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# Soil moisture is a primary driver of comammox *Nitrospira* abundance in New Zealand soils



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#### HIGHLIGHTS

#### GRAPHICAL ABSTRACT

- Comammox *Nitrospira* was found to be most abundant under the irrigated dairy land use, suggesting they may be copiotrophic.
- Comammox *Nitropsira* abundance was positively correlated with annual rainfall and soil moisture.
- Comammox Nitrospira abundance was negatively correlated with mean annual air temperature and soil pH.
- All the comammox *Nitrospira amoA* sequences found belonged to clade B, with 17 % belonging to a newly distinguished subclade, clade B.2.

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#### ABSTRACT

The objectives of this study were to investigate the abundance and community composition of comammox Nitrospira under: (i) pasture-based dairy farms from different regions, and (ii) different land uses from the same region and soil type. The results clearly showed that comammox Nitrospira were most abundant  $(3.0 \times 10^{6} \text{ copies})$  under the west coast dairy farm conditions, where they were also significantly more abundant than canonical ammonia oxidisers. This was also true in the Canterbury dairy farm. The six land uses investigated were pine monoculture, a long term no input ecological trial, sheep + beef and Dairy, both irrigated and non-irrigated. It was concluded that comammox Nitrospira was most abundant under the irrigated dairy farm  $(2.7 \times 10^{6} \text{ copies})$ . Contrary to the current industry opinion, the relatively high abundance of comammox Nitrospira under fertile irrigated dairy land suggests that comammox Nitrospira found in terrestrial ecosystems may be copiotrophic. it was also determined that comammox Nitrospira was more abundant under irrigated land use than their non-irrigated counterparts, suggesting that soil moisture is a key environmental parameter influencing comammox abundance. Comammox abundance was also positively correlated with annual rainfall, further supporting this theory. Phylogenetic analysis of the comammox Nitrospira detected determined that 17 % of the comammox community belonged to a newly distinguished subclade, clade B.2. The remaining 83 % belonged to clade B.1. No sequences from clade A were found.

#### 1. Introduction

\* Corresponding author. *E-mail address*: hong.di@lincoln.ac.nz (H.J. Di). Nitrification, the microbial oxidation of ammonia ( $NH_3$ ) via nitrite ( $NO_2^-$ ) to nitrate ( $NO_3^-$ ) is an important process in the terrestrial nitrogen (N) cycle, as it contributes to the production of two environmentally significant products, nitrous oxide ( $N_2O$ ), and nitrate. Traditionally, nitrification

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was thought to be a two-step process, where ammonia is first converted to nitrite by ammonia-oxidizing bacteria (AOB) before nitrite oxidizing bacteria (NOB) complete the oxidation to nitrate (Costa et al., 2006). Later, it was discovered that a group of archaea (ammonia oxidizing archaea, AOA) could also undertake ammonia oxidation, typically in oligotrophic/extreme conditions such as low ammonia availability and pH (Könneke et al., 2005; Francis et al., 2007; Di et al., 2009; Di et al., 2010).

The separation of nitrification into two steps involving different microorganisms has puzzled scientists as a single organism completing both steps of nitrification was theoretically assumed to be more efficient (Costa et al., 2006). Therefore, Costa et al. (2006) theorised that there was a single organism with lower growth rates but higher yields that could undertake both steps of nitrification. The presence of complete ammonia oxidisers (comammox) was later confirmed by Van Kessel et al. (2015) and Daims et al. (2015) who cultivated them from an aquaculture system and deep oil well, respectively. It was found that comammox belongs to lineage II of the genus *Nitrospira*, a group of bacteria traditionally thought to be responsible for nitrite oxidation (Daims et al., 2015). Comammox *Nitrospira* and canonical *Nitrospira* can be distinguished by the presence of the gene responsible for ammonia oxidation, known as the ammonia monooxygenase gene (*amo*). Furthermore, comammox *Nitrospira* can be separated into clade A and clade B based on the phylogeny of this gene.

