

ANALYSIS OF ARCHAEOAL DIVERSITY AND ITS INFLUENCING FACTORS IN THE SOIL OF NY-ÅLESUND MELTWATER AREAS IN ARCTIC NORWAY

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Abstract. Ecosystem and material circulation in the Arctic region are significantly affected by global warming. In this study, the meltwater areas near different geomorphological features in the Ny-Ålesund region of Norway were taken as the research object. The purpose of this study was to analyze the diversity of archaeal community in meltwater soil and the soil physicochemical factors affecting the diversity of archaeal community. The 16sRNA of Archaea in soil samples were sequenced by high-throughput sequencing technology. At the phylum level, Halobacterota was the highest in relative abundance. At the genus level, the relative abundance of *Rice_Cluster_II* was the highest. The soil physicochemical factors that significantly affected the archaeal community were studied by redundancy analysis (RDA) and weighted gene co-expression network analysis (WGCNA). According to the results of RDA and WGCNA, NH₄⁺-N, SiO₃²⁻-Si and MC were important physicochemical factors. *WoeseArchaeales* was identified as a hub genus by WGCNA. The combination of the two methods can accurately and comprehensively analyze the correlation between archaeal community and physicochemical properties.

Keywords: Arctic Ny-Ålesund, soil archaeal community, soil physicochemical properties, high-throughput sequencing, weighted gene co-expression network analysis

Introduction

Climate change had a profound impact on the global landscape, especially in the Arctic (Kerr, 2009; Lutz et al., 2016). In the polar regions, rising temperatures had led to many environmental problems, such as the expansion and change of ice sheets (Jenkins et al., 2018; Kingslake et al., 2018), glacier collapse, and the increasing of meltwater (Bell et al., 2018; Edwards et al., 2019). One of the most significant changes caused by global warming was the increase in the area of meltwater (Rühland et al., 2013). The abundance of the meltwater lake is gradually increasing, and the microbial community structure in the meltwater lakes is responding rapidly (Michelutti et al., 2020).

As decomposers, microorganisms participate in biogeochemical cycles in ecosystems. At present, the diversity of bacterial communities in the Arctic has been extensively studied. However, Archaea can survive in extreme environments, and changes in the diversity of archaeal communities in time and space also have important feedback effects on the extreme ecosystems (Naylor et al., 2020). Archaea play an important role in carbon and nitrogen cycling in Arctic soil ecosystems (Lv et al., 2019; Qin et al., 2023). The metabolic activities of Archaea affect the physicochemical properties of environmental factors. The metabolic activity of ammonia-oxidizing archaea will leach nitrate in the soil, resulting in the decrease of nitrogen content in the soil (Beeckman et al., 2018). Methanogenic Archaea produce methane under hypoxic conditions, increasing the amount of organic carbon in the environment (Wen et al., 2017).

In order to better study the diversity of archaeal community structure and the effects of soil physicochemical factors on their distribution in Arctic meltwater areas, we collected soils from meltwater areas with different characteristics and used weighted gene co-expression network analysis (WGCNA) to analysis. WGCNA combined with redundancy analysis (RDA) can more accurately analyze the correlation between archaeal community and environmental physicochemical factors. WGCNA is a R package (<https://cran.r-project.org>), it was originally used to search for key genes for diseases and traits in medicine (Miller et al., 2008; Fuller et al., 2007; Li et al., 2020a). Because of the similarity between Amplicon Sequence Variant (ASVs) and genes, some researchers applied it in microbial groups to find the hub ASVs (Qin et al., 2023). Hub ASVs refers to a series of ASVs with high connectivity and high co-expression with other ASVs. By calculating the adjacency and connectivity, the correlations of hub microbes with physicochemical factors can be intuitively found.

In order to understand the archaeal community diversity in soil samples from the meltwater areas of the Arctic region and its relationship with soil physicochemical properties, RDA and WGCNA were combined for analysis. Soil samples were taken from six different types of meltwater areas in the Ny-Ålesund region of the Arctic (*Figs. 1* and *2*). Intertidal soils that were sometimes exposed to the water, sometimes submerged under the water, and subtidal soils that remained submerged were collected and analyzed. WGCNA revealed the correlation between soil physicochemical properties and archaeal community diversity, and the hub Archaea related to soil physicochemical properties were found. These data provide the basis for a better understanding of the response of archaeal communities to environmental changes in Arctic meltwater areas.

