




Archaea in boreal Swedish lakes are diverse, dominated by Woesearchaeota and follow deterministic community assembly

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Summary

Despite their key role in biogeochemical processes, particularly the methane cycle, archaea are widely underrepresented in molecular surveys because of their lower abundance compared with bacteria and eukaryotes. Here, we use parallel high-resolution small subunit rRNA gene sequencing to explore archaeal diversity in 109 Swedish lakes and correlate

archaeal community assembly mechanisms to large-scale latitudinal, climatic (temperate to arctic) and nutrient (oligotrophic to eutrophic) gradients. Sequencing with universal primers showed the contribution of archaea was on average 0.8% but increased up to 1.5% of the three domains in forest lakes. Archaea-specific sequencing revealed that freshwater archaeal diversity could be partly explained by lake variables associated with nutrient status. Combined with deterministic co-occurrence patterns this finding suggests that ecological drift is overridden by environmental sorting, as well as other deterministic processes such as biogeographic and evolutionary history, leading to lake-specific archaeal biodiversity. Acetoclastic, hydrogenotrophic and methylotrophic methanogens as well as ammonia-oxidizing archaea were frequently detected across the lakes. Archaea-specific sequencing also revealed representatives of Woesearchaeota and other phyla of the DPANN superphylum. This study adds to our understanding of the ecological range of key archaea in freshwaters and links these taxa to hypotheses about processes governing biogeochemical cycles in lakes.

Introduction

Lakes around the globe receive, transport and transform sizable amounts of carbon (Battin *et al.*, 2009; Tranvik *et al.*, 2009). Yet the magnitude of their role in global carbon cycling remains uncertain and may rest to a large extent on poorly understood microbes that drive ecosystem-scale processes. Moreover, these freshwater systems are thought to be particularly sensitive to climate warming (Schneider and Hook, 2010) enhancing microbial productivity (Prowse and Stephenson, 1986; Rouse *et al.*, 1997) and ultimately biogeochemical processes (Thornton *et al.*, 2015; Wik *et al.*, 2016) and water quality (Roulet and Moore, 2006; Weyhenmeyer *et al.*, 2016).

To construct accurate models for water quality and predict the role of lakes in global biogeochemical cycles, such as the production of greenhouse gases (GHGs)

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including carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), it is essential to understand the microbes underpinning these processes. Most studies specific to freshwater archaea have focused on sediments and wetlands because these habitats are important CH₄ sources (Bastviken *et al.*, 2004; Bridgham *et al.*, 2013). Pelagic and oxygenated freshwaters are, however, also a source of CH₄ (Grossart *et al.*, 2011; Bogard *et al.*, 2014; Angle *et al.*, 2017; Bižić *et al.*, 2020) and show considerable archaeal diversity (Auguet *et al.*, 2011; Hugoni *et al.*, 2013).

In contrast to extensive studies of bacterial (e.g. Newton *et al.*, 2011; Eiler *et al.*, 2012; Savio *et al.*, 2015; Ruiz-González *et al.*, 2017) and to a lesser extent eukaryotic microbial diversity (Debroas *et al.*, 2017) in freshwater systems, archaeal diversity is largely under-sampled in freshwaters. Because archaea form only a minor fraction of the microbial community, their diversity is poorly represented in studies using PCR primers that amplify both bacterial and archaeal 16S rRNA genes (Caporaso *et al.*, 2012; Wang *et al.*, 2012; Klindworth *et al.*, 2013; Thompson *et al.*, 2017). Despite their low abundance, archaea are expected to be a critical component of the microbial community in freshwaters, due to their role in CH₄ and nitrogen cycling and the unknown metabolic potential of the recently described archaeal lineages.

The best-known archaea in the pelagic zones of freshwaters are CH₄ producing archaea (methanogens) and ammonia-oxidizing archaea (AOA). The activity of methanogens is the main source of biogenic CH₄, and nitrification by AOA contributes to N₂O production. Methanogens often found in lakes include Methanobacteriales, Methanosarcinales and Methanomicrobiales (Earl *et al.*, 2003; Borrel *et al.*, 2011) that are particularly abundant in the anoxic bottom waters of dystrophic lakes (Peura *et al.*, 2015). AOA comprise the phylum Thaumarchaeota and groups such as Nitrosoarchaeum-like (group I.1a) and Nitrosotalea-like (SAGMGC-1) archaea. AOA have been shown to inhabit in particular oligotrophic surface freshwaters (Pouliot *et al.*, 2009; Hu *et al.*, 2010; Auguet and Casamayor, 2013; Berdjeb *et al.*, 2013; Hugoni *et al.*, 2013; Mukherjee *et al.*, 2016). Much less is known about the distribution and drivers of planktonic freshwater archaea other than methanogens or AOA. In a survey of high-altitude lakes, the main archaeal groups were Woesearchaeota and Parvarchaeota, recently described lineages with poorly defined functions (Ortiz-Alvarez and Casamayor, 2016). In a comparison of two alpine lakes differing in trophic status, environmental factors explained considerably less spatio-temporal variation of the archaeal community than in a parallel study on bacteria (Berdjeb *et al.*, 2013). In