Since its initial discovery, comammox Nitrospira has been found in a variety of terrestrial environments including forest, pastoral and arable ecosystems (Shi et al., 2018; Li et al., 2019; Wang et al., 2019a; Wang et al., 2019b; Xu et al., 2020; Osburn and Barrett, 2020; Li et al., 2020; Wang et al., 2021a, b; Sun et al., 2021a; Sun et al., 2021b; Lin et al., 2022). Typically, clade A.2 has been shown to be the dominant clade in these ecosystems, with the exception of paddy and forest soils (Hu and He, 2017; Xia et al., 2018; Li et al., 2019; Wang et al., 2019b; Xu et al., 2020; Osburn and Barrett, 2020). Investigations into the environmental factors influencing comammox Nitrospira abundance and community composition have provided new insights into their ecological preference. While preferences are thought to be clade specific, factors such as soil pH, soil NO<sub>3</sub><sup>-</sup> concentrations, soil moisture, and average air temperature are thought to be key drivers of their distribution in terrestrial ecosystems (Xu et al., 2020; Li et al., 2021; Wang et al., 2021a). However, these associations are typically made in environments dominated by comammox clade A and have not been investigated in New Zealand soils. Therefore, the presence and distribution of comammox Nitrospira in different soils and relationships with soil and environmental conditions are not fully understood. A better understanding of the factors and conditions that determine the distribution and role of comammox is important for not only understanding its importance in the nitrogen cycle, but also for developing potential strategies to manage the nitrification process to minimise the potential adverse environmental impacts stated above (Di and Cameron, 2016).

The objectives of this study were to: (i) study the distribution, abundance, and phylogenetic diversity of comammox *Nitrospira* in different dairy pasture soils under different climatic conditions across New Zealand; and (ii) to determine the effect of different land uses on comammox *Nitrospira* abundance and diversity on the same soil type and under the same climate conditions. Based on the observations of previous studies discussed above, it was hypothesised that comammox *Nitrospira* would be widespread in New Zealand soils, with their abundance typically less than that of canonical ammonia oxidisers. The comammox *Nitrospira* community was predicted to be dominated by clade A in most of the sampling sites, with clade B preferring the pine monoculture.

#### 2. Materials and methods

#### 2.1. Soil sampling and characterization

#### 2.1.1. Soil samples from different regions

The dairy farm soil samples were taken in autumn 2021 from six different regions throughout New Zealand: Northland (North), Waikato (Wai), and Rotorua Lakes (Roto) in the North Island, Canterbury (Cant), West Coast (West), and Southland (South) in the South Island. These locations are shown in Fig. 1. The soil parent material ranges from sedimentary to volcanic and their physical and chemical properties vary widely (Table 1). All sites were located on intensively grazed, high fertility pasture-based dairy farms where dairy cows graze outdoor pastures (predominantly perennial ryegrass/white clover (Lolium perenne/Trifolium repens)). The selected regional dairy farm sites provide a wide range of environmental conditions as shown in Table 1. In summary, soil pH, annual rainfall, and annual temperature ranged from 5.4 (West) to 6.1 (Roto), 729 mm (Cant) to 4768 mm (West), and 7.7 °C (Cant) to 15.8 °C (North), respectively. Typically, New Zealand dairy farms receive approximately 200 kg N ha  $^{-1}$  yr  $^{-1}$  and 40 kg P ha  $^{-1}$  yr  $^{-1}$  to maximise pasture production (Edmeades et al., 2016). Dairy pasture soils were chosen for this study because this landuse represents some of the most intensive pastoral systems, with significant associated water quality and greenhouse emissions issues reported (Di and Cameron, 2016).

For each site, soil samples were collected from 10 random locations to a depth of 10 cm. Care was taken to avoid areas that were not representative, such as entranceways, fence lines, urine, and dung patches. The samples taken were bulked into a single sample, packed with ice packs, and transported to the laboratory for analysis. Each sample was sieved (2 mm) to remove any non-soil material and stored at -80 °C until ready for DNA extraction. A portion of the samples taken from each site was used to quantify the soil physicochemical properties (Table 1). Each of the regional dairy farm sites were analysed using four replicates.