Materials and methods

Sampling site and sample collection

Samples were collected in the Ny-Ålesund region which is located in Norway's Svalbard archipelago within the Arctic Circle (*Figs. 1* and *2*). The Ny-Ålesund region is dominated by glaciers and slopes (Berthling et al., 2020). Due to the circulation of the atmosphere, the region is affected by the flow of warm air from the low latitudes of the Atlantic Ocean, giving the Ny-Ålesund region a warm and humid climate (Dahlke and Maturilli, 2017). In July 2018, a total of 36 samples were collected (*Table 1*). They were collected in subtidal and intertidal zones of meltwater area near glaciers (BZS, BZX), sand (SZS, SZX), tundra (TZS, TZX), bays (WZS, WZX), London Lake (LZS, LZX)

and reservoir bay (KZS, KZX). 50 grams of soil were dug out at a depth of about 5 cm into a sterile bag using a sterile spoon. The collected samples were temporarily stored at -20°C and then transferred to -80°C for long-term storage in ultra-low temperature refrigerators.



Figure 1. Map of sampling sites



Figure 2. Geomorphological picture of the sampling sites. The meltwater area near the different landforms contains two sampling sites, with the soil samples located in the intertidal zone marked as 'S' and the soil samples located in the subtidal zone marked as 'X'

Analysis of soil physicochemical property

A total of nine soil physicochemical properties were determined. Soil pH was measured by weighing 2 g of soil and adding 5 mL deionized water, shaking for 1 min, and then using a pH meter (PHS-3C, Rex Instrument Factory, Shanghai, China) after

30 min. The water content (MC) of soil was obtained by measuring the weight difference between the soil before and after drying. The specific operation was to dry the weighed soil sample at 105°C to constant weight, weigh the obtained sample, and finally calculate the weight difference. Total organic nitrogen (TON) and total organic carbon (TOC) in soil were measured in the same way. First, the collected sample was ground into a powder, then decalcified with 10% HCl, rinsed with deionized water to wash away the remaining HCl and dried overnight at 60°C, finally analyzed with an elemental analyzer (EA3000, Euro Vector, Milan, Italy) (Hu et al., 2012). The content of NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, PO₄³⁻-P and SiO₃²⁻-Si were measured by a nutritional automatic analyzer (QuAatro, SEAL Analytical, Darmstadt, Germany) with a relative standard deviation of less than 5%. The soil samples were processed as follows. Soil samples were added with deionized water at a ratio of 1:10 (g mL⁻¹) after lyophilization and grinding to powder (Liu et al., 2016).

Table 1. Sample collection sites

Sample code	Location profile	Coordination
BZS BZX	Intertidal soil in the meltwater area near the Glacier Subtidal soil in the meltwater area near the Glacier	78.9036°N 12.0447°E
SZS SZX	Intertidal soil in the meltwater area near the Sand Subtidal soil in the meltwater area near the Sand	78.9579°N 11.7050°E
TZS TZX	Intertidal soil in the meltwater area near the Tundra Subtidal soil in the meltwater area near the Tundra	78.9652°N 11.6013°E
WZS WZX	Intertidal soil in the meltwater area near the Bay Subtidal soil in the meltwater area near the Bay	78.9664°N 11.5792°E
LZS LZX	Intertidal soil in the meltwater area near the Lake London Subtidal soil in the meltwater area near the Lake London	78.9626°N 12.0592°E
KZS KZX	Intertidal soil in the meltwater area near the Reservoir bay Subtidal soil in the meltwater area near the Reservoir bay	78.9194°N 11.8766°E

DNA extraction, PCR amplification, and sequencing

According to Mobio PowerSoil DNA Isolation Kit description (Mobio, laboratories, Inc., Carlsbad, CA, USA and QIAGEN, Inc., Germantown, MD, USA) (Magoc and Salzberg, 2011), total DNA (10-30 ng uL⁻¹) was extracted from soil samples. Then the extracted DNA were diluted to 1 µgµL⁻¹ with sterile water depending on the concentration. Specific primers Arch519F (5'-CAGCCGCCGCGGTAA-3') and Arch915R (5'-GTGCTCCCCGCCAATTCCT-3') were used to amplify the V4 and V5 highly variable regions of the archaeal 16S rRNA gene. 15 µL of Phusion® Hi-Fi PCR Master Mix (New England Biolabs), 10 ng of template DNA, 0.2 µM of forward and reverse primers were used for PCR reactions. The thermal cycle were sequentially composed of initial denaturation (98°C, 1 min), denaturation (98°C, 10 s), annealing (50°C, 30 s), extension (72°C, 30 s) and heating (72°C, 5 min) (Wang et al., 2022). The Qiagen gel extraction kit (Qiagen, Germany) was used to purify PCR bands. Sequenced libraries were generated by using the TruSeq®DNA PCR-Free Sample Preparation Kit (Illumina San Diego, CA) and indexed codes were added as recommended by the manufacturer. Finally, sequencing was performed on the Illumina NovaSeq platform (Ma et al., 2022).

Processing and analysis of sequencing data

For the Effective Reads obtained above, DADA2 method (Callahan et al., 2016) in QIIME2 system was used for noise reduction to filter out abundance less than 5 sequences (Li et al., 2020b). Thus, the final Amplicon Sequence Variant (ASVs) and feature list were obtained (Callahan et al., 2017). The resulting ASVs were then compared with the Silva138.1 database using the classify-sklearn algorithm (Bokulich et al., 2018; Bolyen et al., 2019) in QIIME2 system to obtain species information for each ASV.

