

Biogeography and niche differentiation of Bathyarchaeia in paddy soils across eastern China

Xingyun Yi¹, Kristian Brandt¹, Shu-Dan Xue², Jingjing Peng³, Yifei Wang², Meng Li⁴, Ye Deng², and Guilan Duan²

¹University of Copenhagen

²Research Centre for Eco-Environmental Sciences Chinese Academy of Sciences

³China Agricultural University

⁴Shenzhen University

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Abstract

Bathyarchaeia (formerly Bathyarchaeota) is a group of highly abundant archaeal communities that play important roles in global biogeochemical cycling. The presence of Bathyarchaeia in arable soils is relatively limited. In this study, we aimed to investigate the spatial distributions and diversity of Bathyarchaeia in paddy soils across eastern China, which is a major rice production region. The relative abundance of Bathyarchaeia among total archaea ranged from 3% to 68%, and Bathy-6 was the dominant subgroup. Bathyarchaeia showed higher migration ability and wider niche. Soil pH and C/N ratio were identified as key factors influencing the Bathyarchaeia composition, whereas C/N ratio and mean annual temperature influenced the relative abundance of Bathyarchaeia. Network analysis showed that specific Bathyarchaeia taxa occupied keystone positions in the archaeal community and co-occurred with some methanogenic and ammonia-oxidizing archaea. This study provides important insights into the biogeography and niche differentiation of Bathyarchaeia in agroecosystems.

1. Introduction

Archaea drives a series of global biogeochemical cycling of carbon and nitrogen (Baker et al. 2020; Bates et al. 2011; Offre et al. 2013). *Bathyarchaeia* belongs to the kingdom archaea, which was initially discovered in hot springs. They were previously placed in the *Miscellaneous Crenarchaeotal Group* (MCG) (Barns et al. 1996; Inagaki et al. 2003). *Bathyarchaeia* has been found in various other environments, including sediments, volcanic mud, termite guts, bioreactors, and soils (Calusinska et al. 2018; Loh et al. 2020; Pan et al. 2019; Xiang et al. 2017; Yu et al. 2017; Zou et al. 2020). *Bathyarchaeia* is highly abundant in marine sediments, making them one of the most abundant groups of microorganisms on the earth (He et al. 2016; Zhou et al. 2018; Zou et al. 2020). However, previous studies on *Bathyarchaeia* have mainly focused on sediments, whereas the distribution of *Bathyarchaeia* in arable soils is not much studied.

To date, pure cultures of *Bathyarchaeia* have not been successfully isolated. However, cultivation-independent studies suggest that this group of organisms possesses high physiological and metabolic diversity (Lewis et al. 2021). Members of *Bathyarchaeia* can grow on different substrates, such as detrital proteins, polymeric carbohydrates, fatty acids, methane, and other organic matter (Evans et al. 2015; Lazar et al. 2016; Pan et al. 2020). Four genomes of *Bathyarchaeia* were reconstructed from White Oak River sediments. They contained genes encoding enzymes involved in acetogenesis using the reductive acetyl-CoA pathway, indicating an anaerobic lifestyle (Lazar et al. 2016). Furthermore, some *Bathyarchaeia* members are likely to perform dissimilatory nitrite reduction to ammonium (Lazar et al. 2016), and a possible role in methane metabolism has also been suggested (Evans et al. 2015). A previous study reported that supplementing rice

paddy soil with fulvic acid significantly increased the relative abundance of *Bathyarchaeia* (Yi et al. 2019). Therefore, *Bathyarchaeia* may play a role in the biodegradation of humus, which is abundantly present in paddy soils due to the slow microbial decomposition of plant and animal residues under flooding conditions. Paddy soil is an active zone of global carbon and nitrogen cycling. Therefore, studying the distribution and activity of *Bathyarchaeia* can be important for food production and climate change regulation.

Previous phylogenetic studies have classified *Bathyarchaeia* into 25 subgroups (Rinke et al. 2021; Zhou et al. 2018), and different subgroups exhibit different ecological functions and distribution. Therefore, elucidating the mechanisms underlying *Bathyarchaeia* biogeography and community assembly in paddy soils can help predict corresponding ecological processes. Generally, the microbial community assembly can be described using the Niche-based theory or Neutral-based theory (Hanson et al. 2012; Zhou & Ning 2017). Niche-based approaches consider that the community structure is influenced primarily by deterministic processes such as environmental filtering and species interactions (Chase and Myers 2011; Jiao et al. 2019). For instance, previous studies have revealed that the specific *Bathyarchaeia* subgroups show niche differentiation and exhibit different habitat preferences. Members of *Bathy-6* grow in suboxic zones and sulfide-depleted shallow layers of sediments, whereas members of *Bathy-8* prefer deeper and anoxic layers (Lazar et al. 2015). Furthermore, *Bathy-8* is considered an indicator of saline environments (Lazar et al. 2015), whereas *Bathy-11* and *Bathy-5* are indicators of freshwater environments (Fillol et al. 2016). Moreover, salinity and total organic matter (TOC) are crucial factors affecting the abundance and composition of the *Bathyarchaeia* community (Pan et al. 2019; Yu et al. 2017; Zou et al. 2020). The neutral theory hypothesizes that all individuals are ecologically identical, and the community structure is primarily influenced by stochastic processes such as random death and dispersal (Hubbell 2004; Tilman 2004). Stochastic processes play crucial roles in influencing microbial community structures in various environments (Chen et al. 2019; Zhou & Ning 2017). However, the assembly processes of the *Bathyarchaeia* community have garnered less attention in arable soils.

