environmental factors that are known to influence process rates (Dandie et al., 2011; Enwall et al., 2010; Philippot et al., 2009) indicating that our understanding of the factors controlling the diversity and function of denitrifying communities is still inadequate. Moreover, there is a need to identify the soil conditions in which the presence and activity of denitrifiers are likely to lead~~s~~ to substantial N_2O emissions, so that appropriate strategies for targeted and effective management can be deployed or developed where they are lacking.

Pastoral dairy farming is a preferred land use in mild and wet climates on relatively fertile soils and flat sites that occupy low-lying positions in the landscape as these locations support high rates of pasture production (Saggar et al., 2013). The combination of periodically anoxic soil conditions, high concentrations of N in cattle excrement patches and relatively high microbial biomass at these sites, combine to favour denitrification as a major oxidative metabolic pathway. Despite this, denitrification rates and potentials as well as N_2O emissions through denitrification vary widely among pasture soils (Cayuela et al., 2013; Giltrap et al., 2011; Groffman et al., 2006).

Soil management practices including the addition of organic amendments such as plant residues, compost, manure, or effluent irrigation can increase soil fertility and microbial biomass, and may lead to structural shifts in ~~shift~~ soil microbial communities, which in turn ~~influences~~ alter soil biochemical processes (Kennedy and Smith, 1995). The addition of crop residues to soils ~~is associated with the~~ increases the ~~d~~ abundance of denitrifier genes and leads to greater denitrification in soils (Barrett et al., 2016; Gao et al., 2016; Henderson et al., 2010; ~~Gao et al., 2016~~). Likewise, increasing soil water content ~~moisture~~ is associated with increasing denitrifier gene abundances in soils (Liu et al., 2012; Mergel et al., 2001). Management practices that alter the size of the denitrifier community in soils are also likely to affect ~~a soil's potential to denitrify available NO_3^- and~~ its denitrification enzyme activity (DEA), as the abundance of denitrifier genes can be a strong determinant of DEA (Deslippe et al., 2014; Hallin et al., 2009; Čuhel et al., 2010; Enwall et al., 2010). However, the geologic origins of a soil can determine its dominant properties over a range of soil C and water contents (Bronick and Lal, 2005)~~,~~ ~~yet detailed knowledge of~~

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~~how these variations in soil properties affect denitrifier populations and denitrification is still limited.~~ Indeed we previously found that soil texture, drainage class, and latitude were powerful regulators of denitrification end products (N_2 vs. N_2O) and that both the forms and quantity of gases emitted could be predicted by the ~~..~~ 16S rRNA gene communities of soil samples (Morales et al., 2015). However, we still lack detailed knowledge of how ~~these~~ variation~~s~~ in soil properties affect denitrifier populations and denitrification ~~is still limited~~. Better information on the role of soil physiochemical characteristics in determining the size and activity of denitrifiers may allow for improved and soil-specific management of N_2O emissions from pastoral agriculture.

Here, w~~W~~e sought a better understanding of the relationships between the structure, abundance, and activity of denitrifiers over a range of New Zealand dairy-pasture soils, which varied widely in soil properties and had different management conditions. We asked if the properties of these soils drove unique denitrifier communities that supported different DEA or were likely to generate different N_2O emissions. We ~~hypothesised~~ expected to find that the size and structure of denitrifier communities' would vary most strongly in accordance with soil moisture and that soil physical properties or ~~and that pasture~~ management practices that ~~alter~~ increase soil water, would ~~carbon and fertility will~~ enhance the size and ~~, structure and~~ activity of ~~the~~ denitrifier~~s community~~. ~~Knowledge about denitrifier activity under contrasting conditions may enhance our ability to promote complete denitrification in order to reduce N_2O emissions from pastoral agriculture.~~

2 Materials and Methods

2.1 Sites and soils

Our aim was to sample soils that would encompass the range of physiocochmical conditions that predominate on New Zealand dairy farms. We therefore targeted ~~The~~ soils ~~were selected~~ on the basis of their geographical location (North or South Island of New Zealand), and ~~variation in~~ mineralogy (allophanic or non-allophanic soils). As soil moisture is a key factor affecting the structure and activity of soil denitrifier communities (Liu et al., 2012; Mergel et al., 2001) it was also important to sample in

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both wet and dry seasons. We therefore sampled soils over a 6-month period from winter to summer. Soil textures varied from a stony silt loam to a fine sandy loam, and the sites ranged from poorly drained to well drained (Table 1). We sampled soils expected to have the greatest soil water contents in winter and those we expect to be driest in summer, with other soils sampled in between these times (see supplementary table 1~~X~~ for soil sampling dates). ~~Our objective in this study was to understand the influence of variability in soil properties on denitrification activity and denitrifier population therefore, pasture soils with varying physical and chemical characteristics were~~ We collected soils from 10 different ~~New Zealand dairy farms (Fig. 1). All the~~ sites ~~New Zealand~~ commercial dairy farms (Fig. S1). All sites were fenced from livestock and none had been grazed within 8 weeks of sampling. All sites ~~the from which the soil samples were collected are commercially managed grazed dairy pastures~~ were dominated by perennial ryegrass (*Lolium perenne)* and white clover (*Trifolium repens*). ~~The soils were selected on the basis of their geographical location (North or South Island of New Zealand), and variation in mineralogy (allophanic or non-allophanic soils). Soil textures varied from a stony silt loam to a fine sandy loam, and the sites ranged from poorly drained to well drained (Table 1).~~ Fertilization regimes varied among the farms and consisted of applications of 150–200 kg N ha^{-1} annually. Detailed descriptions of the individual fertiliser applications at the 10 farms are described in the supplementary information.

~~*Insert Fig. 1*~~

Insert Table 1

2.2. Sampling and analysis of soil properties

At each ~~For the collection of soil samples, on each~~ farm, we randomly selected six ~~random location~~blocks of 100 m^2 ~~area were identified~~for the collection of soil samples.~~.~~ At randomly selected locations within each block, t~~Soil samples were collected between August and December 2010 once from each site. T~~wenty–five soil cores (25 mm diameter × 100 mm long) were ~~collected~~ obtained ~~from the 0–100 mm depth~~ using a steel corer. The 25 cores from each block were pooled to form a single composited sample per block (n = 6 composited soil samples per farm). ~~The 25 cores from each location were pooled, but the 6 replicates from each farm~~ All s~~were stored separately (n = 6).~~ Soil samples were collected between August and December 2010 once from each site. Soil ~~cores~~ samples were taken to the laboratory,

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individually ~~while keeping the replicates separately soils from each replicate were~~ homogenised, sieved to 2 mm~~,~~ and stored at 4ºC in plastic bags (10 sites × 6 replicates = 60 samples). ~~All of the pastures sampled in this study were fenced from livestock and none had been grazed within 8 weeks of sampling. Twenty five soil cores (25 mm diameter × 100 mm long) were collected from the 0–100 mm depth using a steel corer from six random locations of 100 m^2 area on each farm (once) between August and December 2010 (10 sites × 6 replicates = 60 samples). The 25 cores from each location were pooled but the 6 replicates from each farm were stored separately (n = 6). Field fresh soil cores were taken to the laboratory, sieved to 2 mm, and stored at 4ºC in plastic bags.~~ A sub-sample of each soil replicate was stored at –20ºC for molecular analysis. ~~The~~ We measured pH, nitrate (NO_3^-) & ammonium (NH_4^+) –N (mineral-N), total nitrogen (TN), total carbon (TC), Olsen phosphorus (P), and soluble C, on the field-moist sieved soils using standard protocols (for details see Jha 2016). Soils were analysed for these parameters within 2 weeks of sampling.

