



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

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- 10 Abstract. Denitrification is an anaerobic respiration process that is the primary contributor of the nitrous oxide (N₂O) 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)
- 15 and assessed community structure by terminal restriction fragment length polymorphism (T-RFLP) of the nitrite reductase (*nirS*, *nirK*) and N₂O reductase (*nosZ*) genes. We quantified denitrifier enzyme activity (DEA) using acetylene inhibition technique.

Differences in the physicochemical characteristics of the soils were driven mainly by soil mineralogy and the management practices of the farms. We found that *nirS* and *nirK* communities strongly structured along the gradients of soil water and

20 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 richness and abundances of *nir*S, *nir*K and *nos*Z genes were significantly positively correlated with DEA. Our data suggest that soil moisture, microbial biomass, and fertility were the primary drivers of the structure and abundance of denitrifier communities across a wide range of geologic and geographical soil origins.

25 1 Introduction

Nitrous oxide (N₂O), 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 (NO₃⁻) to nitrite (NO₂⁻) and into the gaseous forms N₂O and benign dinitrogen (N₂). It is the major global contributor to N₂O production in grassland soils (Saggar et al., 2013) and is responsible for a significant fraction of agricultural greenhouse gas





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(NIR), nitric oxide (NO) reductase (NOR), and N₂O reductase (N₂OR) (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 N₂ and are said to be 'complete' denitrifiers.

- Those denitrifiers that lacks N₂OR emit N₂O, as the final product of denitrification are called 'incomplete' denitrifiers. *NirS*, *nirK*, and *nosZ* 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 N₂O: N₂ produced during denitrification (Philippot et al., 2011;Bakken et al.,
- 10 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. However, 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). Moreover, 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.

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 shift soil microbial communities, which in turn influences soil biochemical processes (Kennedy and Smith, 1995). The addition of crop residues to soils is associated with

- 20 the increased abundance of denitrifier genes and greater denitrification in soils (Barrett et al., 2016;Henderson et al., 2010;Gao et al., 2016). Likewise, increasing soil 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
- 25 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 how these variations in soil properties affect denitrifier populations and denitrification is still limited.

We 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.

30 We collected 10 soils from dairy-grazed pastures with contrasting soil textures, drainage classes, management strategies (effluent irrigated or non-irrigated), and geographic locations in New Zealand. The differences in the physicochemical properties of soils enabled us to integrate the information about variability in denitrifier communities and denitrification activities in different pasture soils. We hypothesised that pasture management practices that alter soil water, carbon and fertility will enhance the size, structure and activity of the denitrifier 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

- 5 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 collected from 10 New Zealand dairy farms (Fig. 1). All the sites from which the soil samples were collected are commercially managed grazed dairy pastures 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.
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Insert Fig. 1

Insert Table 1

Twenty-five soil cores (25 mm diameter \times 100 mm long) were collected from the 0–100 mm depth using a steel corer from six random locations of 100 m² area on each farm (once) between August and December 2010 (10 sites \times 6 replicates \times 2 depth = 120 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 4°C in plastic bags. A sub-sample of each soil replicate was stored at -20° C for molecular analysis. The extracts for both chemical and molecular analyses were prepared within 6 months of collection of the soil samples. The physicochemical characteristics of the field-moist sieved soils were analysed using standard protocols described previously (Morales et al., 2015).

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 method of gas sampling and analysis are described previously (Morales et al., 2015). 2.3 DNA extraction from soils
- 30 DNA was extracted from 0.25 g of each replicate soil sample 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* in soil samples. T-RFLP for *nir*S and *nos*Z genes was conducted as described in
Deslippe et al. (2014) except the reaction conditions for *nir* genes were as follows.

PCR amplification of *nir*S gene was performed in a total volume of 25 μl reaction mixture containing 2.5 μl of 10×PCR buffer (1 mM MgCl₂), 0.5 mM MgCl₂, 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

- 10 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 ×
- 15 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 T-RFs per electropherogramme was taken to indicate genotype

20 richness per sample. We then 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 *nirS*, *nirK*, and *nosZ* 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. The reactions were linear over 7 orders of magnitude and sensitive down to 10^2 copies.