A recent global meta-analysis reported that *Bathyarchaeia* is globally distributed in paddy soils with high abundance, and the predominant subgroup is *Bathy-6* (Xue et al. 2023). The meta-analysis showed that the mean annual precipitation and the mean annual temperature could be associated with the relative abundance of *Bathyarchaeia* and *Bathyarchaeia* community structure, respectively (Xue et al. 2023). However, this meta-analysis had some limitations, such as the limited availability of soil physicochemical parameters data and distribution. Therefore, studying niche differentiation governed by soil type-related factors was difficult. Furthermore, it is important to note that this meta-analysis could potentially be affected by various additional factors associated with soil management practices, including irrigation, anthropogenic interventions like flooding, the specific growth stages of rice, and the absence of uniform approaches for soil sampling procedures (e.g., sampling depth), DNA extraction techniques, and primer selection for sequencing. Such disparities can introduce potential biases into the results. Consequently, our approach involved the sampling of paddy soils from contrasting pedoclimatic regions across eastern China, all at the same stage of rice growth. We then conducted an examination of their taxonomic composition through Illumina sequencing of the 16S rRNA genes. The primary objectives of this study encompassed characterizing the composition and diversity of *Bathyarchaeia* in paddy soils across eastern China, exploring the mechanisms governing the assembly of *Bathyarchaeia* communities in paddy soil, and delving into the differentiation of ecological niches and potential ecological functions of *Bathyarchaeia* within paddy soils.

2. Materials and methods

2.1. Soil sampling and property measuring

Paddy soil samples ($n = 57$) with different chemical characteristics were collected from 19 sites (3 samples/site) in June 2020 across eastern China (Fig. 1a), which are the main rice-producing areas in China. While sampling, the paddy soils were flooded for over a month, with rice plants at the tillering stage. Three composite samples were collected from each site, indicating three replicates. These composites were created by combining five surface (<10 cm) paddy soil cores. The samples were kept under ice and transported to the lab. Each soil sample was divided into the following two aliquots: one was freeze-dried for DNA extraction,

and the other was stored at 4 °C for further soil chemical analysis.

Soil chemical parameters were evaluated, as reported previously (Yuan et al. 2016). Soil pH was detected with air-dried soil at a water ratio of 1:2.5 using a pH meter. Total carbon (TC) and total nitrogen (TN) were analyzed using an Elemental Analyzer (Vario EL, Elementar, Germany), and the resulting data was used for the calculation of the C/N ratio. Soil organic matter (SOM) was measured using a Muffle furnace (VULCAN 3–1750A, NEYTECH, America). Nitrate (NO_3^-) and ammonium (NH_4^+) were analyzed using a Continuous Flow Analytical System (AA3, SEAL, Germany). Total iron (Fe) and manganese (Mn) analyses were performed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The physicochemical properties of the soils are shown in Table S1. The soil properties differed significantly among different sites; for instance, the soil pH ranged from 4.8 to 8.1 (Fig. 1b), the C/N ratio ranged from 8.6 to 19.8, and the SOM content ranged from 2.81% to 39.37% (Table S1). Climatic indexes of different sites were obtained from the WorldClim database (www.worldclim.org), and the mean annual temperature (MAT) differed from 6.2 °C to 21.4 °C.

2.2. DNA extraction and Illumina sequencing of the 16S rRNA gene

DNA was extracted following the manufacturer’s protocol using the FastDNA SPIN kit (MP bio, CA, USA). The DNA was dissolved in 100 μL of sterilized deionized water and stored at -18°C until further analysis. NanoDrop (ND-1000, USA) was used to detect DNA concentration and OD260/OD280. For the Illumina sequencing, the archaeal 16S rRNA genes were amplified using the primer pair 524F10extF/Arch958RmodR (5'-TGYCAGCCGCCGCGGTAA-3'/5'-YCCGGCGTTGAVTCCAATT-3') (Liu et al. 2016), with 2 μL of DNA samples used as the template. Purified amplicons were sequenced using Illumina MiSeq PE300 (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.3. Statistical analyses

Raw sequence data were analyzed using the R 4.0.1 platform using “dada2” packages for the analysis of the 16S rRNA gene sequence (<https://benjjneb.github.io/dada2/tutorial.html>) (Callahan et al. 2016). Briefly, the adapters and primer sequences were first removed from raw sequence data using “cutadapt.” Moreover, clean sequences underwent trimming and merging. Amplicon sequence variants (ASVs) were derived following the removal of chimeric sequences, and their categorization was achieved using the Silva database release 138 to attain taxonomic insights (Quast et al. 2013; Yilmaz et al. 2014). The ASV table was subsampled to the minimum requisite sequence count for subsequent statistical assessments. Calculation of α -diversity (Shannon and Chao1 indices) was executed with the “microeco” and “vegan” packages (Liu et al. 2021, Oksanen J et al. 2022). The α -diversity and community composition visualizations were produced using Origin 2020 and the “ggplot2” packages in R (Pingram et al. 2019). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances was performed using “microeco” packages to visualize the similarity between samples.

To analyze the community composition of *Bathyarchaeia*, a phylogenetic tree was constructed employing reference sequences from a prior study to classify the *Bathyarchaeia* subgroup (Zhou et al. 2018). The outgroup sequences belonged to Crenarchaeum (*Cenarchaeum symbiosum*) and Nitrosoarchaeum (*Nitrosoarchaeum koreensis*). These reference sequences encompassed 15 *Bathyarchaeia* subgroups (Zhou et al. 2018). ASVs affiliated with *Bathyarchaeia*, as per the Silva 138 database, were also selected. The construction of the phylogenetic tree was executed within the MEGA11 platform (Tamura et al. 2021). The alignment of all sequences was performed using ClustalW, and the Maximum Likelihood tree was employed for the construction, with a Bootstrap analysis (1000) being carried out to evaluate tree topology (Zhou et al. 2018). Based on the tree, the subgroup information of *Bathyarchaeia* ASVs was obtained and used for downstream statistical analyses. ArcMap software was used to predict and visualize the large-scale distribution pattern of *Bathy-6* across eastern China paddy soils for the analysis of predictive atlas maps. The Kriging interpolation method was used to estimate the relative abundance of *Bathy-6* across the whole map after the input of site information, including geographical coordinates and the relative abundance of *Bathy-6*. Further, the predictive maps were obtained using a province mask. For the heatmap of *Bathyarchaeia* ASVs, the figure

was constructed using Evolvview (Subramanian et al. 2019).

