



Comammox bacteria and ammonia oxidizing archaea are major drivers of nitrification in glacier forelands

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ABSTRACT

This study investigated the abundance of comammox bacteria and canonical ammonia-oxidizing bacteria (AOB) and archaea (AOA), and their relative contribution to nitrification along a chronosequence of deglaciated forelands. The results showed that nitrification related gene abundance tended to increase with glacier retreat, with comammox bacteria and AOA appearing to be the most critical drivers for soil nitrification rates. These findings provide new evidence for the presence of comammox bacteria in glacier forelands and enhance our understanding of the niche differentiation of canonical nitrifier and comammox bacteria.

Glaciers cover 10 % of the Earth's land surface and are highly vulnerable to climate change (Milner et al., 2017). Retreating glaciers due to global warming offer an ideal habitat for studying primary succession, as they present a chronosequence of soil development from the initial accumulation of bare substrates to the formation of complex plant communities (Cazzolla Gatti et al., 2018). Considering that nitrogen (N) is a limiting factor for plant and microbial growth during primary succession, microbial colonization involved in N transformation is essential for primary production and ecological functioning of glacial ecosystems (Ollivier et al., 2011).

Nitrification is a key process in the global N cycle and is crucial for N availability. Nitrification is traditionally believed to be driven by ammonia oxidizers (ammonia-oxidizing bacteria, AOB and ammonia-oxidizing archaea, AOA) converting ammonia to nitrite in the rate-limiting step of ammonia oxidation, followed by nitrite-oxidizing bacteria (NOB) converting nitrite to nitrate (He et al., 2007; Prosser and Nicol, 2012). However, the discovery of comammox *Nitrospira*, capable of oxidizing ammonia to nitrate within a single cell (Daims et al., 2015;

van Kessel et al., 2015), challenges this long-standing paradigm of nitrification. This breakthrough offers a new opportunity to examine the relative contributions of comammox and canonical ammonia oxidizers to nitrification in different terrestrial ecosystems (Hu and He, 2017), and gain insights into their niche differentiations in terrestrial ecosystems.

Analyses of glacier ecosystems resulted in conflicting findings regarding the dominant ammonia-oxidizing microorganisms at glacier fronts. Some studies have indicated the prevalence of AOA due to their adaptability to ammonia deficiency (Nicol et al., 2005; de Vries et al., 2021), while Zeng et al. (2016) suggested that AOB are dominant because of their strong association with ammonium concentration. Additionally, the finding of comammox bacteria suggested their preference in oligotrophic environments due to their high affinity for ammonia, and they competitively outcompete AOB and AOA in such habitats (Kits et al., 2017). This suggests that comammox bacteria may have greater ecological significance in N-limited ecological zones. Glacier forelands have been widely used for exploration of the succession of nutrient and microbial diversity, but empirical evidence for the

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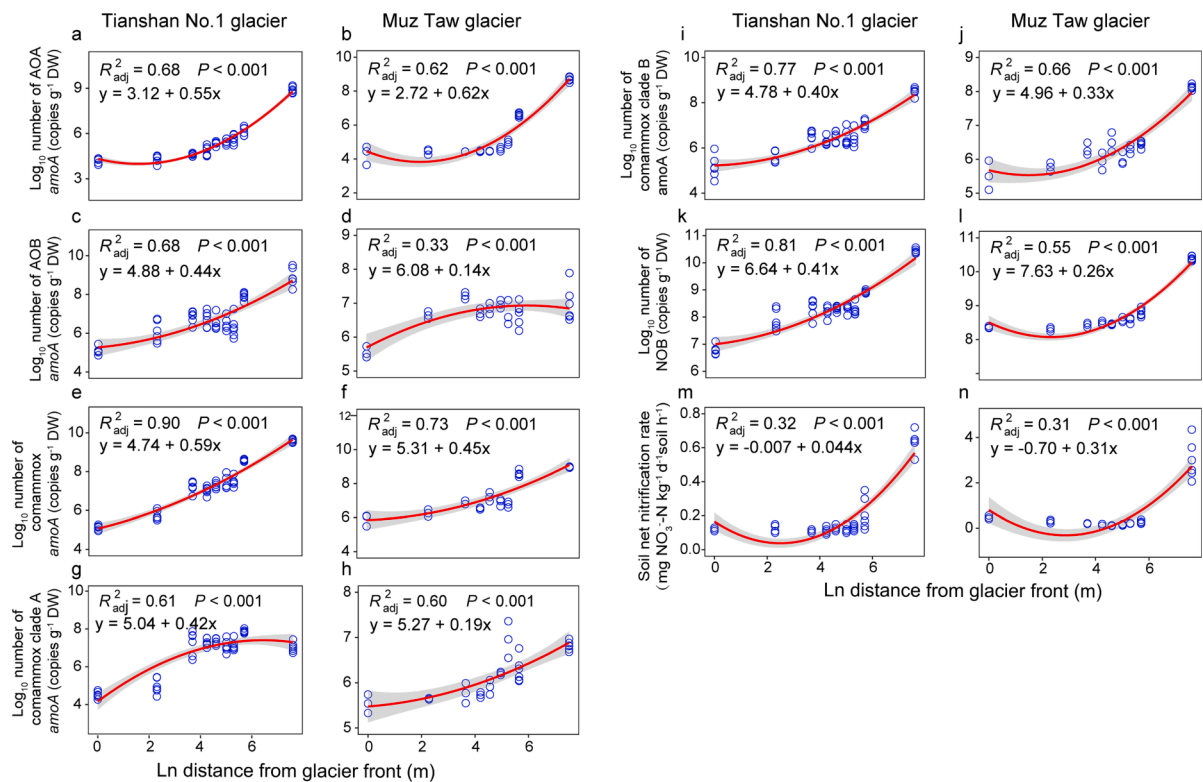