#### 2.1.2. Soil samples from different land uses

To further understand how different land uses may affect comammox Nitrospira in the soil. Six sites with the same soil type in 'Cant' were further selected to determine the effect of land use on comammox Nitrospira abundance and community composition, with each site representing a different land use. The land uses in this study include: an irrigated sheep and beef farm, S + B Irr +; a non-irrigated sheep and beef farm S + B Irr -; a pine (Pinus radiata) monoculture, Pine; a long-term no input biological organic site, BOS; an irrigated dairy farm, Dairy Irr +; and a non-irrigated dairy farm; Dairy Irr -. The BOS and Pine sites are no input, undisturbed ecosystems. The BOS site contained several pasture species including perennial ryegrass, browntop (Agrostis capillaris), cocksfoot (Dactylis glomerata) plantain (Plantago lanceolata) with several weed species. All the Dairy and Sheep + Beef sites were covered in perennial ryegrass/ white clover with a minor weed component. The Sheep + Beef sites also contained browntop. Both of these land uses receive fertiliser inputs to maintain soil nutrient levels in accordance with the optimum ranges given in Edmeades et al. (2016). However, the dairy farm receives an additional 190 kg N ha<sup>-1</sup> yr<sup>-1</sup> to maximise pasture production.

All sites were located on a Templeton silt loam, a Typic Immature Pallic soil in the New Zealand soil classification (Hewitt, 2010). These soils are characterised as well-drained, deep, moderately structured, and loamy. All sites were within 2 km of each other, where the mean annual temperature and rainfall is 11.5  $^{\circ}$ C and 630 mm, respectively.

Five separate samples were collected per site to a depth of 10 cm, where each sample served as a replicate. Samples were collected, stored, and transported as described in Section 2.1.1. The physicochemical properties of the soils are reported in Table 2.

#### 2.2. Physicochemical analysis of soil samples

Soil pH was determined by mixing soil and water at a ratio of 1:2.5. Samples were then shaken for 1 h and left overnight. pH was measured using a Mettler-Toledo pH meter (Mettler-Toledo, Switzerland) the following morning. Olsen P was determined using the methodology explained in Olsen (1954). Soil cations (K, Ca, Mg and Na) were quantified using a soil solution ratio of 1:20 1 M ammonium acetate at pH 7 (Rayment and Higginson, 1992). Total N was analysed by combustion method using an Elementor Vario Max Cube Analyser. The methods used to analyse Organic C and CEC are explained in Blakemore (1987) and Brown (1943),



Fig. 1. Regional dairy farm locations throughout New Zealand, indicated by arrows. The red circle located in Canterbury indicates the area where the land use samples were taken.

respectively. Soil gravimetric water content was calculated by drying 10 g of soil at 105  $^\circ \rm C$  overnight.

#### 2.3. DNA extraction and qPCR

The genomic DNA was extracted from each sample using a NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions.

The extracted DNA was diluted 10-fold using deionised water in a CAS-1200 Robotic liquid handling system (Corbett Life Science, Australia). Real-time quantitative PCR (qPCR) analysis was carried out using a Rotor-Gene® 6000 real-time rotary analyzer (Corbett Life Science, BioStrategy, Auckland, New Zealand). Each qPCR reaction had a final volume of 16 µL containing 8 µL TB Green Premix Ex Taq (Tli RNaseH Plus) (TaKaRa Biotechnology co., Dalian, China), 4.5 µL Invitrogen UltraPure Distilled H<sub>2</sub>O (Life Technologies, New York USA), 1 µL of each primer

#### Table 1

Soil physicochemical properties and environmental parameters of the regional sample sites. North, Northand; Wai, Waikato; Roto, Rotorua; Cant, Canterbury; West, West Coast; South, Southland.

Sample site	Soil type	Annual temperature (°C)	Annual rainfall (mm)	pН	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Olsen P (mg kg <sup>-1</sup> )	CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	Exch Mg (cmol <sub>c</sub> kg <sup>-1</sup> )	Exch K (cmol <sub>c</sub> kg <sup>-1</sup> )	Exch Ca (cmol <sub>c</sub> kg <sup>-1</sup> )	Exch Na (cmol <sub>c</sub> kg <sup>-1</sup> )	Base saturation (%)
North	Waikare silty clay loam	15.8	1353	5.7	84.7	7.3	53	32	2.74	0.57	20.8	0.27	75.3
Wai	Horotiu silt loam	13.6	1495	5.9	40.4	3.5	17	13	0.27	0.19	7.1	0.13	60.6
Roto	Oropi silt loam	12.2	1285	6.1	36.5	3.3	48	17	0.79	0.29	12	0.11	76.8
Cant	Lismore stony silt loam	11.1	729	5.9	36.5	3.5	53	17.5	0.53	0.71	10.3	0.17	69.7
West	Harihari recent silt loam	7.7	4768	5.4	68.8	5.6	47	15	0.94	0.25	5	0.18	40.4
South	Mataura recent sandy	9.9	1194	5.6	62.6	5.7	21	19	0.9	0.24	6.7	0.27	42.5
	loam												