To investigate the determinism and stochasticity in influencing archaeal and Bathyarchaeal community structure, the Sloan neutral community model (NCM) was used to determine the effect of stochasticity on the archaeal and Bathyarchaeal community assembly using the “Hmisc” package (Sloan et al. 2006, Harrell & Dupont 2019). The “spaa” package was used to evaluate the width and overlap of the niche (Zhang 2016). A cognitive assessment was employed to ascertain the connection between environmental factors and microbial communities with the utilization of the “linkET” package. Structural equation modeling (SEM) was employed to quantify the direct and indirect influences of environmental factors on the shaping of both the archaeal and Bathyarchaeal communities, utilizing SPSS and AMOS software. To elucidate the correlational association between environmental factors and the relative abundance of Bathyarchaeal subgroups, Pearson’s correlation analysis was conducted through the “microeco” packages. The graphical representations were generated using Origin 2020.

For the co-occurrence network analysis, Spearman’s correlation coefficients between ASVs were initially calculated through the “microeco” packages on the R platform. The Spearman’s correlation threshold was set at a coefficient > 0.7 or < -0.7 with a significance level of $p < 0.01$. Subsequently, the networks were visualized using Gephi.

2.4. Accession number

The 16S rRNA gene sequence data is deposited at the GenBank with BioProject accession number PRJNA1023015.

3. Results

3.1. Archaeal community composition and diversity

Archaeal α -diversity (Shannon and Chao1 indices) varied among different soil types (Fig. S1, $p < 0.05$). Generally, soils with medium pH ($6.0 < \text{pH} < 7.0$) showed higher archaeal α -diversity than soils with pH over 7.5. Archaeal community composition also differed between the different sites (Fig. S1). *Crenarchaeota* was the most abundant in most samples at the phylum level, accounting for 20% to 70% of total archaea, followed by *Halobacterota* (1% to 70%) as the second most abundant phylum (Fig. S2). The relative abundance of *Euryarchaeota* and *Thermoplasmata* was 2%–33% and 5%–20%, respectively. *Asgardarchaeota* and *Micrarchaeota* were also detected in some samples with lower relative abundance. *Bathyarchaeia* and *Nitrososphaeria* were the two most dominant classes in most samples at the class level (Fig. 2a). However, the relative abundance of *Bathyarchaeia* differed drastically among different sites, ranging from 3% to 68% of total archaea. *Methanosarcinia* and *Methanobacteria* were also detected at relatively high abundance in most samples, which belong to *Halobacterota* and *Euryarchaeota*, respectively.

3.2. Distribution and diversity of Bathyarchaeia in paddy soils

The α -diversity of *Bathyarchaeia* in various paddy soils exhibited significant differences among distinct paddy soils, with the Shannon index ranging from 2.16 to 5.63 (refer to Fig. S3, $p < 0.05$). Notably, soils characterized by a pH > 7.5 displayed a reduced Bathyarchaeal α -diversity when compared to soils with a pH < 7.5 . Furthermore, the NMDS analysis indicated that *Bathyarchaeia* within paddy soils clustered distinctly based on the sampling sites and pH (see Fig. S3).

In the aggregate, five subgroups (*Bathy-6*, *Bathy-11*, *Bathy-15*, *Bathy-17*, and *Bathy-5bb*) were identified within all the paddy soils (Fig. 2b). *Bathy-6* constituted the predominant subgroup across most samples, accounting for 70% to 80% of the total *Bathyarchaeia* (as depicted in Fig. 2c). Conversely, *Bathy-11*, *Bathy-17*, and *Bathy-5bb* were detected as less prevalent groups in numerous samples. It is noteworthy that paddy soils in Southeast China exhibited a higher relative abundance of *Bathy-6* in comparison to their counterparts in Northwest China (Fig. S4).

The heatmap, based on the 40 most abundant Bathyarchaeal ASVs, was generated with a focus on soil pH (as demonstrated in Fig. 2d). Out of the 40 most abundant Bathyarchaeal ASVs, 36 ASVs were affiliated with *Bathy-6*, while three ASVs were associated with *Bathy-15*, and one ASV was linked to *Bathy-17*. Furthermore, soil pH had a substantial impact on the distribution of these dominant ASVs (see Fig. 2d). Notably, two ASVs (ASV1970 and ASV1998) affiliated with *Bathy-6* were present at elevated levels in all the samples. Nevertheless, most other ASVs were solely detected in samples with acidic soil conditions (pH < 7.0).

3. 3. Bathyarchaeal community assembly in paddy soils

The NCM results at ASV levels indicated that stochastic processes were dominant in archaea community assembly, with an R^2 value of 0.613 (Fig. 3a). We found that 86.2% of archaeal ASVs and 87.0% of Bathyarchaeal ASVs fitted to the neutral model, indicating that a majority of archaeal ASVs assembled in paddy soils following stochastic processes. However, the neutral model fitted ASVs accounted for only 40.9% and 47.0% relative abundance of total archaeal and Bathyarchaeal community, respectively. Approximately 12.6% of Bathyarchaeal ASVs deviated from the neutral expectation, accounting for more than 45.0% relative abundance. These results indicate that most low-abundance *Bathyarchaeia* assembled following stochastic processes, whereas high-abundance *Bathyarchaeia* mainly assembled following deterministic processes. This phenomenon can be further supported by the assembly process of the highest abundant *Bathy-6* (Fig. 2), exhibiting a higher percentage of the deterministic process (about 38.5%) than total archaea (13.8%) and total *Bathyarchaeia* (13%, Fig. 3b).