Fig. 1. The variation of nitrifier abundance and soil nitrification rates with increasing distance from the glacier terminus representing successional ages of 0–361 years in Tianshan Mountain No.1 glacier and 0–275 years in Muz Taw glacier. a/b: AOA, ammonia-oxidizing archaea; c/d: AOB, ammonia-oxidizing bacteria; e/f: comammox *Nitrospira*; g/h: comammox *Nitrospira* clade A; i/j: comammox *Nitrospira* clade B; k/l: NOB, nitrite-oxidizing bacteria; m/n: soil nitrification rates.

niche of comammox bacteria remains scarce, particularly in barren glacial ecosystems. Therefore, it is crucial to investigate the ecological niche differentiation between comammox bacteria and canonical nitrifiers to enhance our understanding of global N cycling dynamics, especially with regard to predicting the potential expansion of deglaciated areas resulting from accelerated global warming.

To quantify the variation in gene abundance of nitrifiers and their potential role in nitrification across a chronosequence of deglaciated forelands, we selected two glacial forelands in the Xinjiang region of China (Fig. S1), including the well-documented east branch of Tianshan Mountain No.1 glacier (43°06'18"N, 86°48'26"E) (Li et al., 2011) and the less disturbed Muz Taw glacier (47°03'44"N, 85°33'40"E) (Huai et al., 2015), situated on China-Kazakhstan border. The retreat of these two glaciers has created new terrestrial habitats characterized as primary succession, encompassing three common stages: bare soil stage, pioneer plant stage, and alpine meadow stage. We employed a 'time-space' substitution approach to collect soil samples from July 28 to August 4, 2021. Nine sampling transects were established in parallel along the direction of glacier retreat at intervals of 0, 10, 40, 70, 100, 150, 200, 300 and 2000 m from the glacier terminus, representing different successional ages up to 361 years. A total of 87 soil samples were collected from the top 0–15 cm layer.

Soil parameters and the abundance of nitrification related genes were determined to identify key factors affecting soil nitrification. The net nitrification rate was calculated as the accumulation rate of the net nitrate nitrogen production before and after cultivation using a modified incubation method (Neill et al., 1997). All soil physicochemical properties are shown in Figure S2. Soil DNA was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals, CA, USA) and quantitative PCR was performed on the CFX96 Optical Real-Time Detection System (Bio-Rad Laboratories Inc., Hercules, CA, USA). Primer sets Arch-amoA/Arch-amoAR, amoA-1F/amoA-2R, Nitro-1198F/Nitro-1423R, comamoA/comamoAR, CA377f/C576r, and CB377f/C576r were used for AOA-

amoA, AOB-*amoA*, NOB, comammox-*amoA* clade A and clade B, respectively (Table S1). It is worth noting that although primers Nitro-1198F/Nitro-1423R are commonly described as being used for NOB, they are rather specific for *Nitrobacter*. Statistical analyses were conducted in R (v4.1.2) using "ggplot2", "corrplot", "tidyverse" and "randomForest" packages to examine the log-transformed gene abundances of various nitrifier groups. Detailed methods could be found in the Supplementary Materials.

We observed the presence of all targeted nitrifier groups throughout the glacial degradation process, with high abundance of the comammox *Nitrospira* in both glacier forelands. The absolute abundance of comammox *Nitrospira* ranged from 7.44×10^4 to 4.19×10^9 copies g⁻¹ dry soil, with comammox *Nitrospira* clade A and B at 2.56×10^4 to 1.32×10^9 and 3.18×10^4 to 4.82×10^8 copies g⁻¹ dry soil, respectively. In contrast, the abundance of typical nitrifiers, including AOA, AOB and NOB, ranged from 3.69×10^4 to 2.88×10^9 , 2.81×10^4 to 1.78×10^9 and 2.28×10^6 to 8.18×10^{10} copies g⁻¹ dry soil, respectively (Fig. 1). To the best of our knowledge, this is the first documented evidence of widespread presence of comammox *Nitrospira* during glacier degradation, despite its well-established occurrence in various terrestrial ecosystems such as forests, grasslands, and farmlands (Li et al., 2023). The glacier forelands characterized with low ammonia availability and a wide range of pH (ranging from 4.89 to 8.61) were dominated by comammox *Nitrospira*, suggesting their significant nitrification potential in low-N and nutrient-poor environments (Wang et al., 2019; Hu et al., 2021).