QIIME2 system was used to calculate the alpha and beta diversity of Archaea in soil samples. Alpha analysis reveals the richness of soil samples, and Beta diversity reveals the species diversity differences among different soil samples (Dalkıran and Zünbülğil-Ünsal, 2023). QIIME2 system was also used for LEfSe analysis to reveal species with significant differences in abundance between groups (Segata et al., 2011). R software (version 3.2.4) was used for redundancy analysis (RDA) and Monte Carlo test to explain the correlation between the distribution of Archaea community and the physical and chemical properties of soil. RDA is analyzed by using vegan packages (Oksanen, 2022). Low abundance ASVs were removed. By using weighted gene co-expression network analysis package, the physicochemical properties and Archaea module heat map were obtained. The hub ASVs network was mapped by Cytoscape (version 3.10.0).

Results

Physicochemical properties of soils

Moisture content (MC), pH, total organic carbon (TOC), total organic nitrogen (TON), and five soluble nutrients (ammonium ion, silicate ion, nitrate, nitrite, and phosphate) of soil samples in 12 sites were measured. In *Figure 3*, it could be seen that the MC of intertidal samples was obviously lower than that of subtidal samples. The oxygen in the intertidal zone is relatively sufficient, which is conducive to the metabolism of ammonia-oxidizing archaea (Kim et al., 2021). Samples from meltwater areas near sand and tundra (SZS, SZX, TZS, TZX) contained significantly more total organic carbon and total organic nitrogen than samples from other landforms. The higher content of total organic carbon and organic nitrogen in the soil samples of meltwater areas near these two landforms may be caused by vegetation input and low organic matter decomposition rate (Schaphoff and Lucht, 2013). The vegetation coverage of glacier, London Lake, bay and reservoir bay is low, so the input of organic carbon is limited. Organic carbon in lakes is easily decomposed by microorganisms or migrated with water, and the deposition rate is low. Therefore, these regions have low content of total organic nitrogen and total organic carbon. The content of ammonium and silicates were the highest in the soil of meltwater areas near sand (BZS, BZX). The content of phosphate was higher in glacier, London Lake, reservoir bay and tundra (TZS, WZS, LZS, KZX). This may be caused by the aquatic plants. Studies have shown that phosphorus in soil is the main limiting factor for the biomass of aquatic plants (Andresen and Lougheed, 2021).

Diversity and composition of archaeal community

High-throughput sequencing was performed on 36 samples from six meltwater areas in the Ny-Ålesund region of the Arctic, and noise reduction was performed on the

effective sequences. A total of 3342 ASVs were obtained. The Good's coverage index of all 36 samples was above 99.9%, indicating the sequencing had sufficient depth. According to alpha diversity analysis, the richness of soil archaeal community in the intertidal zone was higher than that in the subtidal zone. High content of organic matter and stable water conditions provide suitable conditions for the formation and persistence of microaggregates. Soil samples from meltwater areas near the tundra contain abundant archaeal communities. It may be due to the abundance of carbon, nitrogen and phosphorus in the soil of the tundra area.