Among the 13% of Bathyarchaeal ASVs that diverged from neutral expectations, 12.6% were more frequently observed than anticipated by the NCM prediction, indicating that they exceeded the neutral prediction. ASVs exceeding the prediction are believed to possess greater migratory capabilities and a heightened ability to relocate to new habitats. Notably, *Bathy-6*, as previously mentioned, was the most prevalent Bathyarchaeal subgroup and exhibited a higher proportion of ASVs surpassing the NCM prediction compared to other archaea and *Bathyarchaeia*. This suggests that *Bathy-6* may have more robust migratory abilities and experience less constraint on dispersal than other subgroups. The breadth of ecological niches at the class level, as determined by the null model, was computed to assess the diversity of resource utilization among *Bathyarchaeia*. The outcomes demonstrated that the niche width of *Bathyarchaeia* in paddy soils surpassed that of other archaea, indicating a greater diversity in resource utilization among Bathyarchaeia in paddy soils (as visualized in Fig. 3c).

3.4. Factors affecting the distribution of the Bathyarchaeal community

Our findings revealed that deterministic processes played a substantial role in the assembly of highly prevalent *Bathyarchaeia*, particularly the *Bathy-6* subgroup. This suggests that environmental filtering is a pivotal factor influencing their community structure. Consequently, we conducted an in-depth exploration of the relationship between environmental parameters and the distribution of *Bathyarchaeia* and *Bathy-6*.

The statistical analysis demonstrated that the relative abundance of *Bathyarchaeia* exhibited a significant negative correlation with soil C/N and a positive correlation with the mean annual temperature (MAT) (as portrayed in Fig. 4a, $p < 0.01$). In the case of the Bathyarchaeal community, soil pH and C/N emerged as the two principal factors shaping the communities of *Bathyarchaeia* and *Bathy-6*. This observation was reinforced by the results of structural equation modeling (SEM), which assessed the direct and indirect impacts of environmental parameters on the Bathyarchaeal community and *Bathy-6* (Fig. 4b). SEM outcomes indicated that the *Bathy-6* community could be directly influenced by soil pH and the Bathyarchaeal community. Moreover, the relative abundance of *Bathy-6* was positively affected by MAT and indirectly influenced by soil C/N and pH.

Furthermore, we performed Pearson's analysis to assess the influence of environmental parameters on the abundance of Bathyarchaeal subgroups detected in this study. The results indicated that soil EC, SOM,

NH_4^+ , NO_3^- , Fe, TN, and TC exhibited predominantly positive correlations with *Bathy-5bb*, *Bathy-15*, *Bathy-11*, and negative correlations with *Bathy-17*. The relative abundance of *Bathy-6* displayed significant negative correlations with pH and C/N and positive correlations with MAT (Fig. 4c).

Moreover, Random Forest analysis provided further insights, indicating that MAT, C/N, and pH are the primary factors driving the distribution of *Bathy-6* (Fig. 5). These results underscore that *Bathy-6* tends to have a higher abundance in environments characterized by higher temperatures, lower C/N ratios, and slightly acidic conditions (Fig. 5).

3.5. Co-occurrence of *Bathyarchaeia* with soil microorganisms

Co-occurrence network analysis was performed to determine the interactions of *Bathyarchaeia* with other archaea to examine the potential ecological functions of *Bathyarchaeia* in paddy soils. *Bathyarchaeia* mainly co-occurred with the members of *Nitrososphaeria*, *Methanosarcinia*, and *Methanobacteria* (Fig. 6d). The co-occurred ASVs were selected to establish a phylogenetic tree (Fig. S5). The phylogenetic tree indicated that the co-occurrence archaea have various potential metabolisms (Fig. S5). Some co-occurrence archaea belonging to methanogens can use acetate and H_2/CO_2 to produce methane. The Zi-Pi plot indicated that *Bathyarchaeia* and *Nitrososphaeria* included keystone “species” (Fig. 6b). The keystone ASVs were ASV 1790 (*Bathyarchaeia*), ASV 2007 (*Bathy-6*), ASV 1248 (*Nitrososphaeria*), indicating their crucial role in maintaining the stability of microbial community structure. ASVs belonging to *Bathyarchaeia* were selected to establish the network (Fig. 6e). *Bathyarchaeia* and *Bathy-6* were divided into different modules. Different modules showed significant correlations with other environmental parameters, indicating a high diversity within *Bathyarchaeia* and *Bathy-6* in paddy soils (Fig. 6f). Niche overlap was evaluated to explore the interaction between *Bathyarchaeia* and other archaea. *Bathyarchaeia* showed a higher niche overlap with archaea, whereas *Nitrososphaeria* showed a lower niche overlap with archaea (Table S2).

4. Discussion

4.1. *Bathyarchaeia* inhabiting paddy soil is highly abundant but not diverse

Bathyarchaeia exhibits a widespread distribution in diverse environments. Past investigations have predominantly concentrated on marine, mangrove, and freshwater sediments, where *Bathyarchaeia* have been notably abundant (Baker et al. 2020; Lazar et al. 2015; Pan et al. 2019; Yu et al. 2017). Nevertheless, the presence of *Bathyarchaeia* in arable soils has received limited scrutiny. Within this study, we have determined that the proportion of *Bathyarchaeia* relative to all archaea varies significantly among distinct paddy soils, constituting an average of approximately 31.2%.