~~2.2 Denitrification enzyme activity (DEA) of soils~~

DEA was determined using ~~sieved (2 mm) soil following~~ the acetylene inhibition method described in Luo et al. (1999), with the exception that we added chloramphenicol to inhibit the *de novo* synthesis of enzymes. Thus the values we report represent only the existing enzyme activity in soils. DEA was assessed for all soil samples within 2 days of collection. DEA incubation conditions and the ~~method of~~ gas sampling methods ~~and analysis~~are ~~are~~ described ~~previously~~elsewhere (Morales et al., 2015). We intended to measure microbial biomass carbon (MBC) within 48 hours of soil sampling but a technical problem with our ~~GC~~ set-up delayed measurements of MBC for nearly 3 months. To standardize this effect across soil samples we monitored changes in the size of the MBC pool in two soils over 7 months. We found that no significant changes in MBC occurred between 3 and 7 months for soils stored at 4ºC (see supplementary table 1~~X~~). We therefore report MBC data for all soils that were stored at 4ºC between 4 and 6 months.

2.3 DNA extraction from soils

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Within six months of soil sample collection, soil samples were thawed on ice and a ~~DNA was extracted from~~ 0.25 g aliquot was obtained. DNA was extracted from ~~of each replicate~~these soil samples using the MoBio PowerSoil™ DNA Isolation Kit (MoBio, Solana Beach, CA, USA) following the manufacturer's instructions. The yield and quality of DNA extracts were verified as described in Deslippe et al. (2014). DNA was stored at –20°C until analysed. ~~Molecular analyses were performed within 6 months of extraction of DNA.~~

2.4 Terminal restriction fragment length polymorphism (T–RFLP) of denitrifier genes

Terminal restriction fragment length polymorphism (T–RFLP) was performed to analyse the community structure and diversity of ~~functional genes~~ *nir* and *nos* genes in soil samples. T-RFLP for *nir*S and *nosZ* genes was conducted as described in Deslippe et al. (2014) except that ~~the reaction~~PCR ~~conditions~~ for *nir* genes occurred in a total volume of 25 µl reaction mixture, which ~~were as follows.~~

~~PCR amplification of *nir*S gene was performed in a total volume of 25 µl reaction mixture~~ containinged 2.5 µl of 10×PCR buffer (1 mM $MgCl_2$), 0.5 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dNTP), 1.25 U of *Taq* polymerase (Fisher *Taq*, Thermofisher Scientific® Inc.), 0.8 mg/ml Bovine Serum Albumin (BSA), 1.0 µM of each primer, and 10 ng DNA template per reaction. The PCR amplification consisted of an initial denaturation of the DNA template at 94°C for 30 s, followed by 35 cycles of 20 s at 94°C, 20 s at 56°C, and 20 s at 68°C. The reaction was completed by 10 min at 68°C.

For T-RFLP of the *nir*K gene we used the primers Copper 583F, 909R (Dandie et al., 2011). The amplifications of *nir*K and *nos*Z genes were achieved under slightly different condition than the *nir*S gene according to the specifications of the reagents used for PCR. The PCR amplification was performed in a total volume of 25-µl reaction mixture containing 10 µl of 2 × NEB Taq master mixes (New England Biolabs® Inc.), 0.4 µM of each primer, and 10 ng DNA template per reaction. PCR consisted of an initial denaturation of the DNA template at 94°C for 2 min, followed by 35 cycles of 30 s at 94°C, 1 min at 56°C, and 1 min at 72°C. The reaction was considered complete after 10 min at 72°C.

The T-RFLP profiles generated for the soil samples were analysed using Peak Scanner® v1 software (Life Technologies) and as described in Deslippe et al. (2014). The total number of terminal restriction fragments (T-RFs) per electropherogramme was taken to indicate genotype richness per sample. We then

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calculated the gene Shannon's diversity index and Pielou's evenness index (Magurran, 1988) per sample and used 1-way analysis of variance (ANOVA) to determine if soils belonging to the three physicochemical groups differed with respect to gene richness, evenness and diversity.

2.5 Quantitative polymerase chain reaction (qPCR) of total bacterial and denitrifier genes

Quantification of bacterial *nir*S, *nir*K, and *nos*Z genes was accomplished using qPCR, following the methodology of Deslippe et al. (2014)~~ as described in Morales et al. (2015)~~. Amplification efficiencies of qPCR reactions for samples were within the ~~expected~~ range of values (E = 90–110%) ~~based on previous reports~~published previously (McPherson and Moller, 2006)~~(CITE)~~. The reactions were linear over 7 orders of magnitude and sensitive down to 10^2 copies. The ratio of ~~nosZ:nirK+nirS~~abundances of denitrifier genes in environmental samples has been interpreted previously as an index of the potential for complete denitrification (Philippot et al., 2009~~CITATIONS NEEDED~~). Here, we calculated the nosZ: (*nir*S+*nir*K)~~ nirK+nirS~~ of soil samples. We expected that soils with low nosZ: (*nir*S+*nir*K)~~ nirK+nirS~~ are more likely to emit a greater N_2O:(N_2O + N_2).

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2.6 Statistical Analysis

The normality and homoscedascity of all~~The~~ ~~data for~~ soil ~~chemical~~physicochemical, ~~characteristics,~~ gaseous ~~-~~emission~~s,~~ and biological datasets were examined using Anderson-Darling (Stephens, 1986) and Levene's tests, respectively in~~denitrifier gene terminal restriction fragments (T-RFs) and abundance was analysed using~~ Minitab® 16 software (Minitab Inc.). ~~Data normality was evaluated using the Anderson-Darling test (Stephens, 1986). As the assumptions of normality of data were violated for some of the parameters, the data sets were transformed to normal using the~~ Box-Cox transformations (Box and Cox, 1964) were applied to data sets as required to conform to model.

~~The data for soil chemical characteristics, gaseous emissions, denitrifier gene terminal restriction fragments (T-RFs) and abundance was analysed using Minitab® 16 software (Minitab Inc.). Data normality was evaluated using the Anderson-Darling test (Stephens, 1986). As the assumptions of~~

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~~normality of data were violated for some of the parameters, the data sets were transformed to normal using the Box-Cox transformations (Box and Cox, 1964).~~

The differences in the means of soil characteristics such as 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, DEA, number of gene T–RFs and gene copy numbers were assessed using a one-way analysis of variance (ANOVA) test with soil type as a factor. Tukey's Studentized Range Test at $\alpha = 0.05$ significance level was used *post hoc* to reveal significant differences among means. The relationships among the soil chemical characteristics pH, nitrate (NO_3^-) & ammonium (NH_4^+) –N, TN, TC, Olsen P, MBC, DEA, number of denitrifier gene T–RFs, and gene copy numbers were determined using Pearson's correlation analysis.