Table 2

Soil physicochemical properties of the Templeton silt loam sample sites. S & B Irr +, irrigated sheep and beef farm; S & B Irr -, non-irrigated sheep and beef farm; Pine, pine monoculture; BOS, a long-term no input biological organic site; Dairy Irr +, irrigated dairy farm; Dairy Irr -, non-irrigated dairy farm.

Sample site	pН	$\frac{\text{Organic C}}{(g \text{ kg}^{-1})}$	$\frac{\text{Total N}}{(\text{g kg}^{-1})}$	Olsen P (mg kg <sup>-1</sup> )	CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	Exch Mg (cmol <sub>c</sub> kg <sup>-1</sup> )	Exch K (cmol <sub>c</sub> kg <sup>-1</sup> )	Exch Ca (cmol <sub>c</sub> kg <sup>-1</sup> )	Exch Na (cmol <sub>c</sub> kg <sup>-1</sup> )	Base saturation (%)
S + B Irr +	6.14	29.1	2.6	13	17	1.54	0.7	6.7	0.27	52.8
S + B Irr -	6.15	31.8	2.7	22	18	1.7	1.08	7.6	0.4	61.5
Pine	5.45	81.3	4	17	26	4.15	0.55	8.3	1.77	56.3
BOS	6.14	29.3	2.3	8	17	7.6	0.62	43.1	0.15	55.2
Dairy Irr +	6.03	31.7	2.7	13	19	1.28	0.34	7.2	0.29	49
Dairy Irr –	6.09	31	2.6	21	17	1.3	0.77	5.9	0.27	47.7

(10  $\mu$ M) and 1.5  $\mu$ L DNA template. Standard curves were generated using 10-fold serial dilutions of plasmids containing correct inserts of the target genes. The primer sets comamoA F/comamoA R (Zhao et al., 2019), Arch–amoA F/Arch–amoA R (Francis et al., 2005), and amoA1F/amoA2R (Rotthauwe et al., 1997) were used to quantify comammox *Nitrospira*, AOA, and AOB *amoA* gene abundances, respectively (Table 3). The amplification conditions are given in Table 3. Melting curve analysis was performed at the end of each amplification cycle to confirm the reactions specificity. Real-time qPCR data analysis was carried out by using Rotor-Gene 6000 series software 1.7 (Corbett Life Science, Biostrategy, Auckland, New Zealand).

#### 2.4. High-throughput sequencing

Before sequencing, the samples underwent two rounds of PCR. The first round used adapted ComamoA F/ComamoA R — overhang primers. The second used Nextera XT Index Kit (Illumina, San Diego, CA, USA) to attach barcodes to the sequences. The resulting PCR products were purified using AMPure XP beads (Beckman Coulter, Brea, CA) and the 49 samples (all sites excluding Pine) were pooled in equimolar ratios. MiSeq Reagent kit v3 was used for library construction. The libraries were sequenced via the MiSeq platform (Illumina, San Diego, CA, USA). Sequencing was undertaken by Massey Genome Services (Palmerston North, New Zealand).

The sequencing reads were imported into QIIME2 (version 2021-11) (Bolyen et al., 2019). Low-quality sequences with a quality score <20, ambiguous nucleotides, short and chimeric sequences were discarded using the DaDa2 pipeline (Callahan et al., 2016). The produced ASVs (alternative sequence variants) were exported into Geneious Prime (version 2022.0.2) where the nucleotide sequences were in silico translated into amino acid sequences. ASV sequences along with reference protein sequences collected from the NCBI database were used to construct a Bayesian phylogenetic tree (Huelsenbeck and Ronquist, 2001). Representative ASV sequences were deposited in NCBI GenBank and can be found under accession numbers ON469828–ON469905.

#### 2.5. Statistical analysis

Prior to statistical analysis, the abundance values were  $\log_{10}$  transformed. A one-way analysis of variance (ANOVA) followed by the LSD test were used to determine if there was a statistically significant difference in abundance between each of the ammonia oxidisers at each sample location. This analysis was also used to detect significant differences in ammonia oxidiser abundance between sample locations. Abundance values are presented in normalised form.