Concerning the community structure of *Bathyarchaeia* in paddy soils, the *Bathy-6* subgroup exclusively predominates across all surveyed sites, accompanied by a smaller representation of ASVs associated with *Bathy-11*, *Bathy-15*, *Bathy-5bb*, and *Bathy-17*. In comparison with our recent meta-analysis (Xue et al. 2023), the subgroup diversity of *Bathyarchaeia* in paddy soils within eastern China (comprising 5 subgroups) appeared lower than the global scale (comprising 7 subgroups), as *Bathy-18* and *Bathy-5b* were not detected in our study. This discrepancy might be elucidated by the greater diversity observed in paddy soils worldwide. Furthermore, previous studies have indicated that *Bathyarchaeia* exhibit a relatively low relative abundance in certain paddy soils (Jiao et al. 2019), while Zheng et al. reported a higher abundance of *Bathyarchaeia* in water-saturated paddy soils compared to cultivar-rotation paddy soils and upland soils (Zheng et al. 2019). All these findings collectively suggest that the abundance and diversity of *Bathyarchaeia* in paddy soils are considerably influenced by pedoclimatic conditions. Therefore, it would be beneficial to explore the specific soil conditions that favor either low or high relative abundance of *Bathyarchaeia* in future research.

4.2. Different assembly progress of abundant and rare *Bathyarchaeia* taxa in paddy soils

NCM results indicated that stochastic processes dominated the archaea community assembly in paddy soils. The results from the NCM revealed that abundant *Bathyarchaeia* ASVs exhibit higher migratory capabilities within paddy soils and experience less dispersal limitation, implying a broader distribution range and

a greater propensity for colonization within these soils (Chen & Wen 2021). The null model also indicated that *Bathyarchaeia* displays a wider niche breadth at the class level compared to other archaea in paddy soil. A broader niche breadth signifies that *Bathyarchaeia* possesses a heightened ability to adapt to various environments, accompanied by metabolic versatility, which in turn results in a widespread and abundant distribution pattern (Chen et al. 2019; He et al. 2022; Pandit et al. 2009). This characteristic is also supported by previous studies indicating that *Bathyarchaeia* were detected in various environments and were suggested to show multiple metabolisms. NCM results also showed that most low abundant *Bathyarchaeia* (87.0% of ASVs) fitted the neutral model. Soil nutrients in arable soils can decrease the effect of environmental filtering for microbial distribution (Shu et al. 2021). Due to fertilization practice, paddy soils contain much higher nutrients than other environmental habitats, which increases the importance of stochastic processes in *Bathyarchaeia* community assembly and affects rare *Bathyarchaeia* taxa.

4.3. Niche differentiation of *Bathyarchaeia* groups in paddy soils

To the best of our knowledge, this represents the inaugural study investigating the niche differentiation of *Bathyarchaeia* in relation to the physicochemical characteristics of arable soils, including pH and the C/N ratio. Our Random Forest analysis unveiled MAT as the preeminent factor influencing the abundance of *Bathyarchaeia* in paddy soil (as depicted in Fig. 5a). The relative abundance of *Bathyarchaeia* and *Bathy-6* exhibits a notably positive correlation with MAT (as seen in Fig. 4). This observation harmonizes with our recent global meta-analysis (Xue et al. 2023), underscoring the influence of temperature on *Bathyarchaeia* subgroups in soils, as similarly documented by previous study (Xiang et al. 2017), who explored the impact of temperature on *Bathyarchaeia* subgroups in soils via multivariate regression tree analysis (Xiang et al. 2017). The discovery of *Bathyarchaeia* in hot springs further accentuates its remarkable adaptability to high-temperature environments (Barns et al. 1996).

We found the significance of the C/N ratio as an important factor in regulating the relative abundance of *Bathyarchaeia* in paddy soils (Fig. 4 and Fig. 5). However, in oligotrophic environments, such as sea and mangrove sediments, TOC was reported to be the major limiting factors for the abundance of *Bathyarchaeia*, regulating the quantity of *Bathyarchaeia* (Pan et al. 2019; Yu et al. 2017). However, in paddy soils, organic matter and ammonia are abundant due to fertilization; therefore, the C/N ratio becomes the major factor associated with the abundance of *Bathyarchaeia*. The dominant subgroup differs in sediments and paddy soils, causing a niche differentiation. These results can elaborate our understanding of the niche preference of *Bathyarchaeia* in different environments and give suggestions for the enrichment of *Bathyarchaeia*.

The results of Mantel analysis suggested that soil pH is also a key factor regulating the *Bathyarchaeia* and *Bathy-6* subgroups. SEM results further supported the important role of soil pH in influencing the community structure. The heatmap results also indicated that the abundance and number of ASVs also significantly correlate with soil pH. pH is a crucial factor in influencing bacterial, archaeal, and fungal community structures in soils (Jiao et al. 2019; Tedersoo et al. 2020; Tripathi et al. 2018), also influencing the community structure of *Bathyarchaeia* in mangrove sediments (Pan et al. 2019).

4.4. *Bathyarchaeia* co-occurred with methanogens and ammonia oxidizers

Genomic analysis suggested that *Bathyarchaeia* may play a crucial role in global carbon and nitrogen cycling (Zhou et al. 2018). In this study, we found the interactions between *Bathyarchaeia* and other archaea were very complex, and *Bathyarchaeia* play an important role in the construction of the archaeal network (Fig. 5a). The co-occurrences of *Bathyarchaeia* and acetate methanogens, including *Methanosarcinia* and *Methanobacteria*, were also found in other environmental habitats. These results suggest that *Bathyarchaeia* can be involved in carbon cycling by producing acetate for the heterotrophic microbes and acetoclastic methanogens, and acetate might be the bridge associating the interactions between *Bathyarchaeia* and *Methanosarcinia* (Xiang et al. 2017; Zou et al. 2020). The co-occurrences of *Bathyarchaeia* and hydrogen Methanogens were also found in paddy soils in this study. These results suggested that *Bathyarchaeia* might be involved in carbon cycling differently and play a crucial role in the carbon cycle in arable soils.