In order to reduce the dimensionality of the many correlated soil physicochemical characteristics we performed principal components analysis (PCA). We included % soil water content (SWC), % SWC at field capacity (% FC SWC), pH, TN, TC, Soluble C, Olsen P, nitrate (NO_3^-) and ammonium (NH_4^+) –N as factors in the PCA. Soils grouped along the first and second ordination axes. We used multiple response permutation procedure (MRPP) to assess the statistical significance of these groupings. MRPP calculates the chance-corrected within-group agreement (A), a measure of within-group homogeneity compared with that expected by chance, where A = 1 corresponds to identical members within each given group (maximum effect of factor), and where $A \leq 0$ corresponds to within-group heterogeneity equal to or larger than that expected by chance (no effect of factor; McCune and Medford 1999). We also calculated Pearson correlations among soil microbial characteristics and the ordination axes, and plotted those that were significantly correlated (tau>0.2) with axis 1 and 2 as vectors on the PCA. ~~In order to assess how denitrification and soil microbial properties varied with the physicochemical characteristic of soils, we calculated Pearson and Kendall correlations among physicochemical characteristics and the ordination axes, and plotted those that were significantly correlated with axis 1 and 2 as vectors on the PCA. A nonparametric Kruskal-Wallis test was performed to examine the differences in soil parameters among various group of soils obtained through PCA.~~

Analysis of the *nir*S, *nir*K and *nos*Z community structure was based on threshold normalised peak heights of T-RFs from electropherogrammes (Deslippe et al., 2014). Non-metric multidimensional scaling (NMS)

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ordinations were performed using Bray & Curtis distance (Bray and Curtis, 1957) in the programme PC-ORD (McCune and Mefford, 1999). In order to illustrate how the structure of denitrifier communities varied with the physicochemical characteristic of soils, we calculated ~~The~~ Kendall's rank correlations among physicochemical and biological characteristics of soils with the NMS ordination axes ~~were calculated~~ in PC-ORD. The significant correlates (tau > 0.2) were overlaid as vectors on the NMS ordination plots.

3 Results

3.1 Variations in soil chemical characteristics

The 10 soils differed significantly with regard to all measured physicochemical characteristics (Table S2~~1~~). The PCA of soil characteristics generated 3 significant axes, of which the first two accounted for 83.4 % of the total variance (Fig. 1~~2~~). Axis 1, which accounted for 53.4% of the variation in soil properties, described primarily a difference in ~~percent moisture at field capacity (~~% FC SWC~~)~~, although total N and total C, and Olsen P also weighed heavily in forming Axis 1. Axis 2, which accounted for 30.0% of the variation in soil properties, described primarily a gradient in mineral N-form, with NO_3^-–N increasing and NH_4^+–N decreasing along Axis 2.

The 10 soils segregated into three groups, with replicates of a soil tending to cluster closely together in the PCA. Firstly, the two allophanic soils OH (Otorohanga silt loam) and HR (Horotiu silt loam) (group 1) were separated from all other soils by their relatively high % FC SWC, their high total N, C and allophane contents. Secondly, the effluent-irrigated soil, MWEI (Manawatu effluent-irrigated fine sandy loam), was separated from all other soils (group 2) due to its high NO_3^-–N content. The seven remaining soils formed a loose cluster~~ed mainly~~ due to their relatively high NH_4^+–N and low Olsen P contents (group 3). MRPP ~~indicated~~ confirmed that these groups differed significantly in soil physicochemical characteristics (A= 0.379, $P < 0.001$, Fig. 1). Table S3~~2~~ summarises the physicochemical characteristics for the 3 groups of soils. Overall, ~~W~~we found that axes 1 and 3 of the PCA were not significantly correlated to ~~any measure of denitrification or to microbial biomass carbon~~DEA or MBC. However, axis

2 of the PCA, which describes a gradient in mineral N-form, was significantly positively correlated to DEA (r^2 = 0.214) and to MBC (r^2 = 0.303; Fig. 1~~2~~).

Insert Fig~~.~~ure 1~~2~~

3.2 Bacterial denitrifiers in New Zealand dairy-grazed pasture soils

3.2.1 ~~Richness, diversity and evenness~~Diversity indices of denitrifier gene T-RF profiles

Across all soil samples, total T-RF richnesses were nirS=52, nirK=53, nosZ=47, which are typical values for T-RFLP studies of functional genes in soils (Deslippe et al., 2014; Rich and Myrold, 2004; Rösch and Bothe, 2005)~~(CITE~~. While the minimum and maximum numbers of T-RFs varied among samples, th~~All three genes varied significantly in richness among the 10 soils (Table 2). Th~~e patterns of richness, evenness, and diversity among soils belonging to the three groups of soils were similar (see supplementary table S4~~X~~), for simplicity, we therefore present only the values for T-RF richness in f~~F~~igure 2~~3~~. *Nir*S communities among the three groups of soils had similar richnesses. By contrast, the allophanic soils of group 1 had significantly lower ~~ies of the~~ *nir*K richness, while the effluent irrigated soils of group 2 had significantly greater richness than all other soils (Kruskal-Wallis H = 13.84, P = 0.0001). ~~*and n*~~*Nos*Z richness was significantly greater in the effluent irrigated soils of group 1 compared with all other soils (Kruskal-Wallis~~communities also differed significantly among the 3 groups of soils as defined by physicochemical properties (Kruskal Wallis test statistics H for *nir*K H = 13.84, P = 0.0001; *nos*Z~~ H = 8.59, P = 0.014). ~~The *nir*K and *nos*Z communities of the group 2 soil, which had higher Olsen P and MBC, were highly rich, diverse and even relative to those of the group 1 and 3 soils. However, diversity metrics for *nir*S communities did not differ significantly among the 3 groups (H = 0.11, P = 0.946).~~

Insert Figure 3

3.2.2 Denitrifier community structure in soils

Ordination of the soil samples in *nirS* T–RF space indicated significant structuring of the *nir*S community according to variation in the physicochemical characteristics of the soils (Fig. 3~~a~~)~~.~~. Interestingly however, the variation in *nir*S community structure was not driven by the same physicochemical characteristics that varied most widely among soils and formed the first and second PCA axes. Consequently the *nir*S

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communities of soil samples did not cluster according to the three ~~Moreover, *nir*S communities did not correspond to the three~~ groups of soils ~~based on major soil physicochemical characteristics.~~in our PCA. ~~In particular~~Rather, Axis 1 ~~of Figure 3a,~~ which accounted for 30.~~2~~0% of the variation in *nir*S community structure, was significantly correlated to SWC and the Olsen P contents of the soils (Fig. 3). This indicates that *nir*S community structure in dairy pasture soils from across New Zealand responded most strongly to moisture and P gradients. Likewise, ~~. Moreover, *nir*S communities did not correspond to the three groups of soils based on major soil physicochemical characteristics. For example the replicates of the PL soil (Paparua Lincoln silt loam), which had lower SWC and Olsen P than did the other group 3 soils, ranked very low on axes 1 and 2 of the NMS ordination, causing them to separate from other group 3 soils.~~

~~Ordination of the soil samples in *nirK* T-RF space indicated that 68.2~~66.9% of the variation in *nir*K community structure (~~NMS~~ axis 1) was significantly correlated to the SWC and Olsen P contents of the soils. Axis 1 of the *nir*K ordination primarily separated two soils (~~HR soil (~~Horotiu silt loam, and ~~) from group 1 and PL (~~Paparua Lincoln silt loam) ~~soil from group 3 (Fig 3b~~from all other soils~~)~~. However, even when these two soils were removed from the dataset, NMS ordination revealed that the *nir*K community was primarily structured according to Olsen P and soil water variables (both SWC and % FC SWC ; (supplementary fig S2-~~X~~a). ~~Similar to the *nir*S T-RF space axis 1 was significantly correlated to SWC and the Olsen P content.~~ An additional 7.7% of the variation in *nirK* community structure ~~formed axis 2 of the ordination on which HR and PL separated from their respective groups. Axis 2~~ was most strongly correlated to *nir* gene abundance (*nir*S + *nir*K) in soils, which was higher in the ~~for the group 1~~ HR soil, an allophanic soil, ~~soil~~ than for ~~the group 3 PL~~any other.~~ soil.~~