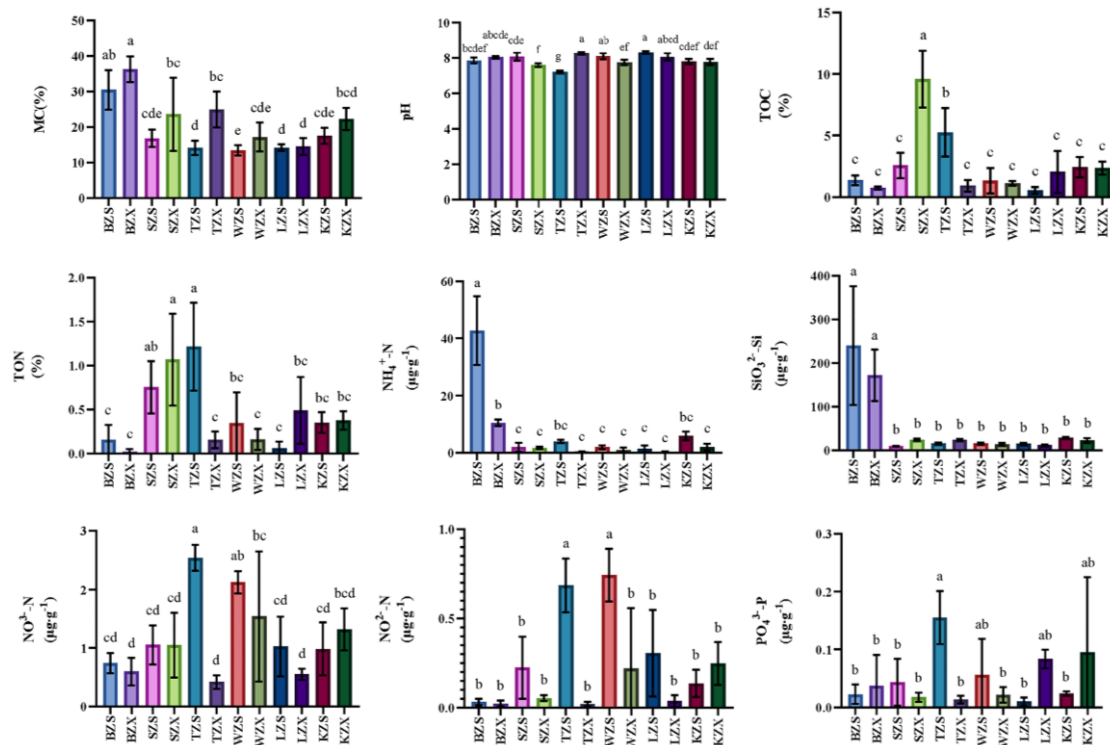


Figure 3. Physicochemical properties of soil samples from the meltwater region of Ny-Ålesund. Significant differences were accepted when $p < 0.05$ between the two groups. The letters a, b, c, d, e and f were used to show statistically significant differences. The error bars represent mean \pm SD, $n = 3$

In Figure 4, the ASVs of all samples were clustered to 8 phyla at the phylum level. Halobacterota, Crenarchaeota, Nanoarchaeota, Euryarchaeota and Aenigmarchaeota were phyla with high relative abundance. The proportion of Halobacterota in meltwater areas near sand was the highest in all meltwater areas. The proportion of Crenarchaeota in soil samples from meltwater areas near glacier (BZX, BZS) was lower than that in other sites. Nanoarchaeota had the highest relative abundance in subtidal soil of meltwater areas near reservoir bay (KZX), whereas it had the lowest relative abundance in intertidal soil of meltwater areas near tundra (TZS). All ASVs of samples were clustered into 36 genera. In Figure 5, the top 10 genera with high relative abundance include *Rice_Cluster_II*, *WoeseArchaeales*, *Candidatus_Nitrocosmicus*, *Methanobacterium*, *Candidatus_Nitrososphaera*, *Halobacterales*, *Methanoregula*, *Methanosaeta*, etc. In subtidal soil of meltwater areas near sand (SZX), the proportion of *Rice_Cluster_II* was much higher than that of other sites. *Candidatus_Nitrocosmicus*

had the highest relative abundance in the meltwater area near the reservoir bay. *Methanobacterium* has the highest relative abundance in subtidal soil of meltwater areas near bay (WZX).