2.6 Statistical Analysis

The data for soil chemical characteristics, gaseous emissions, denitrifier gene terminal restriction fragments (T-RFs) and 30 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).

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

- 5 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 (NO₃⁻) & ammonium (NH₄⁺) –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, 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 significance of these groups. In order to assess how denitrification and soil microbial properties varied with the physicochemical characteristic of soils, we calculated
- 15 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 *nirS*, *nirK* and *nosZ* community structure was based on threshold normalised peak heights of T-RF from electropherogrammes (Deslippe et al., 2014). Non-metric multidimensional scaling (NMS) ordinations were performed using

20 Bray & Curtis distance (Bray and Curtis, 1957) in the programme PC-ORD (McCune and Mefford, 1999). 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

25 The 10 soils differed significantly with regard to all measured physicochemical characteristics (Table S1). The PCA of soil characteristics generated 3 significant axes, of which the first two accounted for 83.4 % of the total variance (Fig. 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 NO₃⁻–N increasing and NH₄⁺–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 clustered mainly due to their relatively high NH₄⁺–N and low Olsen P contents (group 3). MRPP indicated that these groups differed significantly in soil physicochemical characteristics (A= 0.379, P < 0.001). Table S2 summarises the physicochemical characteristics for the 3 groups of soils. We found that axes 1 and 3 of the PCA were not

5 significantly correlated to any measure of denitrification or to microbial biomass carbon. However, axis 2 of the PCA, which describes a gradient in mineral N-form, was significantly correlated to DEA ($r^2 = 0.214$) and to MBC ($r^2 = 0.303$; Fig. 2).

Insert Fig. 2

3.2 Bacterial denitrifiers in New Zealand dairy-grazed pasture soils

10 3.2.1 Richness, diversity and evenness of denitrifier gene T-RF profiles

All three genes varied significantly in richness among the 10 soils (Table 2). The richness, evenness, and diversities of the nirK and nosZ communities also differed significantly among the 3 groups of soils as defined by physicochemical properties (Kruskal-Wallis test statistics H for nirK H = 13.84, P = 0.0001; nosZ H = 8.59, P = 0.014). The nirK and nosZ 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 nirS communities did not differ significantly among the 3 groups (H

15 = 0.11, P = 0.946).

Insert Table 2

3.2.2 Denitrifier community structure in soils

Ordination of the soil samples in nirS T-RF space indicated significant structuring of the nirS community according to 20 variation in the physicochemical characteristics of the soils (Fig. 3a). In particular, Axis 1 of Figure 3a, which accounted for 30.2% of the variation in nirS community structure, was significantly correlated to SWC and Olsen P content of the soils. This indicates that *nirS* community structure in dairy pasture soils from across New Zealand responded most strongly to moisture and P gradients. Moreover, nirS 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

25 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% of the variation in nirK community structure (NMS axis 1) separated HR soil (Horotiu silt loam) from group 1 and PL (Paparua Lincoln silt loam) soil from group 3 (Fig 3b). Similar to the nirS T-RF space axis 1 was significantly correlated to SWC and the Olsen P content. An additional 7.7% of

30 the variation in nirK community structure formed axis 2 of the ordination on which HR and PL separated from their





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respective groups. Axis 2 was most strongly correlated to *nir* gene abundance in soils, which was higher for the group 1 HR soil than for the group 3 PL 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 patterns in *nosZ* community structure that corresponded to the physicochemical characteristics of the soils (Fig. 3c).