Ordination of the soil samples in *nosZ* T-RF space revealed little clustering of soil samples by origin or group~~, indicating a weak structuring of the *nosZ* community~~. Likewise, we detected no significant patterns of correlation among the first and second ordination axes and the soil physiochemical characteristics. However axis 1 of the NMS ordination, ~~which accounts for~~in ~~30.3% of the variation in *nosZ* community structure,~~ was also most strongly correlated to SWC and Olsen P (supplementary fig S2~~X~~b). ~~that corresponded to the physicochemical characteristics of the soils (Fig. 3c).~~

Insert Figure ~~3~~4

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3.2.3 Denitrifier gene abundance

The ~~average~~ number of *nir*S and *nir*K gene copies varied ~~significantly~~ widely among the 10 soils; *nir*S gene copies ranged from 2.5 × 10^7 to 3.9 × 10^8 copies g^{-1} soil, while *nir*K gene copies varied from 2.3 × 10^8 to 5.9 × 10^8 g^{-1} soil ~~(Fig. 4a).~~. Overall soils, *nir* genes ~~The genes encoding nitrite reductase (*nirS+nirK)*~~ were on average an order of magnitude more abundant than those encoding the final step of denitrification. ~~(*nosZ*) in all soils.~~ ~~*The abundance of n*~~*N*os*Z* gene copies varied ~~over~~ most widely ~~two orders of magnitude among the soils in three groups~~ (7.1 × 10^6 to 4.8 × 10^7 g^{-1} soil), a much greater range than for *nir*S+*nir*K gene copies. The sum of *nir* gene (*nirS+K)* copies was significantly greater in the allophanic soils of group 1 than in the soils of group 3 (*P*<0.005, Fig. 4~~3~~a), with the effluent irrigated (group 2) soil having intermediate values. Despite large variability, t~~The abundance of *nosZ* gene copies was significantly higher in t~~he group 2 soil had ~~soil~~ significantly more *nosZ* gene copies than the soils in group 1 and 3, ~~(*P*<0.005)~~ while the group 1 soil had significantly fewer than the other two groups (P<0.005, Fig. 4~~3~~a). ~~and *nir* gene abundance was significantly higher in group 1 soils than in the soils of group 3 (*P*<0.005).~~ Consequently the ratio of *nosZ*:*nirS+K* genes, which may indicate of the relative abundance of complete denitrifiers, varied significantly among the three groups of soils (Fig. 4b), with the ~~MWEI~~ effluent irrigated group 2 soil~~s~~ harbouring the ~~proportionally greater~~highest, the allophanic soils of group 1 the lowest and the group 3 soils intermediate ratios of *nos:nir* genes. ~~community of complete denitrifiers.~~

~~**The abundance of *nir* and *nos* genes also varied with the major physicochemical characteristics of the soils. The allophanic soils of group 1 were similar in harbouring relatively large *nir*S+K communities but were among the smallest *nosZ* communities. Consequently these soils had similar and low *nos:nir* ratios, with complete denitrifiers comprising approximately 1% of all bacterial denitrifiers. The group 2 effluent irrigated soil had relatively large *nir* and *nos* communities, with complete denitrifiers comprising about 7% of the denitrifier community.**~~

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~~However, we found the size of denitrifier communities, like other soil characteristics, to be quite variable among replicates of group 2.~~ *Insert Figure ~~4~~5*

3.3 Denitrification enzyme activity (DEA)

DEA varied considerably among the pasture soils, but also among replicates ~~of~~ within a soil~~, as evident in the high SEM values~~ (Table S3~~2~~). DEA varied by a factor of five among the soil groups, with the group 2 soil (~~was highest in the~~ effluent irrigated ~~(~~MWEI) ~~soil that formed group 2~~achieving significantly higher DEA than other groups, while the soils of ~~(H = 12.09, *P* = 0.02) while~~ group 1 (HR & OH) ~~soils~~ had ~~the lowest~~significantly lower DEA values than other groups ~~of all soils ((~~H = 12.09, *P* = 0.02; Table S3). DEA varied considerably in soils belonging to group 3. Overall, DEA was mostly strongly positively correlated soil NO_3^-–N contents and was mostly strongly negatively correlated to soil NH_4^+–N contents, driving its significant correlation with axis 2 of our PCA (Fig ~~3~~1). All significant physicochemical ~~and biological~~ correlate~~d~~s to DEA are given in Table S5.

~~was highest in the effluent irrigated (MWEI) soil that formed group 2 (H = 12.09, *P* = 0.02) and while group 1 (HR & OH) soils) had the lowest DEA values of all soils (Table S2). DEA varied considerably in soils belonging to group 3. Overall, DEA of the soils of three groups werewas mostly strongly significantly positively correlated to the pH, Olsen P, MBC, soluble C,soil NO_3^- N contents and was mostly strongly negatively correlated to *nos*Z gene copy numbers of the soils and negatively correlated to the soluble C,soil NH_4^+ N contents, driving its significant correlation with axis 2 of our PCA (Fig 2). All significant physicochemical and biological correlated to DEA are given in , and *nir*S+K gene copy numbers (Table S3).~~

3.4 Relationships among denitrification and denitrifier community size and structure across a range of soil moistures

Given that the structure of *nir*S, *nir*K and *nos*Z communities varied primarily in response to soil water content and Olsen P (Fig ~~3~~4), we wished to know if unique relationships between the richness and size of the denitrifier gene community and DEA exist at different SWCs. ~~Soils with similar~~ To address this question, we categorized soils according to coarse-scale SWC (high, moderate and low) and examined Pearson's correlations among these variables within soil SWC categories. ~~were analysed separately to~~

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~~determine the relationship between DEA, and denitrifier gene abundance and richness across a range of SWC. This revealed~~F ~~that~~ or ~~in~~ soils in the highest ~~with high~~ SWC category (MWEI, OH, and HR), we found that strong and significant positive correlations existed between denitrifier gene copy numbers and DEA ~~was significantly correlated to their denitrifier gene abundance~~ [*nos*Z (r = 0.643, *P* = 0.049), *nir*K (r = 0.821, *P* = 0.007)]~~, and *nir*S (r = 0.887, *P* = 0.001)] and~~. Likewise, strong and significant positive correlations existed between DEA and the~~denitrifier gene~~ T-RF richness of denitrifier genes [*nos*Z (r = 0.801, *P* = 0.010), *nir*K (r = 0.783, *P* = 0.013), and *nir*S (r = 0.793, *P* = 0.011)]. However, these patterns of correlation were not present in the soils categorized as moderate or low SWC.