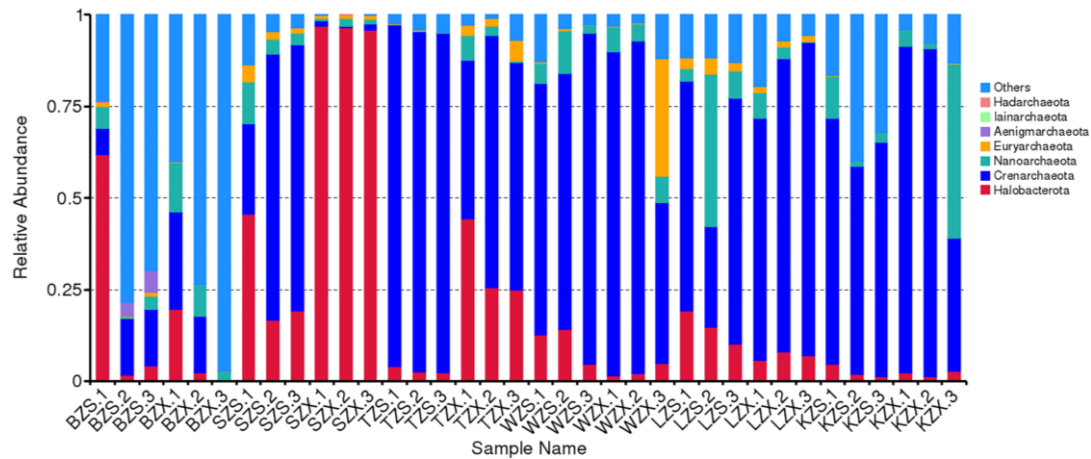


Figure 4. Bar chart of relative species abundance of Archaea at phylum level

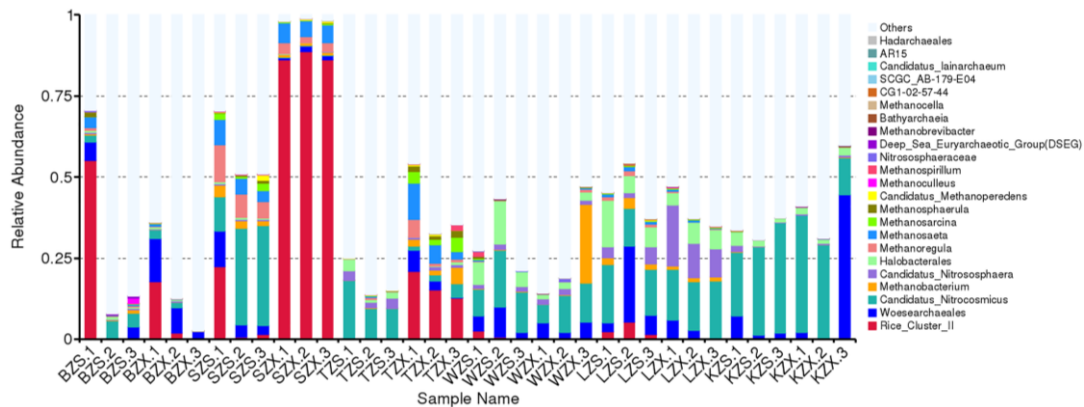


Figure 5. Bar chart of relative species abundance of Archaea at genus level (b)

Correlation between soil physicochemical properties and archaeal community structure

In this study, redundancy analysis (RDA) and Monte Carlo test (Table 2) were used to investigate the relationship between archaeal diversity and nine soil physicochemical factors. According to the results, MC ($r^2 = 0.4111$, $Pr = 0.0005$), SiO_3^{2-} -Si ($r^2 = 0.6028$, $Pr = 0.0010$), NH_4^+ -N ($r^2 = 0.8566$, $Pr = 0.0005$) and TOC ($r^2 = 0.4436$, $Pr = 0.0045$) had great influence on the overall diversity and distribution of archaeal community. BZS is the most affected by four physicochemical factors among all sampling sites (Fig. 6).

In addition to environmental physicochemical properties, we also examined Archaea that differ greatly in abundance. According to LefSe results, there were significant differences in 9 of the 12 sites (Fig. 7). In terms of genus, *Rice_Cluster_II* in SZX and *Candidatus_Nitrosomicus* in KZS had the highest LDA scores (>5.0). WGCNA was performed for all ASVs and physicochemical properties at the sampling sites, and the

correlation heat maps of each module and physicochemical properties were obtained (Fig. 8). The module MEturquoise showed strong positive correlation with NH_4^+ , SiO_3^{2-} and MC. The results of the module MEturquoise were imported to Cytoscape to obtain the network diagram. In Figure 9a, a total of 10 hub Archaea were identified, all of which were unclassified Archaea. Among them, ASV761 was the most pivotal and had strong correlation with other Archaea in the figure. The module MEblue showed strong positive correlation with PO_4^{3-} . The results of the module MEblue was imported to Cytoscape to obtain the network diagram. In Figure 9b, a total of 6 hub Archaea were identified. Among the hub Archaea obtained, four belong to the genus *WoeseArchaeales* and the rest are unclassified.

Table 2. A Monte Carlo permutation test of relationship between physicochemical properties and composition of archaeal communities

	RDA1	RDA2	r ²	Pr	
NH4 +- -N	0.4048	0.9144	0.8566	0.0005	**
SiO32--Si	0.4708	0.8823	0.6028	0.0010	**
NO3--N	-0.6738	-0.7389	0.1348	0.0855	.
PO43--P	-0.9989	0.0476	0.1531	0.0915	.
NO2--N	-0.9073	-0.4205	0.1813	0.0440	.
TON	0.5200	-0.8542	0.1460	0.0835	.
TOC	0.8285	-0.5600	0.4436	0.0045	**
MC	0.6633	0.7483	0.4111	0.0005	**
pH	-0.8003	-0.5995	0.0015	0.9690	.