Insert Figure 3

3.2.3 Denitrifier gene abundance

The average number of *nir*S and *nir*K gene copies varied significantly among the 10 soils; *nir*S gene copies ranged from 2.5 $\times 10^7$ to 3.9 $\times 10^8$ copies g⁻¹ soil, while *nir*K gene copies varied from 2.3 $\times 10^8$ to 5.9 $\times 10^8$ g⁻¹ soil (Fig. 4a). The genes

- 10 encoding nitrite reductase (*nir*S+*nir*K) were on average an order of magnitude more abundant than those encoding the final step of denitrification (*nos*Z) in all soils. The abundance of *nos*Z gene copies varied over 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 abundance of *nos*Z gene copies was significantly higher in the group 2 soil than the soils in group 1 and 3 (*P*<0.005) 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
- 15 nosZ:nirS+K genes varied significantly among the soils (Fig. 4b), with the MWEI effluent irrigated group 2 soils harbouring the proportionally greater 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 *nos*Z communities. Consequently these soils had similar and low *nos:nir* ratios, with complete denitrifiers comprising

20 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. However, we found the size of denitrifier communities, like other soil characteristics, to be quite variable among replicates of group 2.

Insert Figure 4

3.3 Denitrification enzyme activity (DEA)

- DEA varied considerably among the pasture soils, but also among replicates of a soil, as evident in the high SEM values (Table S2). DEA was highest in the effluent irrigated (MWEI) soil that formed group 2 (H = 12.09, P = 0.02) and group 1 (HR & OH soils) had the lowest DEA of all soils (Table S2). DEA varied considerably in soils belonging to group 3. DEA of the soils of three groups were significantly positively correlated to the pH, Olsen P, MBC, soluble C, NO₃⁻–N content and *nos* Z gene copy numbers of the soils and negatively correlated to the soluble C, NH₄⁺–N contents, and *nir*S+K gene copy
- 30 numbers (Table S3).





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

Soils with similar SWC (high, moderate and low) were analysed separately to determine the relationship between DEA, and denitrifier gene abundance and richness across a range of SWC. This revealed that in soils with high SWC (MWEI, OH, and HR), 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 denitrifier gene T-RF richness [*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)].

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

10 4.1 Physicochemical and biochemical characteristics of soils

In order to understand the influence of variability in soil properties on denitrification activity and denitrifier population, in this study we selected pasture soils that varied widely in geologic and geographical origins and in the management practices applied to the pasture. While variability in soil properties was generally greater among than within soils, we also noted significant variability in soil characteristics among the soil replicates of some soils. Soil characteristics can vary significantly

15 within a paddock due to uneven grazing and excretal deposition, which lead to a non-uniform distribution of nutrients on farms (Baily et al., 2012;Hutchings et al., 2007).

The greatest variation in soil physicochemical properties was due to the volcanic origin of the two allophanic soils sampled. The allophanic soils of group 1 were well drained and had similar silt loam textures. The soil physicochemical characteristic that caused these soils to differ most strongly from all others was their high moisture contents at field capacity; however,

20 they also had relatively high TC, TN, and soluble C. The group 1 soils had relatively low Olsen P contents, which is likely to reflect the high affinity of allophane for phosphates (Hashizume and Theng, 2007). The soils that formed group 1 had the lowest DEA of the 3 groups which could possibly be due to binding of adenosine by allophane thus limiting enzyme activity (Hashizume and Theng, 2007).

The second greatest source of variation in soil properties was driven by effluent irrigation of the MWEI soil. This soil had

- 25 received repeated annual effluent applications for the last 16 years and had the highest Olsen P, MBC, pH, NO₃⁻-N, and the highest DEA of all the soils tested. Regular effluent application leads to higher availability of labile C, P and N in soils and induces physical changes as well as changes in organic matter, nutrients and salts (Lado and Ben-Hur, 2009;Truu et al., 2009). Along with enhancing the labile C and substrate availability (van der Weerden et al., 2016), effluent application increased the size and activity of the denitrifier community in our study. It is noteworthy that the MW (with no effluent
- 30 application) and MWEI (with regular effluent application) soils showed a similar magnitude of variation in soil properties,





which was much greater than that of any other soil. This suggests that effluent irrigation increases soil NO_3^- and Olsen P concentrations and MBC without altering the spatial heterogeneity of the soil.