~~In particular, the group 1 soil (MWEI), which had the highest Olsen P, microbial biomass, soil NO_3^- N content, *nos*Z gene copy abundance, and *nos*Z gene phylotypes richness, diversity, and evenness also had higher DEA of other two groups.~~

4 Discussion

Despite its relatively small total land area, New Zealand is geologically diverse and the 1.8 million hectares of land that were managed as dairy pasture in 2015 (DairyNZ, 2017), have soils derived from a wide range of parent materials. Here we studied 10 dairy pasture soils that varied widely in texture, drainage class and management strategies. We found that the % FC SWC and a gradient in mineral N-form accounted for the greatest variation in soil physiochemical characteristics, and that key microbial parameters for denitrification such as MBC and DEA were significantly positively correlated with higher soil NO_3^-–N. In our study, these patterns were driven primarily by only three soils; the two allophanic soils, which had high % FC SWC (group 1), and the effluent irrigated soil, which had very high NO_3^-–N (group 2). The effluent irrigated soil, which had the highest MBC, likely harboured a larger population of nitrifiers, whose activities generated the NO_3^- required by denitrifiers, and supported the highest rates of DEA we observed. Nonetheless, our results are consistent with previous reports that soil microbial biomass is a key indicator of denitrification process rates (Drury et al., 1991). From this perspective, across a wide range of soil properties, the size of the MBC pool may be an important coarse-scale indicator of soil N_2O emissions under both anoxic (denitrification) and oxic (nitrification) conditions.

Allophanic soils have high water content ~~moisture~~ at field capacity, but they adsorb copper, and are therefore likely to select against *nir*K denitrifiers, whose periplasmic nitrite reductase requires six copper atoms to maintain its trimeric structure. This was reflected in our data by the very low richness, evenness and diversity of *nir*K T-RFs, as well as in the very low numbers of *nir*K gene copies, relative to the other soils. We expected this to also reduce the overall number of genes encoding nitrite reductase in group 1 soils, but didn't observe this. Instead we found that *nir*S denitrifiers replaced nirK denitrifiers in allophanic soils, so that the total number of nir gene copies was equivalent to that in the effluent irrigated soil and significantly greater than the number of nir copies in all other soils. Interestingly, despite the large size of the nirS community, allophanic soils did not, on average, have more diverse *nir*S communities than other soils. However, the size and diversity of nirS communities in allophanic soils was more variable than for other soils. These findings suggest that allophanic soils support relatively few microsites where denitrification, driven by nirS denitrifers, is the dominant respiratory pathway. New Zealand's allophanic soils are porous and free-draining with relatively low bulk densities Molloy, 1998. As such, aerobic microsites conducive to denitrification are expected to be few. Likewise, fewer active microsites for denitrification fits with the low to moderate DEA we observed in the allophanic soils. Allophanic soils are known to adsorb P (Hashizume and Theng, 2007), and the binding of adenosine by allophanes may have limited DEA in these soils despite their relatively large *nir* populations (Hashizume and Theng, 2007). Nonetheless, we also found far fewer copies of nosZ genes, relatively low nosZ diversities, and the lowest *nos:nir* gene ratios in the allophanic soils, suggesting that complete denitrifiers are relatively rare in these soils. Consequently, where and when it occurs, denitrifcation in allophainc soils is likely to lead to significant N_2O emissions. This result fits with other work from our group, which indicates that allophanic soils emit greater N_2O:(N_2O + N_2) relative to other soil types (MacMillan et al 2016). Taken together, these results suggest that targeted management of nirS denitrifers in allophanic soils during wet seasons may be an effective strategy to combat greenhouse gas emissions from pastoral agriculture in this region.

The effluent irrigated soil (MWEI), with physiochemical properties that separated it from all other soils, was characterised by very high nitrate and Olsen P concentrations, relatively high pH (5.9) and high MBC,

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which supported very high DEA. This moderately drained, fine sandy loam had the highest SWC at the time of sampling. MWEI had the largest number of *nir*K gene copies, but only moderate numbers of nirS, leading to intermediate total numbers of *nir* genes. Likewise, it had the greatest diversity of *nir*K genotypes, but only moderate diversity of *nir*S genotypes. These findings emphasise the potential for effluent irrigation to increase denitrification enzyme activity, likely through increasing both the size of the total microbial community (MBC), SWC and nitrate availability, which in turn selects for denitrifiers. However, MWEI supported a significantly larger population of nosZ denitrifers than the other soils and this led to the highest *nos:nir* of any soil. Overall, these findings suggest that MWEI is likely to support a large and active community of denitrifiers but that complete denitrification may limit N_2O emissions from this soil. Consequently, management of greenhouse gas emissions from highly fertile pasture soils like MWEI may benefit from strategies that limit NO_3 availability in soils, such as the application of nitrification inhibitors (e.g. DCD).

When considered in isolation, the seven soils of group 3 still varied significantly with regard to physiochemical characteristics. In particular MW, PS and PL, which ranked higher on Axis 2 of the PCA, differed from the four remaining soils. For example, they had on average three times the nitrate and half the ammonium as the other group 3 soils. These soils also had the three highest pH values (6.0-6.4), and high MBC, but the relatively low SWCs. They supported relatively large numbers of nirS and nosZ denitrifiers, but only average numbers of nirK genes, leading to overall intermediate *nir:nos*. Likewise, these soils had intermediate diversities of nirS, nirK and nosZ genes and ordinations of the nirS, nirK and nosZ gene T-RFs, failed to distinguish these soils from those in the other groups. Despite this when incubated under non-limiting conditions, these three soils, together with MWEI, supported the highest DEAs. These findings indicate that denitrification responds quickly to SWC in moderately fertile soils. Thus, careful management of NO_3 loads by limiting dairy stock or the use of nitrification inhibitors or both, is also likely to be useful for limit greenhouse gas emissions from these soils during wetter periods of the year.

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The four remaining soils of group 3 were the least fertile and the most acidic (pH-4.8-5.7) with the lowest MBC in our study. They were also among the driest. Despite moderately high total numbers of *nir*S, *nir*K and *nos*Z genes, the nos:nir in these soils were equivalent to the other soils of group 3 and intermediate overall. With the exception of the two allophanic soils, the four remaining soils of group 3 had the lowest DEA, which was on average about a quarter of that measured in the other group 3 soils. Taken together, these results suggest lower risk of N_2O emissions from these soils, as aerobic conditions and low concentrations of substrates are likely to limit denitrification much of the time, and moderately high numbers of nosZ denitrifiers will favour some complete denitrification of this smaller total N pool.

Overall, ordinations of T-RFLP data revealed no structuring of the nirS, nirK or nosZ communities according the three groups that defined the major physicochemical characteristics of the soils, a gradient in soil moisture at field capacity and a gradient in mineral N forms. Rather SWC at the time of soil sample collection, and Olsen P were the primary drivers of the structure of denitirfier communities. Given the high correlation of SWC and Olsen P overall in soil samples, this result is likely to indicate considerable plasticity of the denitrifier community in response to ambient soil moisture. In the wettest soils, we found strong and significant positive correlations between the diversity of nirS, nirK and nosZ genes and DEA. We also found strong and significant positive correlations between nirK and nosZ gene copy numbers and DEA in those soils. However, these relationships broke down for soils with moderate or low SWCs. While the aim of our study was to sample pasture soils over a wide range of physiochemical characteristics in order gain insight to the properties of the denitrifier communities they support, seasonal variation in the structure of nirS and nirK denitrifiers in cultivated and pasture soils is well established (Wertz et al., 2016; Tatti et al., 2017; Bent et al., 2016;Yu et al., 2016;Smith et al., 2010). The plasticity of the denitrifier community in response to ambient soil moisture, together with the strong correlations between the size, diversity and activity of denitrifying communities in very wet soils suggests that future work toward characterising the denitrifier communities most likely to contribute to greenhouse gas emissions from pastoral soils should focus sampling efforts on the wettest times of the year.