**Correlation is significant at the 0.01 level

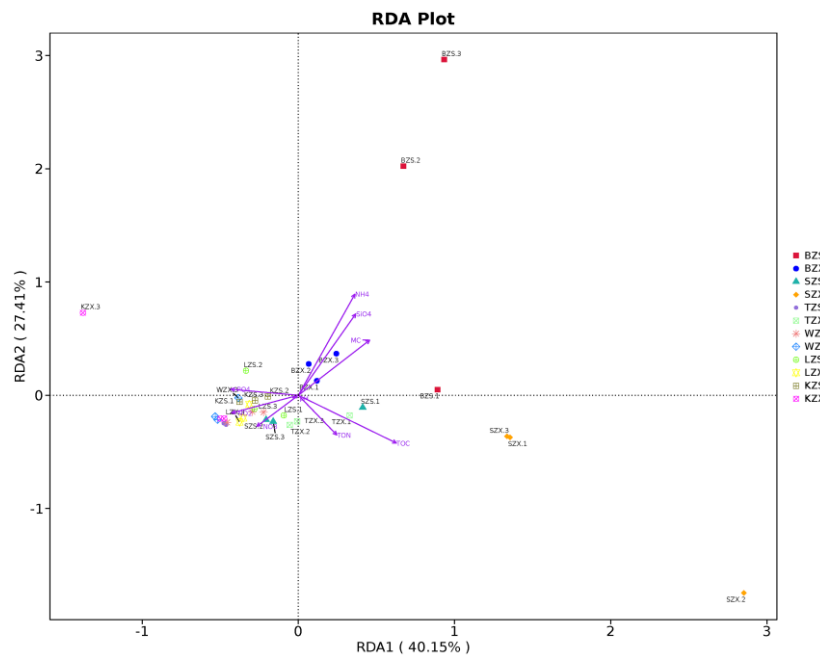


Figure 6. Redundancy analysis showed that the correlation between archaeal communities and physicochemical properties of 36 soil samples. The length of the arrow line represents the degree of correlation between soil physicochemical factors and community distribution, and the longer the line, the higher the correlation

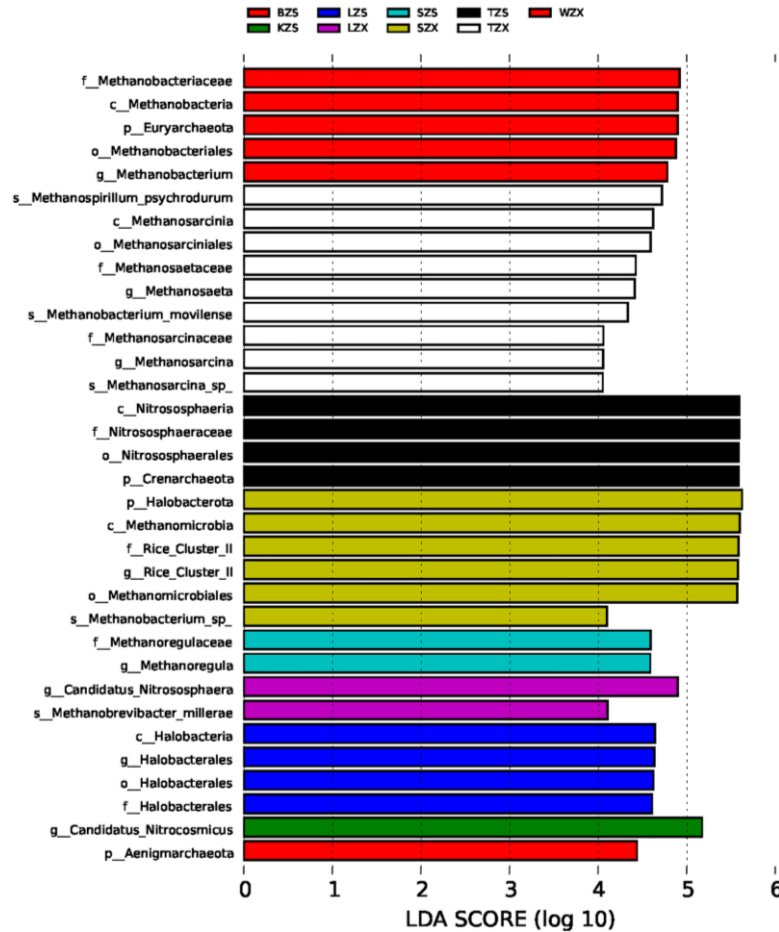


Figure 7. The taxa showing different relative abundance among the sites using the LDA score. The color of the bar chart represents the group, and the length represents the LDA score, which is the degree of influence of significantly different species

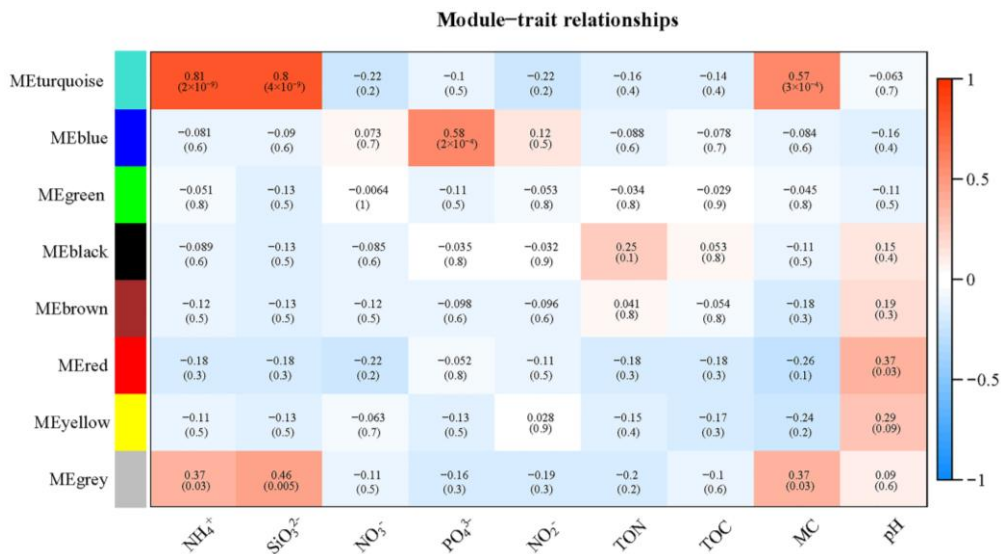


Figure 8. Archaeal modules associated with nine physicochemical properties. The darker the color, the stronger the correlation between the two. Red represents positive correlations, blue represents negative correlations