Bathyarchaeia also frequently co-occurred with members of *Nitrososphaeria* (Fig. 5d), which is consistent with the results of the global meta-analysis (Xue et al. 2023). *Nitrososphaeria* is a group of ammonia-oxidizing archaea highly abundant in rice rhizosphere soil with fertilization (Tang et al. 2019). Ammonia oxidizing archaea plays an important role in the soil nitrogen cycle, catalyzing the first step of the ammonia oxidation process (Baker et al. 2020). Genomic studies suggest that *Bathyarchaeia* may be involved in the nitrogen cycle, and genes involved in ammonia and urea production were found in *Bathyarchaeia* MAGs (Pan et al. 2020). Thus, *Bathyarchaeia* was suggested as a transfer station for nitrogen compounds in the global nitrogen cycle (Pan et al. 2020). Additionally, the metagenomic analysis showed that *Nitrososphaeraceae* contain genes involved in urea degradation, indicating that *Bathyarchaeia* may interact with *Nitrososphaeraceae* via urea production and transformation (Wu et al. 2021). Agricultural systems depend on significant nitrogen fertilizer inputs for farm yield; therefore, the role of *Bathyarchaeia* in agricultural soils on the nitrogen cycle warrants further research.

We also found that *Bathyarchaeia* indicated higher niche overlap with other archaea than other archaea. Higher overlap means higher association with other microorganisms, whereas no higher competition was found in paddy soils. This view is supported by network analysis that *Bathyarchaeia* plays a crucial role in the structure of a network (de Boer & Prins 1990; Holt 1987). In the future, more research is needed to investigate the ecological function of *Bathyarchaeia* in paddy soils.

4.5. *Bathy-6* is the dominant subgroup in paddy soils with broad environmental adaptation

Bathy-6 exhibits a widespread presence in terrestrial environments, including soil, freshwater sediments, and mangrove sediments (Pan et al. 2019; Xiang et al. 2017; Zou et al. 2020), although it has also been detected in certain marine sediments (Yu et al. 2018). In numerous prior studies, *Bathyarchaeia* were primarily observed in anaerobic sediments, characterizing their anaerobic lifestyle. Notably, Lazar *et al.* identified genes encoding enzymes responsible for responding to oxidative stress in *Bathy-6* (AD8-1), suggesting that *Bathy-6* members possess an ability to adapt to fluctuations in oxygen levels (Lazar et al. 2016). Additionally, Pan *et al.* reported the presence of oxygen-dependent metabolic pathways within certain *Bathy-6* genomes, hinting at a microaerophilic lifestyle for *Bathy-6* (Pan et al. 2020). In our study, the results underscored that *Bathy-6* stands as the predominant subgroup within the *Bathyarchaeia* in all the paddy soils (see Fig. 3a). Flooded paddy soils are characterized by microaerophilic conditions due to the presence of dissolved oxygen in soil porewater and oxygen released by rice roots (Chen et al. 2008; Li et al. 2007). This microoxic nature of paddy soil could elucidate why the anaerobic *Bathyarchaeia* subgroups are less prevalent, while *Bathy-6* becomes dominant in these environments. Collectively, these findings collectively indicate that *Bathy-6* possesses wide-ranging environmental adaptability, accommodating both microoxic and anaerobic conditions. This suggests that *Bathy-6* may potentially play a distinctive role in the evolutionary transition of life from anaerobic to aerobic environments. Further investigations are necessary to unravel the mechanisms underpinning the high prevalence of *Bathy-6* in paddy soils.

Furthermore, beyond adapting to diverse oxygen conditions, certain *Bathy-6* ASVs exhibit the capability to thrive across a broad pH range from 5 to 8. *Bathy-6* demonstrates resilience not only in oligotrophic marine sediments (Yu et al. 2018) but also in eutrophic paddy soils with elevated TC. Our network analysis indicated that *Bathy-6* members are subdivided into several groups. These results collectively underscore that *Bathy-6* possesses versatile metabolic capabilities, thrives in diverse habitats, and exhibits varied lifestyles, consistent with genomic predictions (Pan et al. 2020).

In addition, genomic analyses have revealed the presence of genes encoding flagella within *Bathy-6* (Lazar et al. 2016). All of these attributes associated with *Bathy-6* suggest that it may symbolize the transition of *Bathyarchaeia* from a marine to a terrestrial ecosystem. Moving forward, the comprehensive characterization and potential role of *Bathy-6* in paddy soils and even within the global ecosystem warrant further research.

5. Conclusion

Bathyarchaeia is highly abundant in most paddy soils across eastern China, which is predominated by *Bathy-*

6 . The abundance and diversity of *Bathyarchaeia* vary considerably among different soils. The relative abundance of *Bathyarchaeia* in paddy soils is majorly regulated by the C/N ratio and MAT, whereas soil pH is the key factor influencing *Bathyarchaeia* structure. *Bathy-6* is assembled mainly by deterministic progress with higher relative abundance in high temperature, low C/N, and slightly acidic environments. The network analysis revealed that *Bathyarchaeia* helps in maintaining the stability of archaea community structure, with high co-occurrence with methanogen and archaeal ammonia oxidizers. Our results further elaborate our knowledge of *Bathyarchaeia* niche differentiation. However, further research is required to reveal the specific functions of these organisms in paddy soils.