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Several lines of evidence collected here suggest that nirK denitrifiers were more sensitive to the range of physiocochemical characteristics in soils than were nirS denitrifiers. For example, gene copy numbers and diversity metrics of nirS communities were fairly uniform across soil groups, but varied significantly for nirK denitrifiers. Likewise, the patterns of nirK diversity across soil groups were mirrored by the patterns of nos:nir, suggesting that changes in the size of the nirK community had a dominant influence on the overall ratio of complete and incomplete denitrifiers in soil groups. Likewise, independent shifts in nirS and nirK community structures in response to common physiochemical characteristics was recently observed in a eutrophic reservoir (Zhou et al., 2016). In contrast, the structure of nosZ communities did not correspond to any physiochemical property measured. Together, these results may suggest that nirS and nosZ genotypes are equivalently adapted to the physiochemical conditions of a wide range of dairy pasture soils, while nirK denitrifiers are more sensitive. Given that our data suggests greater N_2O emissions in allophanic soils where nirK denitrifiers are few, it may be the microbial communities dominated by nirS denitrifiers should be the target of efforts to reduce GHG emissions from pasture soils. However, further work is necessary to confirm whether microbial communities dominated by nirS denitrifiers support greater N_2O emissions than nirK denitrifier communities of equivalent size.

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5 Conclusions

Here we characterise the size, structure and diversity of *nir*S, *nir*K and *nos*Z 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 N_2O 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 N_2O emissions from such soils. However, our results for allophanic soils suggest that even relatively low rates of denitrification may lead to significant N_2O emissions given their relatively low *nos:nir*. Consequently, we conclude that management strategies to limit N_2O 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 *nir*S denitrifiers may be the most effective for limiting N_2O emissions through denitrification across a wide range of soil types.

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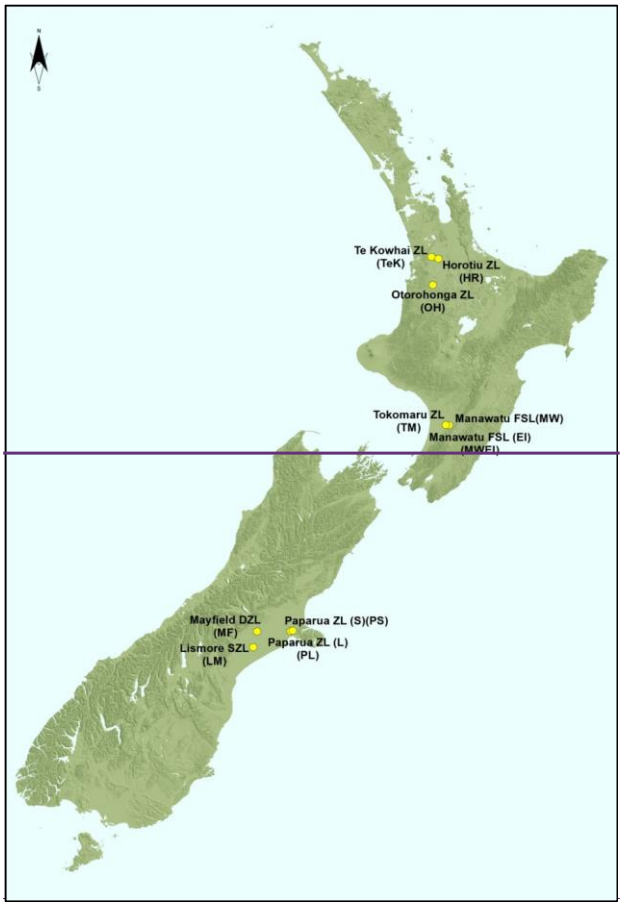


~~**Figure 1: New Zealand map representing the location of the collected dairy pasture soils. Letters adjacent to soil name describe texture class. ZL = silt loam, FSL = fine sandy loam, DSL = deep stony silt loam, SZL = shallow silt loam.**~~

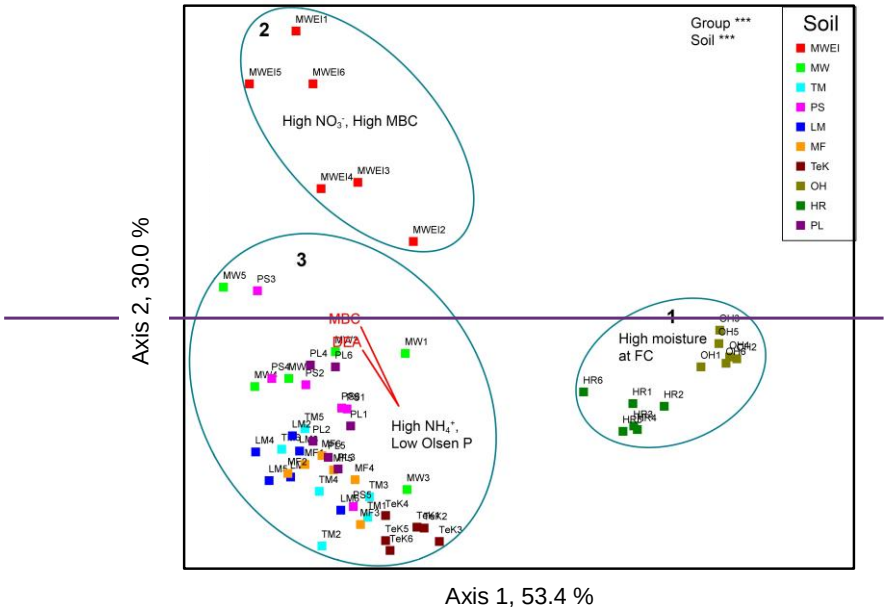
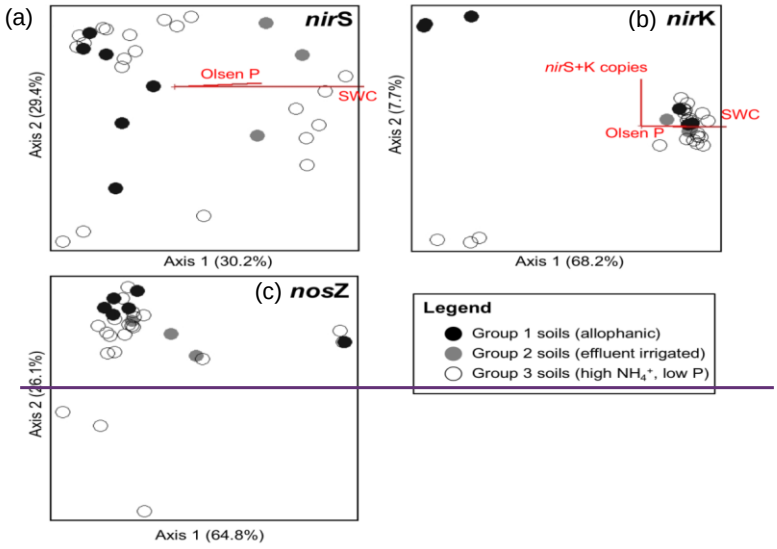


Figure 2: Ordination of Soil Characteristics in 1st and 2nd PC axes (0–100 mm depth). MWEI = Manawatu fine sandy loam (effluent irrigated); MW = Manawatu fine sandy loam; TM = Tokomaru silt loam; TeK = Te Kowhai silt loam; OH = Otorohanga silt loam; HR = Horotiu silt loam; PS = Paparua silt loam (Springston); LM = Lismore stony silt loam; MF = Mayfield silt loam; PL = Paparua silt loam (Lincoln). NO_3^- = Nitrate-N content, NH_4^+ = Ammonical-N content, MBC = Microbial biomass content, FC = Field capacity. Numbers adjacent to soil codes represent replicate number. Numbers in bold inside the ovals indicate group numbers.