Correlations between environmental parameters and ammonia oxidiser abundance were calculated using Pearson's correlation in R with the "Hmisc" package (Harrell and Harrell, 2019). Samples, in which a particular gene abundance was below the qPCR detection limit were excluded from analysis.

Alpha- and Beta-diversities were calculated in R with the "Vegan" package, using Shannon index (H), Pielou's Evenness, and Bray-Curtis indices, respectively (Dixon, 2003).

Table	3
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Primers and	amplification	conditions	used	in qPCR.
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I I I I		1 .		
Target gene	Primer name	Primer sequence (5'-3')	Amplification conditions	Reference
Comammox amoA	comamoA F comamoA R	AGGNGAYTGGGAYTTCTGG CGGACAWABRTGAABCCCAT	95 $^\circ$ C 2 min, 35 cycles 95 $^\circ$ C 30 s, 64–55 $^\circ$ C — 0.5 $^\circ$ C increments for 30 s, 55 $^\circ$ C for remaining 17 cycles. 72 $^\circ$ C 30 s.	Zhao et al. (2019)
AOA amoA	Arch-amoA F Arch-amoA R	STAATGGTCTGGCTTAGACG GCGGCCATCCATCTGTATGT	94 °C 2 min, 40 cycles of 94 °C 20 s, 55 °C 20 s, 72 °C 30 s, 80 °C 10 s.	Francis et al. (2005)
AOB amoA	amoA-1F amoA-2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	94 °C 2 min, 40 cycles of 94 °C 20 s, 55 °C 30 s, 72 °C 30 s, 85 °C 15 s.	Rotthauwe et al. (1997)



**Fig. 2.** The abundance of comammox (COM), AOB and AOA under the various regional dairy farms. Error bars represent the standard error of the mean. Stars above the error bars indicate whether the difference between COM and AOB or AOA is significant within the site (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001). Values above the bars represent comammox/AOA or AOB ratio. North, Northand; Wai, Waikato; Roto, Rotorua; Cant, Canterbury; West, West Coast; South, Southland.

#### 3. Results

## 3.1. The regional variation of comammox Nitrospira, AOB, and AOA amoA gene abundance under dairy pasture

The abundance of the comammox *Nitrospira amoA* gene under regional dairy farms ranged from  $3.0 \times 10^6$  copies (West) to  $3.2 \times 10^5$  copies (North)/g dry soil. Both the AOA and AOB *amoA* gene abundances were highest in Wai, with  $1.5 \times 10^6$  copies/g dry soil and  $1.2 \times 10^6$  copies/g dry soil, respectively, and lowest in Cant, with  $8.2 \times 10^4$  copies/g dry soil and  $1.4 \times 10^4$  copies/g dry soil, respectively (Fig. 2). The comammox *Nitrospira amoA* gene abundance was significantly higher (P < 0.05) than canonical ammonia oxidisers in the West and Cant dairy farm sites. Results from the West

coast dairy farm show that the comammox *Nitrospira amoA* gene is over one order of magnitude more abundant than canonical ammonia oxidisers.

The ratio of comammox *Nitrospira* to AOB and AOA ranged from 0.33 to 14.4 and 0.44 to 28.9, respectively (Fig. 2). Ratios were highest in the West, Cant, and South dairy farms.

# 3.2. The abundance of comammox Nitrospira, AOB, and AOA amoA gene under different land use

Comammox *Nitrospira* and AOA were found at all sites except for Pine. Where comammox *Nitrospira* was detected, abundance ranged from 4.7  $\times$  10<sup>4</sup> copies/g dry soil (BOS) to 2.7  $\times$  10<sup>6</sup> copies/g dry soil (Dairy Irr +) (Fig. 3). The abundance of AOB and AOA amoA gene was similarly



**Fig. 3.** The abundance of comammox (COM), AOB and AOA under the various land uses. Error bars represent the standard error of the mean. Stars above the error bars indicate whether the difference between COM and AOB or AOA is significant within the site (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001). Values above the bars represent comammox/AOA or AOB ratio. S + B Irr +, irrigated sheep and beef farm; S + B Irr -, non-irrigated sheep and beef farm; Pine, pine monoculture; BOS, long-term no input biological organic site; Dairy Irr +, irrigated dairy farm; Dairy Irr -, non-irrigated dairy farm.