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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Figures

Fig. 1

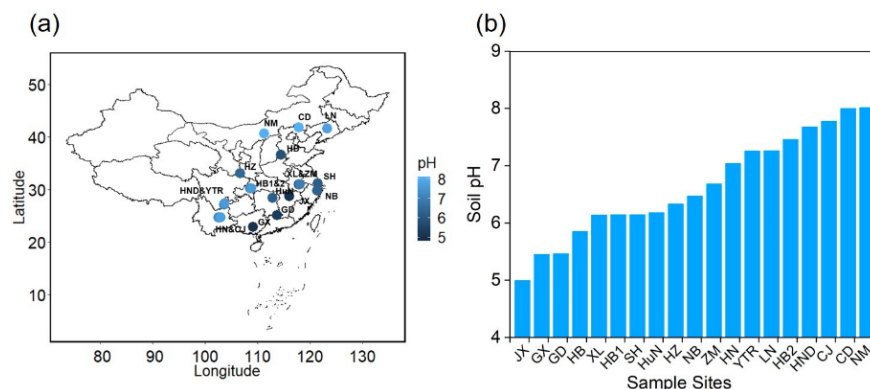


Fig. 2

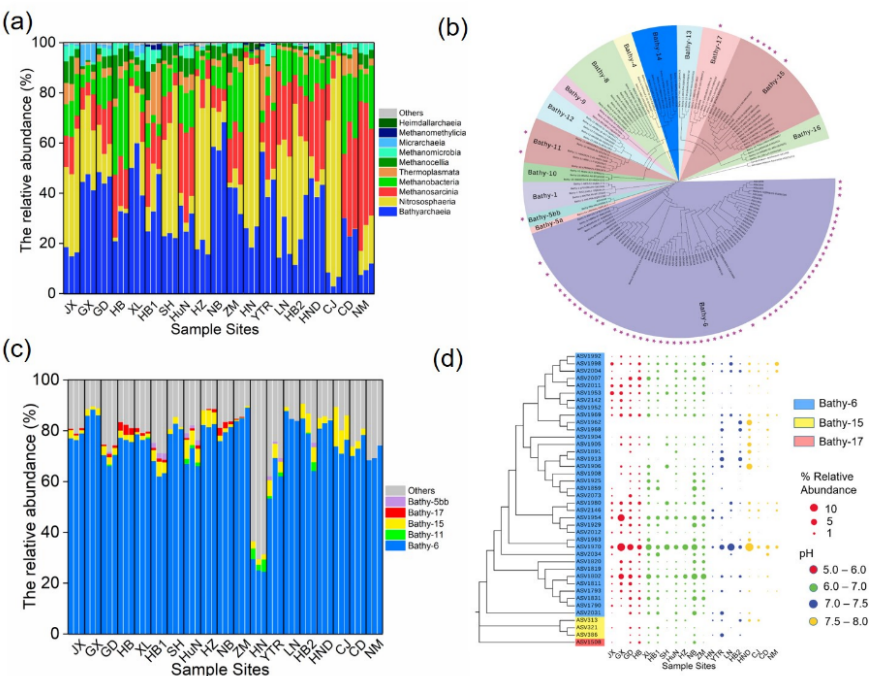


Fig. 3

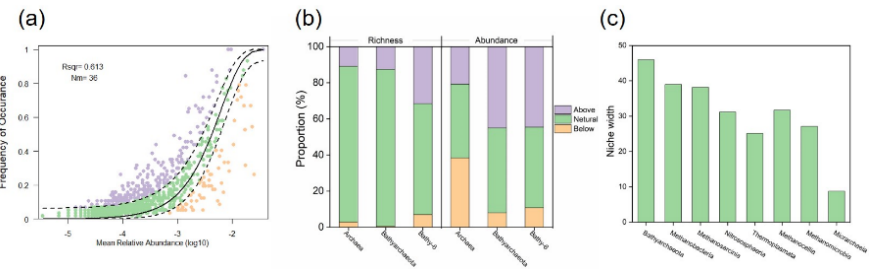


Fig. 4

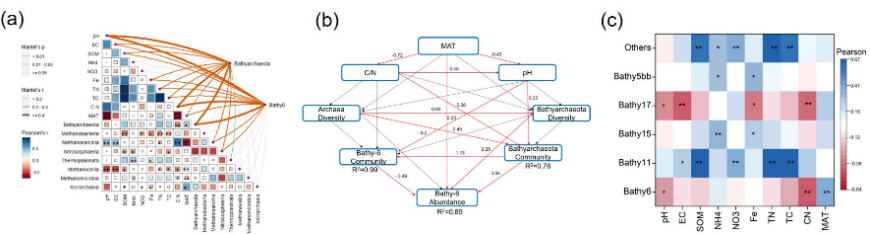


Fig. 5

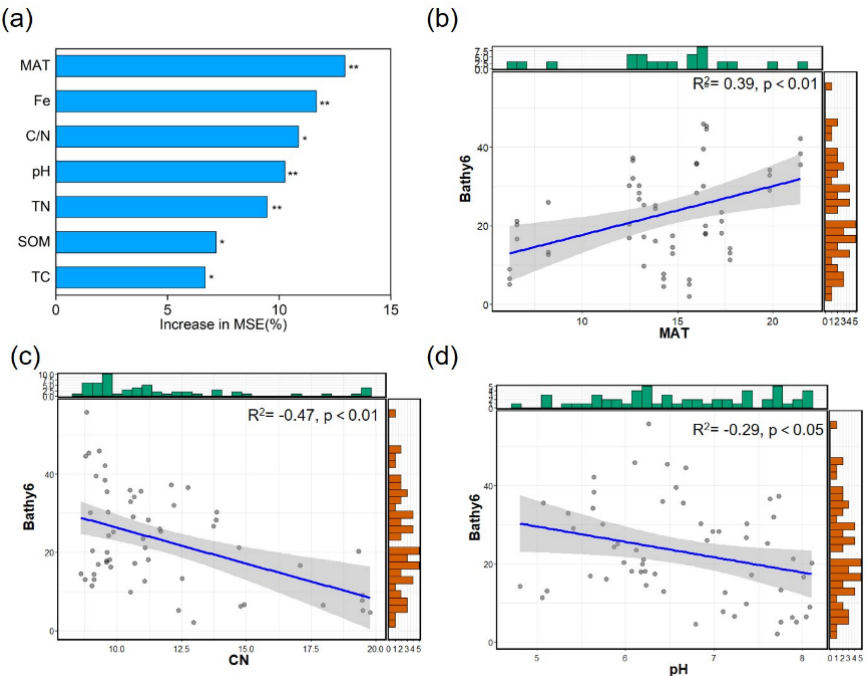
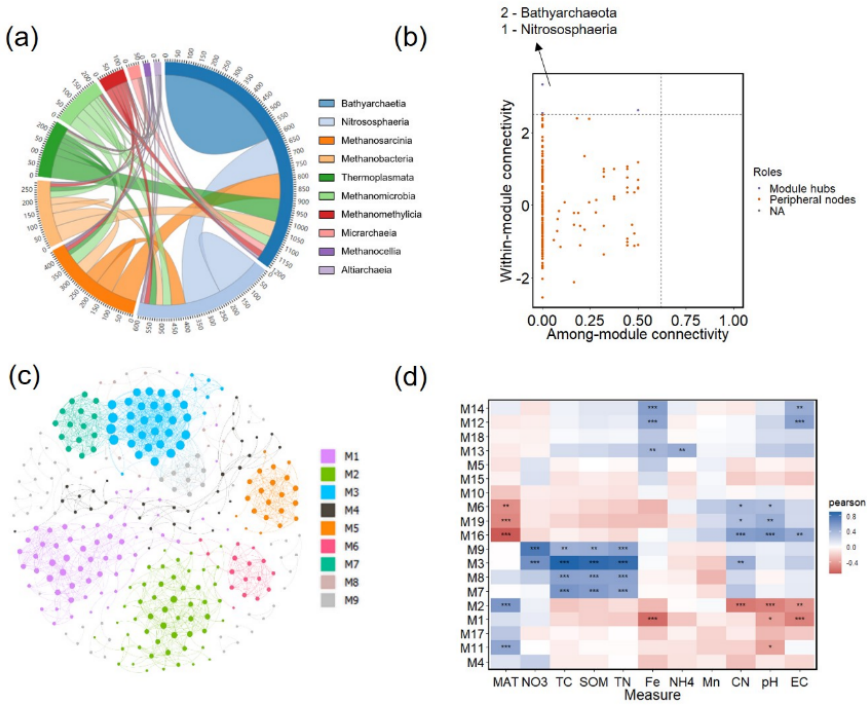


Fig. 6



Legend

Fig. 1. Paddy soils selected for the study.

Map showing the geographical location of all sampling sites across eastern China (a). The color of the points represents the pH of the soil samples. pH of all the paddy soil samples (b).

Fig. 2. The composition and diversity of archaea and *Bathyarchaeia* community in paddy soils.

The composition of the archaea community at class level (a). Phylogenetic tree of Bathyarchaeal amplicon sequence variants (ASVs) (b). The star represents Bathyarchaeal ASVs from paddy soils in this study. The composition of the Bathyarchaeal subgroup in paddy soils (c). The heatmap of Bathyarchaeal ASVs (d). The 40 top abundant Bathyarchaeal ASVs were selected. The size of bubbles represents the relative abundance of ASVs. The color of the bubbles represents the pH of the soil samples. The color of ASV ID represents the subgroup of *Bathyarchaeia*.

Fig. 3. Community assembly progress of archaea and *Bathyarchaeia* in paddy soils.

Neutral community model (NCM) of archaea (a). The solid blue lines mean the best fit to the NCM, and the dashed blue lines represent fit to the model under 95% confidence intervals. Nm means the metacommunity size times immigration, and R² indicates the fit to this model. The proportions of richness (Amplicon sequence variants [ASV] number) and abundance (sequence number) of archaea and *Bathyarchaeia* based on the fitting to the model (b). Null model of Niche width of archaea at class level (c).