~~**Figure 3: Non-metric multidimensional scaling (NMS) ordination of soil samples in (a) *nirS* genotype space, vectors represent the factors that were significantly correlated to *nirS* community structure at tau = 0.2. (b) *nirK* genotype space, vectors represent those factors that were significantly correlated to *nirK* community structure at tau = 0.2. (c) *nosZ* genotype space, no soil physicochemical characteristics were significantly correlated to *nosZ* community structure. Soil abbreviations denote same soil names as described in Fig. 2. SWC = Soil water content, NirS+K = Nitrite reductase (*nirS+K*) gene copy numbers, Olsen P= Olsen Phosphorus. Group 1 soils -OH and HR, group 2 soil -MWEI, group 3 soils – MW, TeK, TM, PL, PS, LM, MF. Abbreviations of soils are described in the Fig. 2 legend.**~~

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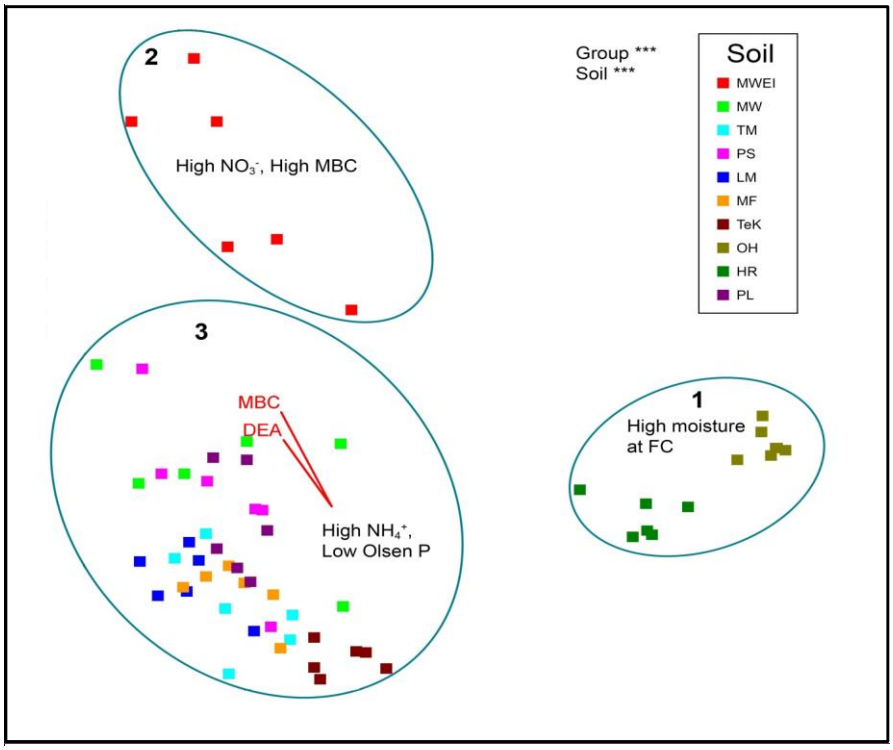

Fig. 1 Ordination of Soil Characteristics

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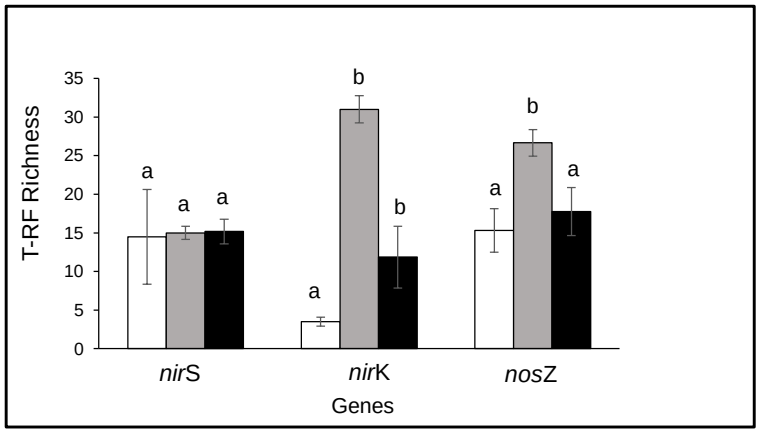

Fig. 2 Gene T-RF richness in different soil groups. White, grey and black columns denote groups I, II and III soils. Mean values are reported ± 1 standard error of the mean. Columns with the same letters are not significantly different.

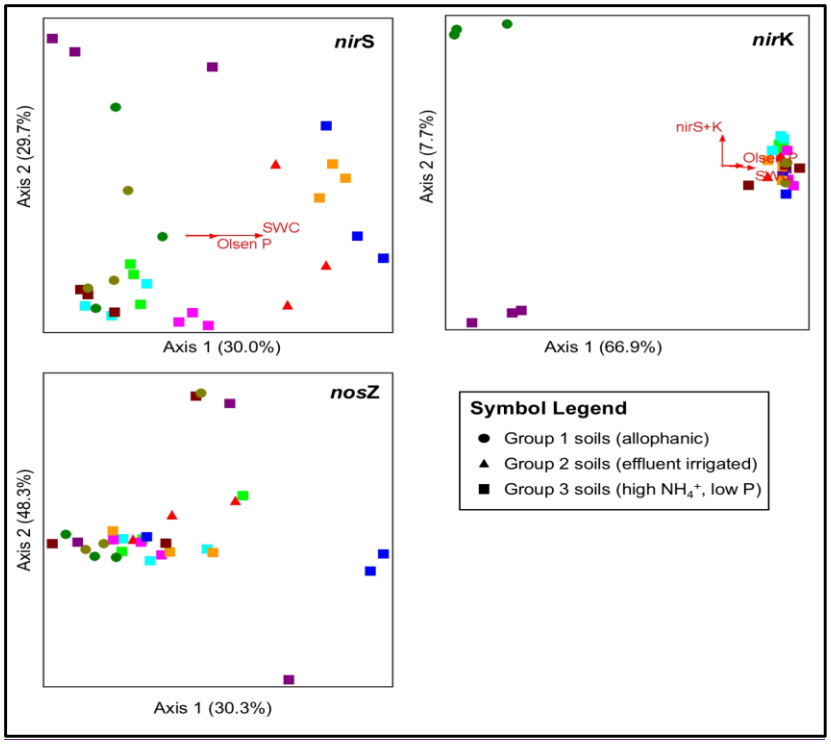

Fig. 3 Non-metric multidimensional scaling (NMS) ordinations illustrating the Bray-Curtis dissimilarities of *nir*S, *nir*K and *nos*Z communities. Vectors represent those factors that were significantly correlated to the first and second ordination axes at tau = 0.2. Symbol colours are the same as in Fig. 2. Symbol shapes represent the three groups of soils as determined by PCA of their physiochemical characteristics (Fig 1).