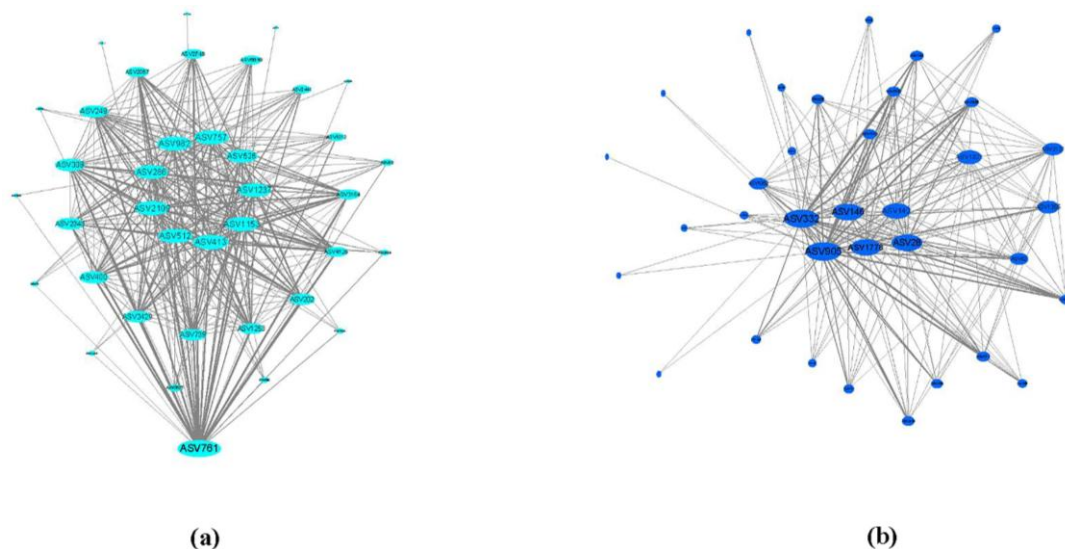


Figure 9. The network diagram of the module *MEturquoise* (a) and the module *MEblue* (b). The nodes represent the ASVs, and the lines show the connections between them. The larger the node and the thicker the line, the more correlated it is with other nodes

Discussion

This paper analyzes the relationship between the physicochemical properties of soil samples from six meltwater areas in Ny-Ålesund region and the diversity of archaeal communities. The environment influences the community structure of Archaea. There were differences in archaeal community composition in different soil samples, and the archaeal communities were most abundant in soil samples from meltwater areas near glacier. According to *Figure 3*, the concentration of $\text{NH}_4^+\text{-N}$ and $\text{SiO}_3^{2-}\text{-Si}$ in the meltwater near glacier were significantly higher than other sites. We speculate that the high concentration of silicate and ammonium ions in the soil of meltwater area near glacier is one of the reasons for the rich species of archaea community (Zhou et al., 2023). At the phylum level, the dominant group in all soil samples are Halobacterota and Crenarchaeota. Studies have shown that Halobacterium can tolerate high salt concentrations and can exist in extreme environments (Shu and Huang, 2021). At the genus level, *WoeseArchaeales* and *Methanobacterium* have a high relative abundance. *Methanobacterium* usually live in oxygen-deprived environments, such as aquatic sediments, flooded soils, peatlands and coastal wetlands (Conrad, 2020; Bridgham et al., 2013). *Candidatus_Nitrososphaera* (Fan et al., 2019) and *Candidatus_Nitrocosmicus* (Wu et al., 2021) were the dominant genera in soil samples and the relative abundance of them in intertidal samples was higher than that in subtidal samples. They belonged to the ammonia-oxidizing archaea (AOA), which were major players in the nitrogen cycle in ecosystems and have high carbon sequestration capacity (Kim et al., 2021). ammonia-oxidizing archaea can oxidize NH_4^+ to NO_2^- and immobilize carbon dioxide (Roland Hatzenpichler, 2008; Zhang et al., 2010) and oxygen is required for this process to proceed (Pester et al., 2011). In addition, NO_2^- could be reduced to N_2O , which was a kind of greenhouse gas (Flores-Santana et al., 2009).