4.2 Influence of soil characteristics on denitrifier gene richness and abundance

Soils with higher MBC, Olsen P, and SWC (especially MWEI) had more diverse and abundant *nir*K and *nos*Z communities.
Thus, higher soil fertilities were associated with bigger, more diverse, and even denitrifier populations in this study. Conversely, the less fertile and drier soils tended to harbour smaller and more uneven denitrifier populations. These findings support our hypothesis that soils with higher C and P contents will harbour larger and more active denitrifier communities. To a lesser extent *nir* richness, diversity and abundance correlated with other measures of soil fertility such as increase in pH, soluble C, and NH₄⁺ contents, these findings agree with previous studies that have found the composition of soil

denitrifier communities to be sensitive to a variety of soil chemical characteristics (Jones and Hallin, 2010;Reyna et al., 2010;Chon et al., 2011;Miller et al., 2008;Tatti et al., 2013).
 Since the S and K variants of the *nir* gene are mutually exclusive (Jones and Hallin, 2010), we expected *nir*S and *nir*K gene

abundance to be negatively correlated however this was not the case for the soils studied. This may suggest that either the prevailing soil conditions did not favour one *nir* variant, or that spatial heterogeneity in the soils sampled allows different

15 populations of denitrifiers to co-exist at small spatial scales (Hallin et al., 2009;Hibbing, 2010;Knapp et al., 2009;Petersen et al., 2012).

4.3 Denitrifier community structure, size and activity

We found that the structure of the *nirS* and *nirK* communities did not correspond to the major gradients in soil physicochemical characteristics (mineralogical and effluent application), but rather were strongly structured along gradients

- 20 of SWC and Olsen P. Conversely the *nos*Z community showed little structure and was unrelated to any of the physiochemical soil properties measured. However when we accounted for soil water content, we found that the composition (*nos*Z:*nir*S+*nir*K), size (gene copies) and activity (DEA) of the denitrifier communities were significantly correlated. In particular the relatively infertile allophanic soils of group 1, had low *nos:nir* and low DEA while the relatively fertile effluent irrigated soil of group 2 had the highest *nos:nir* and supported the greatest DEA. This is in agreement with other
- studies suggesting sensitivity of denitrifier communities to land use and soil types (Hallin et al., 2009;Philippot et al., 2009;Enwall et al., 2010;Smith et al., 2010), but underscores the dominant role of soil moisture in determining the structure of denitrifier communities.

Changes in SWC can cause the structure of denitrifier communities to diverge (Mergel et al., 2001;Reyna et al., 2010;Chon et al., 2011;Liu et al., 2012). In our study, the *nir*K communities of the driest soils diverged most strongly from all others,

30 ranking very low on axis 1 in the NMS ordination. Surprisingly, the wettest soil, from group 1 MWEI (effluent applied Manawatu fine sandy loam), harboured similar *nir*S communities to two other soils (MF and LM) of group 3. This may suggest that the presence of key genotypes in divergent soils might ultimately lead to variations in DEA in soils. Likewise,





with a smaller *nir* community and very low *nir*S richness the PL soil sustained comparatively high DEA relative to the other soils in group 3. This may suggest that, although small in size, the unique denitrifier community of the PL soil had the capacity to perform denitrification even in relatively unfavourable dry conditions, again pointing to the potential for unique genotypes to drive denitrification under the specific conditions that prevail in a given soil environment.

5 4.4 Influence of soil characteristics on DEA

DEA tends to show high spatial variability due to changes in available nutrients and microbe-soil interactions at small spatial scales (Jahangir et al 2016). We found the highest DEA in the group 2 effluent irrigated soil. DEA was positively correlated to soil pH, Olsen P, MBC and NO_3^- -N contents. Even soils that were not sufficiently high in NO_3^- -N to group with the group 1 soil, but which had relatively high MBC and Olsen P tended to have greater DEA than other soils. These findings

10 emphasise the potential for effluent irrigation to increase potential denitrification enzyme activity, likely through increasing both the size of the microbial community (MBC) and nitrate availability. These results highlight that soil management plays an important role in shaping denitrifier assemblages, leading to more active denitrifying communities across soils of contrasting physicochemical characteristics.