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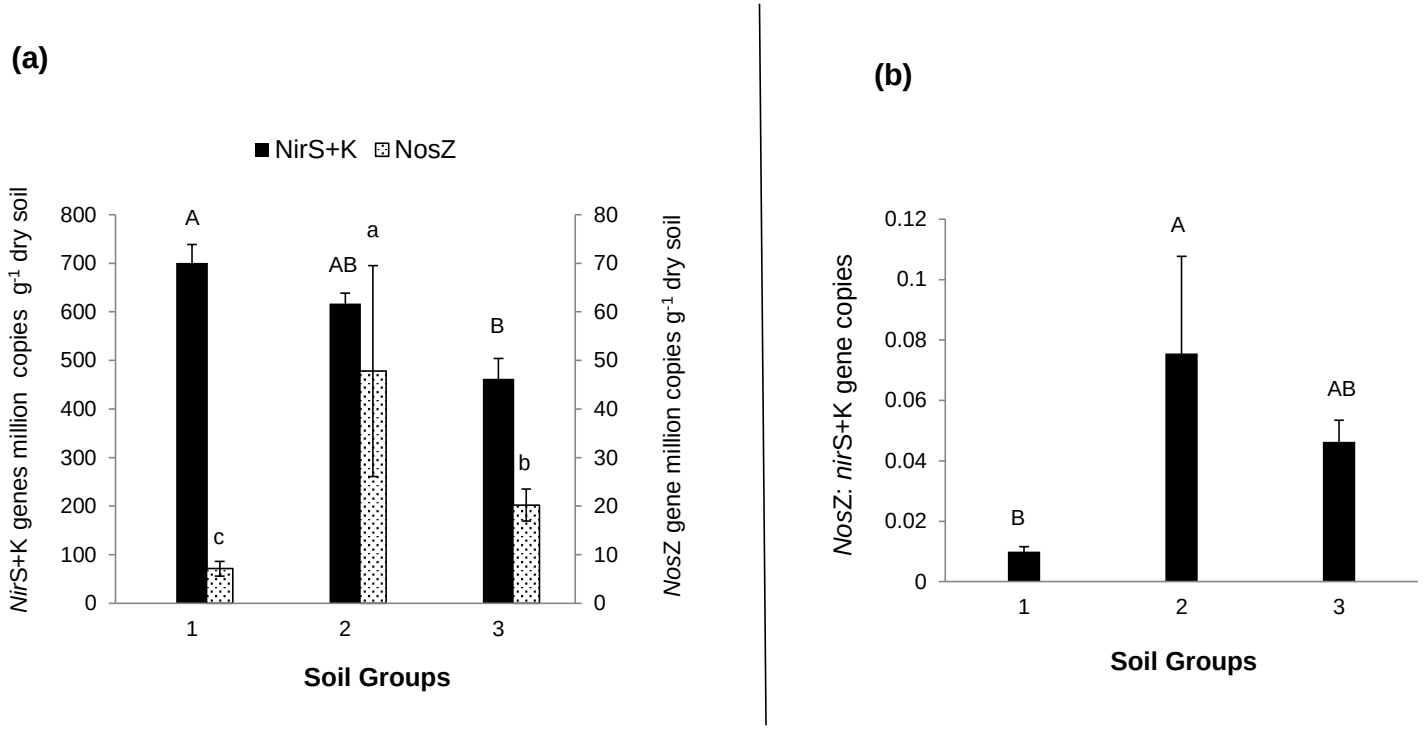

Fig. 4 (a) Denitrifier gene (*nir*S, *nir*K, *nos*Z) copy numbers in different soil groups, error bars denote S.E.M. (b) Denitrifier gene abundance ratio (*nos*Z : *nir*S, *nir*K) in different soils groups. Mean values are reported ± 1standard error of the mean. Columns with the same letters are not significantly different. Letter values with same case or font denote one test (one test for each of the genes). Group 1 soils -OH and HR, group 2 soils -MWEI, group 3 soils – MW, TeK, TM, PL, PS, LM, MF. Abbreviations of soils are described in the Fig. 2 legend

Table 1: Description of soils

Soil	Location of the dairy farm	Geographical Location	Soil Abbreviation	Soil Classification	Mineralogy Class	~~Date of sampling~~
Te Kowhai Silt Loam	AgResearch Ruakura, Waikato	37°44'57.55"S 175°10'27.06"E	TeK	Typic Orthic Gley	GlassyVolcanic , Kaolinitic	~~August 2010~~
Otorohanga Silt Loam	Tokanui, Waikato	38°11'19.70"S 175°12'35.67"E	OH	Typic Orthic	Allophanic	~~August 2010~~
Horotiu Silt Loam	AgResearch Ruakura, Waikato	37°46'30.80"S 175°18'23.27"E	HR	Typic Orthic Allophanic	Allophanic	~~August 2010~~
Tokomaru Silt Loam	Massey University, Palmerston North	40°22'58.50"S 175°36'31.01"E	TM	Argillic-fragic Perch-gley Pallic	Vermiculitic	~~September 2010~~
Manawatu Fine Sandy Loam	Longburn, Palmerston North	40°22'56.99"S 175°32'24.49"E	MW	Weathered fluvial recent	Illitic	~~November 2010~~
Manawatu Fine Sandy Loam (Effluent irrigated)	Longburn, Palmerston North	40°22'58.26"S 175°32'21.65"E	MWEI	Weathered fluvial recent	Illitic	~~December 2010~~
Paparua Silt Loam (Springston)	Springston, Christchurch	43°38'15.97"S 172°28'13.81"E	PS	Weathered Orthic recent	Illitic	~~December 2010~~
Paparua Silt Loam (Lincoln)	Lincoln, Christchurch	43°38'43.91"S 172°25'21.86"E	PL	Weathered Orthic recent	Illitic	~~December 2010~~
Lismore Stony Silt Loam	Ashburton, Canterbury	43°53'17.44"S 171°38'28.43"E	LM	Pallic Orthic Brown	Vermiculitic	~~December 2010~~
Mayfield Deep Silt Loam	Methven, Canterbury	43°38'30.12"S 171°43'47.28"E	MF	No data	No data	~~December 2010~~

Table 2: Richness, Pielou's evenness index, and Shannon's diversity index and of denitrifier gene terminal restriction fragments (T-RFs) in soils

	Richness			Pielou's Evenness Index			Shannon's Diversity Index		
Group	*nirS*	*nirK*	*nosZ*	*nirS*	*nirK*	*nosZ*	*nirS*	*nirK*	*nosZ*
1	14.5 ± 3.5^{a}	3.5 ± 0.9^{c}	15.3 ± 0.5^{b}	0.6 ± 0.1^{a}	0.3 ± 0.1^{c}	0.7 ± 0.0^{b}	2.5 ± 0.3^{a}	1.1 ± 0.3^{c}	2.7 ± 0.0^{b}
2	15.0 ± 0.6^{a}	31.0 ± 4.0^{a}	26.7 ± 1.2^{a}	0.7 ± 0.0^{a}	0.9 ± 0.0^{a}	0.8 ± 0.0^{a}	2.7 ±0.0^{a}	3.4 ± 0.1^{a}	3.3 ± 0.1^{a}
3	15.2 ± 1.6^{a}	11.8 ± 1.8^{b}	17.8 ± 1.0^{b}	0.7 ± 0.0^{a}	0.6 ± 0.0^{b}	0.7 ± 0.0^{b}	2.6 ± 0.1^{a}	2.2 ± 0.1^{b}	2.8 ± 0.1^{b}

Letters denote one way ANOVA test. Values sharing same letter are not significantly different in the column they are present in.
Where MWEI = Manawatu fine sandy loam (Effluent irrigated), HR = Horotiu silt loam, OH = Otorohanga silt loam, MF = Mayfield silt loam, MW = Manawatu fine sandy loam, TM = Tokomaru silt loam, TeK = Tekowhai silt loam, PS = Paparua silt loam (Springston), LM = Lismore stony silt loam, PL = Paparua silt loam (Lincoln); *nir*S and *nir*K = nitrite reductase gene, *nos*Z = nitrous oxide reductase gene.