Local soil factors, rather than global factors, are considered to be the main direct shaping force of the Arctic microbial community (Shi et al., 2015). Common soil

physicochemical factors include pH, water content, carbon, nitrogen, phosphorus and trace elements (Poosakkannu et al., 2017; Wang and Jia, 2016). According to the results of RDA, MC ($r^2 = 0.4111$, $Pr = 0.0005$), $\text{SiO}_3^{2-}\text{-Si}$ ($r^2 = 0.6028$, $Pr = 0.0010$), $\text{NH}_4^+\text{-N}$ ($r^2 = 0.8566$, $Pr = 0.0005$) and TOC ($r^2 = 0.4436$, $Pr = 0.0045$) were significantly correlated with the archaeal community in meltwater. From the RDA results, it can be analyzed that soil moisture and organic carbon have become significant influencing factors on microbial community structure. In the Arctic, the changes in soil organic carbon and moisture content are mainly caused by glacier melting. Studies have shown that as the climate rises, the melting of glaciers leads to the exposure of glacier-covered vegetation and animal remains, and the content of organic carbon and organic nitrogen in the soil will increase significantly (Groff et al., 2023). High organic matter input and stable water conditions may provide suitable conditions for the formation and persistence of archaeal communities (Jílková et al., 2021). The erosion and leaching of sedimentary rocks and sand by meltwater from the glacier, which forms a high concentration of $\text{SiO}_3^{2-}\text{-Si}$ at the bottom of the meltwater area (Hinsa-Leasure et al., 2010). Global warming causes melting of glaciers and degradation of permafrost (Tan and Zhuang, 2015), and then changes the distribution of organic matter in soil. This study shows that carbon and nitrogen content and humidity in soil have significant effects on microbial community richness and community structure. Therefore, archaeal community structure changes with environment caused by climate.

Through WGCNA, we found that two modules were strongly correlated with physicochemical properties, and the hub Archaea in each module were also obtained. $\text{NH}_4^+\text{-N}$, $\text{SiO}_3^{2-}\text{-Si}$, MC and $\text{PO}_4^{3-}\text{-P}$ were determined to be the main influencing factors. In the module MEturquoise, combined with the abundance of sampling sites, it could be seen that the hub Archaea were concentrated in the sampling sites near the glaciers, and were more abundant in the intertidal zone. In the WGCNA results, $\text{NH}_4^+\text{-N}$ ($r = 0.81$, $p < 0.01$) and $\text{SiO}_3^{2-}\text{-Si}$ ($r = 0.8$, $p < 0.01$) were both important physicochemical factors. The hub Archaea appeared in the sampling sites with the richest content of ammonium and silicate ions. Therefore, $\text{NH}_4^+\text{-N}$ and $\text{SiO}_3^{2-}\text{-Si}$ had significant positive correlation with the distribution of the Archaea. Among the 10 hub ASVs, ASV761 had the most and strongest correlation with other Archaea, and its quantity distribution affected the quantity distribution of other Archaea. In the RDA results, $\text{PO}_4^{3-}\text{-P}$ had no significant effect on the overall diversity and distribution of the archaeal community. However, in the module MEblue, $\text{PO}_4^{3-}\text{-P}$ was significantly correlated with the hub Archaea in that module. Two-thirds of the hub Archaea were identified as *WoeseArchaeales*, which were primarily distributed in sampling sites with high content of phosphorus (KZX). Liu et al. (2018) had found that *WoeseArchaeales* had a potential symbiotic relationship with methanogens, which have a higher abundance in places rich in phosphorus (Juttonen et al., 2020). Thus, this may be the reason why $\text{PO}_4^{3-}\text{-P}$ had a significant correlation with the hub Archaea of the module MEblue.

By combining the two methods, we can more accurately and comprehensively analyze the soil physicochemical properties that have a significant impact on the composition of the archaeal community. These results indicate that the variation of nutrients in soil of meltwater areas significantly affects the diversity of archaeal community structure under the background of climate warming. Analyzing the interaction between archaeal community diversity and environmental factors is important for predicting the impact of future environmental changes on Archaea in microorganisms.

Conclusions

Under the influence of climate warming, meltwater areas have emerged near different Arctic landforms. This paper analyzes the diversity of Archaea in the soil of Arctic meltwater regions and reveals the soil physicochemical properties that influence the diversity of Archaea. The community structure and diversity of Archaea in the meltwater areas varied with sampling sites and physicochemical factors. $\text{NH}_4^+\text{-N}$, $\text{SiO}_3^{2-}\text{-Si}$ and MC were important physicochemical factors related to archaeal communities. This paper will provide reference for future studies on the changes of microbial community structure with physicochemical factors. The hub Archaea were obtained by WGCNA, such as *WoeseArchaeales*. This gives us a revelation that we can combine WGCNA with the sandwich agar plate method (Zhang et al., 2024). WGCNA can quickly screen out auxiliary bacteria that have significant correlation with difficult to culture Archaea. This will greatly improve the cultivation efficiency.

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Conflicts of interests. The authors declare no conflict of interests.

Data availability. Raw data have been deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRR23261392- SRR23261427).

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