Fig. 4. Polynomial relationship between comammox Nitrospira amoA gene abundance and soil gravimetric moisture content above 20 %.

distributed, with ranges of  $1.9 \times 10^5$  copies/g dry soil (BOS)– $4.0 \times 10^6$  copies/g dry soil (Dairy Irr +) and  $8.9 \times 10^5$  copies/g dry soil (BOS)– $1.7 \times 10^6$  copies/g dry soil (Dairy Irr –), respectively. Compared to AOB and AOA, comammox *Nitrospira* was the least abundant in S + B Irr + and S + B Irr – (P < 0.05).

The ratio of comammox *Nitrospira* to AOB and AOA ranged from 0.04 to 0.67 and 0.04 to 2.1, respectively (Fig. 3). Ratios were highest under sites that received irrigation, and except for the BOS site, they were lowest under the non-irrigated sites.

#### 3.3. Irrigated vs non-irrigated

The abundance of the comammox *Nitrospira amoA* gene was significantly higher (P < 0.05) in irrigated land compared to their non-irrigated counterparts. This trend was not observed in AOB or AOA *amoA* gene abundances. To investigate this further, soil moisture content and the species' *amoA* gene abundance was compared in Fig. 4 and Table 4. The comammox *Nitrospira amoA* gene was seen to have a strong positive correlation ( $R^2 = 0.88$ ) with a gravimetric soil moisture content above 20 % (Fig. 4). Comparatively, AOA and AOB showed a negative or no correlation with soil moisture, respectively (Table 4).

Overall, comammox *Nitrospira* abundance was positively correlated with annual rainfall and moisture and negatively correlated with pH and annual average temperature (Table 4). Comparatively, AOA was negatively associated with annual rainfall and soil moisture, while AOB was positively correlated with soil pH and negatively associated with annual rainfall.

#### 3.4. Diversity of comammox Nitrospira

After (quality) filtering and in silico translation, a total of 603,460 highquality comammox *Nitrospira amoA* sequences were obtained from 48 soil

#### Table 4

Pearson's correlation between environmental factors and the abundance of comammox (COM), AOA, and AOB. – indicates the correlation was not significant. Full table is given in the supplementary information (Supplementary Table 1).

	Soil pH	Annual rainfall	Annual temperature	Soil moisture
COM	-0.34*	0.53***	-0.38**	0.59***
AOB	0.42**	$-0.32^{*}$	-	-
AOA	-	-0.40**	-	-0.58***

\* P < 0.05.

\*\* *P* < 0.01.

\*\*\* P < 0.001.

samples. These were grouped into 78 unique ASVs based on sequence similarity. Alpha and beta diversity analysis revealed that there was no significant difference in diversity within (Shannon's H, P = 0.749; Pielous evenness, P = 0.925) (Supplementary Fig. 3) and between sampling sites (Supplementary Fig. 4). 25 of the 78 ASVs were selected to represent the original tree (Fig. 5).

Sequence analysis concluded that 82 % of comammox *Nitrospira amoA* sequences within all sites belonged to clade B.1. The remaining 17 % was made up of clade B.2 (Supplementary Fig. 1). Clade A sequences were not detected. Three ASVs accounted for 87.9 % of the total comammox *amoA* sequences, with ASV009 (65 %), ASV012 (9.3 %) and ASV019 (13.6 %) (Supplementary Fig. 2).

#### 4. Discussion

In this study, the distribution and community composition of comammox Nitrospira under regional dairy farms was determined using qPCR and high-throughput sequencing. It was concluded that comammox *Nitrospira* were most abundant  $(3.0 \times 10^6 \text{ copies})$  in the west coast dairy farm, where they were significantly more abundant than canonical ammonia oxidisers. This was also true in the Canterbury dairy farm (Fig. 2). The West coast site has an annual rainfall of >4000 mm, while the Canterbury site received irrigation during the warmer seasons. These results clearly point to a major soil moisture/water input/rainfall effect on the abundance comammox Nitrospira in New Zealand soils. This is supported by the highly significant correlations between the abundance of comammox Nitrospira, soil gravimetric water content, and annual rainfall (see below). These results are contrary to those presented by Xu et al. (2020) who concluded that overall, comammox Nitrospira were less abundant than canonical ammonia oxidisers in Chinese agricultural soils. These contrasting results could be caused by: i) differences in environmental conditions; and ii) differences in comammox Nitrospira community composition.