Fig. 4. The effects of environmental parameters on the distribution of *Bathyarchaeia* and *Bathy-6*.

The relationship between *Bathyarchaeia* and *Bathy-6* communities and environment parameters (a). SEM analyses reveal the direct and indirect effects of climate parameters, pH, and C/N on the relative abundance of *Bathy-6* (b). Red lines represent a significant correlation, and grey lines represent no significant correlation. R² represents the proportion of variance explained. Pearson's correlations between *Bathyarchaeia* and environment parameters at Subgroup level (c). * and ** represent the significance at 0.05 and 0.01 level.

Fig. 5. Drivers of distribution of *Bathy-6* were estimated.

Environmental parameters predicting the relative abundance of *Bathy-6* according to Random Forest analysis (a). * and ** represent the significance at 0.05 and 0.01 level. The high value of the increase in MSE indicates more importance in the Random Forest Model. Linear least-squares regression analysis of the relative abundance of *Bathy-6* and MAT (b), C/N (c), and pH (d) was performed. The green and red bar charts overlaid on the axes represent the marginal distributions.

Fig. 6. Co-occurrence network analyses of archaea and *Bathyarchaeia* in paddy soils.

Co-occurrence network of archaea community based on the archaeal amplicon sequence variants (ASVs) with relative abundance higher than 1% (a). The numbers outside the circular plot represent the number of edges or linkages related to the class. Zi-Pi plot showing the distribution of archaeal ASVs based on their topological roles (b). Co-occurrence network of Bathyarchaeal community based on the Bathyarchaeal ASVs (c). Heatmap of the relationship between *Bathyarchaeia* modules and environmental parameters (f). * and ** represent the significance at 0.05 and 0.01 level.