5 Summary and conclusions

- 15 We studied the relationships among soil physiochemical characteristics and the structure, size and activity of denitrifier communities in 10 dairy pasture soils from across New Zealand. The soils varied in physicochemical characteristics, management, mineralogy, geographic location, and climatic conditions. We found that minerology (allophanic or non-allophanic) and management (effluent irrigated or not) determined the dominant gradients in soil physiochemical characteristics among soils. However, SWC and Olsen P were primary determinants of the structure, size and activity of the
- 20 denitrifier community. In wet soils, we found strong relationships between DEA and the abundance of denitrifier genes, with allophanic soils harbouring the lowest proportional abundance of complete denitrifiers while the effluent irrigated soil sustained the highest DEA and harboured the greatest proportional abundance of complete denitrifiers. Most denitrification occurs during the winter period when pasture soils in New Zealand are frequently saturated (Saggar et al., 2013). Our data therefore suggest that during this time fertile soils, with greater microbial biomass are likely to promote the complete
- 25 reduction of N oxides to N_2 . Under laboratory incubations saturated allophanic soils are likely to be significant sources of N_2O (McMillan et al., 2016;Jha et al., 2016). Allophanic soils adsorb copper an essential co-factor for N_2OR by allophanehumic complexes. Due to this sorption lesser N_2OR is available for reduction of N_2O to N_2 and thus more N_2O is produced (McMillan et al., 2016). If these soils are managed to increase microbial biomass and fertility, this could lead to the reduction of N_2O to N_2 thus reducing harmful greenhouse effects.





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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.







Axis 1, 53.4 %

5 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). NO₃⁻ = Nitrate-N content, NH₄⁺ = 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 5 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 10 soils are described in the Fig. 2 legend.







Fig. 4 (a) Denitrifier gene (*nirS*, *nirK*, *nosZ*) copy numbers in different soil groups, error bars denote S.E.M. (b) Denitrifier gene abundance ratio (*nosZ* : *nirS*, *nirK*) 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

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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	НО	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	Sd	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	ΓM	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

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Table 1: Description of soils

() (c)

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	ı's Evenness Ind	ex	Shan	non's Diversity l	ndex
Group	nirS	nirK	nosZ	nirS	nirK	nosZ	nirS	nirK	Ison
1	$14.5\pm3.5^{\rm a}$	$3.5\pm0.9^{\mathrm{c}}$	$15.3\pm0.5^{\mathrm{b}}$	$0.6\pm0.1^{ m a}$	$0.3 \pm 0.1^{\circ}$	$0.7\pm0.0^{\mathrm{b}}$	$2.5\pm0.3^{\mathrm{a}}$	$1.1 \pm 0.3^{\circ}$	$2.7\pm0.0^{\mathrm{b}}$
2	$15.0\pm0.6^{\rm a}$	$31.0\pm4.0^{\rm a}$	$26.7\pm1.2^{\rm a}$	$0.7\pm0.0^{\mathrm{a}}$	0.9 ± 0.0^{a}	$0.8\pm0.0^{\mathrm{a}}$	2.7 ± 0.0^{a}	3.4 ± 0.1^{a}	3.3 ± 0.1^{a}
3	15.2 ± 1.6^{a}	$11.8\pm1.8^{\rm b}$	$17.8\pm1.0^{\rm b}$	$0.7\pm0.0^{\mathrm{a}}$	$0.6\pm0.0^{\mathrm{b}}$	$0.7\pm0.0^{\mathrm{b}}$	2.6 ± 0.1^{a}	$2.2\pm0.1^{\mathrm{b}}$	$2.8\pm0.1^{\mathrm{b}}$

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, TM = Tekowhai silt loam, PS = Paparua silt loam (Springston), LM = Lismore stony silt loam, PL = Paparua silt loam (Lincoln); *nirS* and *nirK* = nitrite reductase gene, *nosZ* = nitrous oxide reductase gene.