The distribution of comammox *Nitrospira* under various land uses was also explored in this study. This is the first study to investigate the abundance of comammox *Nitrospira* across several land uses. The results showed that comammox *Nitrospira* was most abundant under irrigated dairy pasture  $(2.7 \times 10^6 \text{ copies})$ , and lowest under the undisturbed ecosystems of BHU  $(4.7 \times 10^4 \text{ copies})$  and Pine (undetected). The ratio of comammox to canonical ammonia oxidisers was also highest under the irrigated dairy farm (AOB, 0.67; AOA, 2.1) (Fig. 3). The relatively high abundance of comammox *Nitrospira* in fertile irrigated dairy land suggests that comammox *Nitrospira* found in terrestrial ecosystems may be copiotrophic (Li et al., 2022).



Fig. 5. Neighbour-joining tree of representative comammox *amoA* amino acid sequences. A sub-sample (25) of the 78 ASVs shown so the tree remains concise. *Nitrosomonas eutropha* used as an outgroup. Full tree is shown in the supplementary information (Supplementary fig. 5).

The results found in this study clearly show that comammox *Nitrospira* are part of the ammonia oxidiser community in New Zealand soils. However, it is not clear whether they actively contribute to the production of nitrate. Both Li et al. (2019) and Wang et al. (2019b) utilised <sup>13</sup>CO<sub>2</sub>-DNA-stable isotope probing to determine if comammox actively contributes to nitrification in terrestrial ecosystems. Both studies concluded that comammox is an active member of the ammonia oxidiser community. However, the comammox community found was dissimilar to those found in this study. Therefore, further investigation is required to determine if comammox *Nitrospira* actively contributes to nitrification in New Zealand soils.

Interestingly, comammox Nitrospira abundance was significantly higher in irrigated land compared to its non-irrigated counterparts. Best management practice for irrigation in New Zealand agricultural systems is to maintain soil moisture at 10 mm below field capacity. Therefore, sites that received irrigation will have a higher mean soil moisture content. This created a more desirable environment for comammox, as evidenced by a positive correlation of comammox abundance with both moisture and annual rainfall (Table 4). The increase in soil water content after rain/irrigation can fill micropores and limit oxygen diffusion, thus creating anaerobic areas within the soil. Several studies have indicated that comammox Nitrospira has an adaptive advantage in a low oxygen environment (Palomo et al., 2019; Li et al., 2021; Liu et al., 2021). This is because comammox Nitrospira utilises the reductive tricarboxylic acid (rTCA) cycle to fix CO2. This cycle is O2 sensitive because it employs 2oxoglutarate:ferredoxin oxidoreductase and pyruvate:ferredoxin oxidoreductase enzymes. Their genome also contains 2/2 hemoglobin type II and cytochrome bd genes that are related to oxidative stress and high O2 affinity, respectively (Palomo et al., 2019; Li et al., 2021; Liu et al., 2021).

Comparatively, both AOB and AOA were negatively associated with annual rainfall. This, along with other contrasting correlations shown in Table 4 suggests that canonical ammonia oxidisers and comammox may prefer different terrestrial environments.

Comammox *Nitrospira* abundance was negatively correlated with annual temperature, which is consistent with previous studies (Daims et al., 2015; Liu et al., 2020; Wang et al., 2022). Zhang et al. (2020) concluded that clade B dominated the comammox community at lower temperatures (9.6  $\pm$  0.4 °C) in the Qinghai-Tibet Plateau soils, suggesting that they are better suited to low-temperature environments. The ability of comammox *Nitrospira* to thrive in a low temperature, high moisture environment could mean they play an integral role in nitrification during late autumn, winter, and early spring, when the risk of nitrate leaching and nitrous oxide emissions is high in New Zealand (Cameron et al., 2013).

Currently, it is unclear whether comammox *Nitrospira* can significantly contribute to the production of nitrous oxide. Kits et al. (2019) determined that comammox *Nitrospira inopinata* cannot directly produce N<sub>2</sub>O. Although, it can emit N<sub>2</sub>O abiotically, at similar levels to AOA. However, comammox *Nitrospira inopinata* belongs to clade A.1 which typically prefers aquatic ecosystems. Therefore, further research is required to understand comammox *Nitrospira*'s contribution to N<sub>2</sub>O production in terrestrial ecosystems, particularly ones dominated by clade B, which are not well understood.