Furthermore, we observed a gradual increase in the absolute abundance of all detected groups, including AOA, AOB, comammox and NOB, with increasing distance from the glacier terminus (Fig. 1a-l; $P < 0.001$). This successional pattern aligns with recent reports suggesting a continuum in the life history strategy of soil bacteria in response to labile nutrients (Stone et al., 2023). In contrast, AOA and AOB exhibited a bimodal pattern in the McMurdo Dry Valleys, indicating that specific

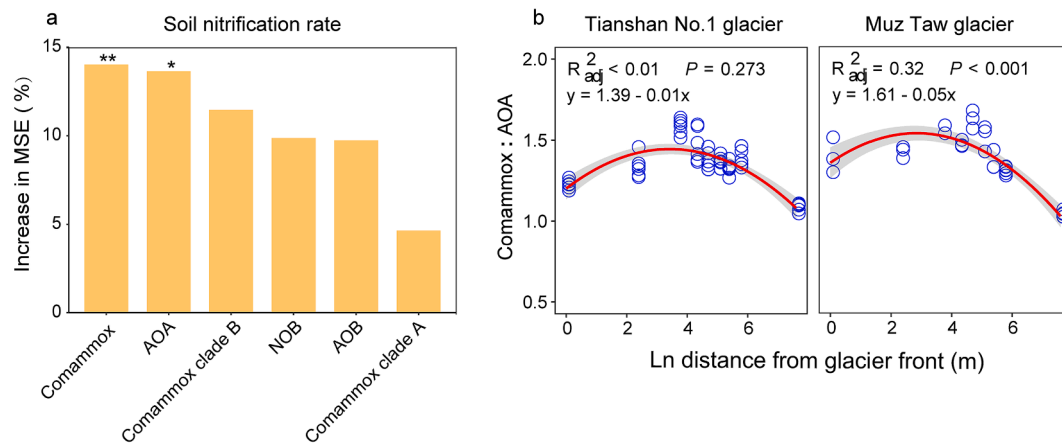


Fig. 2. Random forest predictor importance, as measured by percent increase in mean square error (MSE) of soil nitrification rates (a) and the variation of comammox *Nitrospira*:AOA abundance (b) with increasing distance from the glacier terminus representing successional ages of 0–361 years in Tianshan Mountain No.1 glacier and 0–275 years in Muz Taw glacier (* $P < 0.05$; ** $P < 0.01$). AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; NOB, nitrite-oxidizing bacteria.

geochemical variables may influence different microbial groups (Magalhães et al., 2014). Additionally, the colonization of vegetation during the later stages of glacier forelands would be a key driving factor (Liu et al., 2023), which was further confirmed by the significant effect of vegetation on all nitrifiers using three-factor analysis (Table S2). Our study also revealed that soil nitrification rates exhibited a gradual increase during the bare soil stage, but accelerated more rapidly upon plant colonization (Fig. 1m–n, $P < 0.001$). This pattern parallels the succession of plants, which was mainly attributed to the inactive nitrifying groups in early stages (Brankatschk, et al., 2011).

Comammox *Nitrospira* was identified as the best predictor for soil nitrification rates during glacial retreat based on random forest analysis, with AOA also demonstrating significant contribution (Fig. 2a). Spearman correlation analysis further confirmed a positive correlation between comammox *Nitrospira*, AOA and soil nitrification rates (Fig. S3). The co-occurrence of comammox *Nitrospira* and AOA can be attributed to their shared preference for an oligotrophic lifestyle (Martens-Habbena et al., 2009; Kits et al., 2017). Strikingly, a higher ratio of comammox *Nitrospira*:AOA was observed at the bare soil stage (approximately 1.17–1.66) compared to other stages (approximately 1.01–1.45) (Fig. 2b). This finding may provide additional evidence that the more energy efficient comammox *Nitrospira* contribute significantly to nitrification in nutrient-deficient areas before vegetation established. In the future, it is necessary to differentiate the relative contribution of ammonia oxidizer groups to nitrification rates using nitrification inhibitors combined with DNA stable isotope labeling methods (Zhu et al., 2022), providing more solid evidence in linking microbial abundance changes and function. Nevertheless, the strong relationship between comammox *Nitrospira*, AOA and nitrification rates indicates the potential key role of comammox *Nitrospira* and AOA in the nitrification process during the primary succession of glaciers, thereby enhancing our understanding of microbial successional dynamics in glacier forelands. These results provide novel insights into the competition and niche specialization within the ammonia oxidizing guilds.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2023.116711>.

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