Comammox *Nitrospira* was negatively correlated with soil pH. This is because soil pH determines the portion of free ammonia within the soil (Potter et al., 2003). At low pH, the ammonia/ammonium equilibrium favours the production of ammonium. This might benefit comammox as they have a high affinity for ammonia, particularly clade B, which possess MEP-type ammonia transporters (Palomo et al., 2018; Koch et al., 2019). The comammox genome also contains kdpABC and kdpDE gene clusters. These encode an uptake system that helps the organism to maintain homeostasis under low pH conditions (Xu et al., 2020). However, Xu et al. (2020) found that comammox *Nitrospira* was positively associated with pH (Xu et al., 2020). This is possibly because clade A.2 was the dominant comammox *Nitrospira* clade in that study, suggesting that pH may be a niche defining parameter between clade A.2 and B abundance in terrestrial ecosystems.

Interestingly, comammox *Nitrospira* were not found under the pine monoculture, despite the soil having a low pH (5.45). This could be because the pine soil had a moisture content of 12.5 % and an annual rainfall of 630 mm. Trees also have a higher transpiration rate than pasture and their canopy shelter the soil from rain (Belluscio, 2009). Therefore, it is likely that the soil beneath the pine monoculture remains relatively dry throughout the year. Because of this result and its associated environmental parameters, it is thought that moisture is a more important driver of comammox abundance than pH in New Zealand soils. Further investigation is required to confirm this hypothesis.

Sequence analysis revealed that all the comammox *Nitropsira* amoA detected within each site belonged to clade B. These results are contrary to the current opinion, which is that comammox clade B live an oligotrophic lifestyle (Wang et al., 2021b). Several studies reported that clade B abundance in agricultural soils was low or beneath the detection limit, while clade A varied in abundance (Li et al., 2019; Xu et al., 2020; Wang et al., 2021b). The difference in the observed community composition may be partly due to the difference in soil pH, with previous studies observing a positive correlation between soil pH and a comammox *Nitrospira* community dominated by clade A (Xu et al., 2020; Wang et al., 2021a). It is unclear why clade A was not detected in New Zealand soils. However, clade A.1 typically prefers aquatic ecosystems (Yuan et al., 2021; Li et al., 2022), which were not investigated in this study.

Alpha and beta diversity analysis determined there was no significant difference in community composition between or within sites. This is likely due to the similar environments investigated, with most being pasturebased agricultural systems. Studies that have found a difference in diversity have typically surveyed environments with contrasting biophysiochemical properties (Wang et al., 2021a).

#### 5. Conclusion

Comammox *Nitrospira* was present in eleven of the twelve sites investigated in this study, indicating that they are widely distributed in New Zealand soils. When the abundance of comammox *Nitrospira* under the different land uses was compared, it was determined that they were most abundant under the irrigated fertile dairy farms, suggesting that terrestrial comammox *Nitrospira* may prefer a wet copiotrophic environment. It was shown that comammox *Nitrospira* favoured an environment with high soil moisture, low soil pH, and low annual average temperature. This could mean they play an integral role in nitrification during late autumn, winter, and early spring, when the risk of nitrate leaching, and nitrous oxide emissions is high, in New Zealand. Based on the phylogenetic analysis, 17 % of the comammox community belongs to a newly distinguished subclade, clade B.2. The remaining 83 % belonged to clade B.1. No sequences from clade A were found.

#### CRediT authorship contribution statement

Chris Chisolm; Conceptualization, investigation, methodology, data curation, analysis, and writing – original draft.

Hong J Di; Leader of the research programme, funding acquisition, project supervision, writing – review & editing.

Keith Cameron; Funding acquisition, supervision, writing – review & editing and funding acquisition.

Andriy Podolyan: Supervision of experimentation, technical advice, review & editing.

Anish Sha: Supervision of experimentation, technical advice, review & editing.

Lisa Shu: Supervision of experimentation, technical advice, review & editing.

Jupei Shen: International collaborator, technical advice, review & editing.

#### Data availability

The data may be made available on request

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2022.159961.

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