summary, there is a need for more extensive phylogenetic sampling and characterization of the habitat preferences of archaea in freshwaters, as well as to develop hypotheses about the assembly of the archaeal community in freshwaters.

The structure of the archaeal community can be assumed to depend on the balance between stochastic and deterministic processes. Stochastic processes (i.e. ecological drift) will result in random combinations of taxa, whereas, if deterministic processes dominate, predictable patterns of taxa distributions and abundances will emerge. Deterministic processes have been shown to override random processes in macroorganisms (Gotelli and McCabe, 2002), and this has also been demonstrated for bacteria and eukaryotes in a number of habitats (Horner-Devine *et al.*, 2007; Ofiteru *et al.*, 2010; Caruso *et al.*, 2011; Eiler *et al.*, 2011; Vanwonterghem *et al.*, 2014). Important deterministic processes that determine community composition are thought to be environmental filtering and species interactions (Diamond, 1975; Gotelli and McCabe, 2002), as well as biogeographic and evolutionary history (Vuilleumier and Simberloff, 1980; Cracraft, 1988). However, to our knowledge, it has not been tested if the dominance of deterministic processes also applies to freshwater archaeal communities.

Here, we use high-throughput amplicon sequencing of (i) a universal region of the small subunit (SSU) rRNA gene (V6-V8) covering all three domains, and (ii) the V3-V5 region of the archaeal 16S rRNA gene (Gantner *et al.*, 2011) to obtain a detailed measure of archaeal diversity in surface water of 109 boreal lakes. We compare phylogenetic diversity of lake archaea to environmental variables such as catchment land use/cover, hydrological, and geochemical properties to determine what variables best correlate with the distribution of archaeal taxa, and the roles random (i.e. ecological drift) and deterministic (i.e. environmental sorting and dispersal) factors in archaeal community assembly.

Results and discussion

Characteristics of surveyed lakes

We explored and quantified variation in the diversity of epilimnic freshwater archaea at latitudes ranging from 55.4° to 68.3° (Fig. S1) representing globally the latitudes with the highest concentration, area and perimeter of inland water bodies (Verpoorter *et al.*, 2014). The sampled lakes span from nemoral to arctic (subalpine) vegetation zones and represent summer conditions as they were all sampled during August 2014. Metadata associated with each of the 119 sampled lakes (from

109 sufficient archaeal sequences were retrieved), or at least a substantial subset thereof, included latitude and longitude, temperature, chlorophyll concentration, nutrients and catchment characteristics (for summary statistics see Table S1).

Besides varying in latitude, catchment characteristics and temperature (range 10.3–24.7 °C), the lakes varied in nutrient content. Total organic carbon (TOC) in the lakes ranged from 0.6 to 31 mg L⁻¹ (median 13 mg L⁻¹), total phosphorus (TP) from 2 to 136 µg L⁻¹ (median 10 µg L⁻¹), total nitrogen (TN) from 60 to 1280 µg L⁻¹ (median 360 µg L⁻¹), and pH from 4.8 to 9.0 (median 6.7). TOC, TP and TN correlated positively with each

other and with turbidity and negatively with latitude (Fig. 1). Lake size varied from 0.03 to 14 km² with a median of 0.44 km² (Table S1).

Archaeal contribution and diversity in freshwater lakes

Based on universal sequence reads of archaeal, bacterial and eukaryotic SSU rRNA genes, archaeal SSU rRNA genes comprised from 0.03% up to 1.5% of the overall diversity (Fig. S2). This range with the average of 0.8% of archaeal reads corresponds to the lower ranges of previous studies of archaeal relative abundances in lakes (Pernthaler *et al.*, 1998; Glöckner *et al.*, 1999; Keough

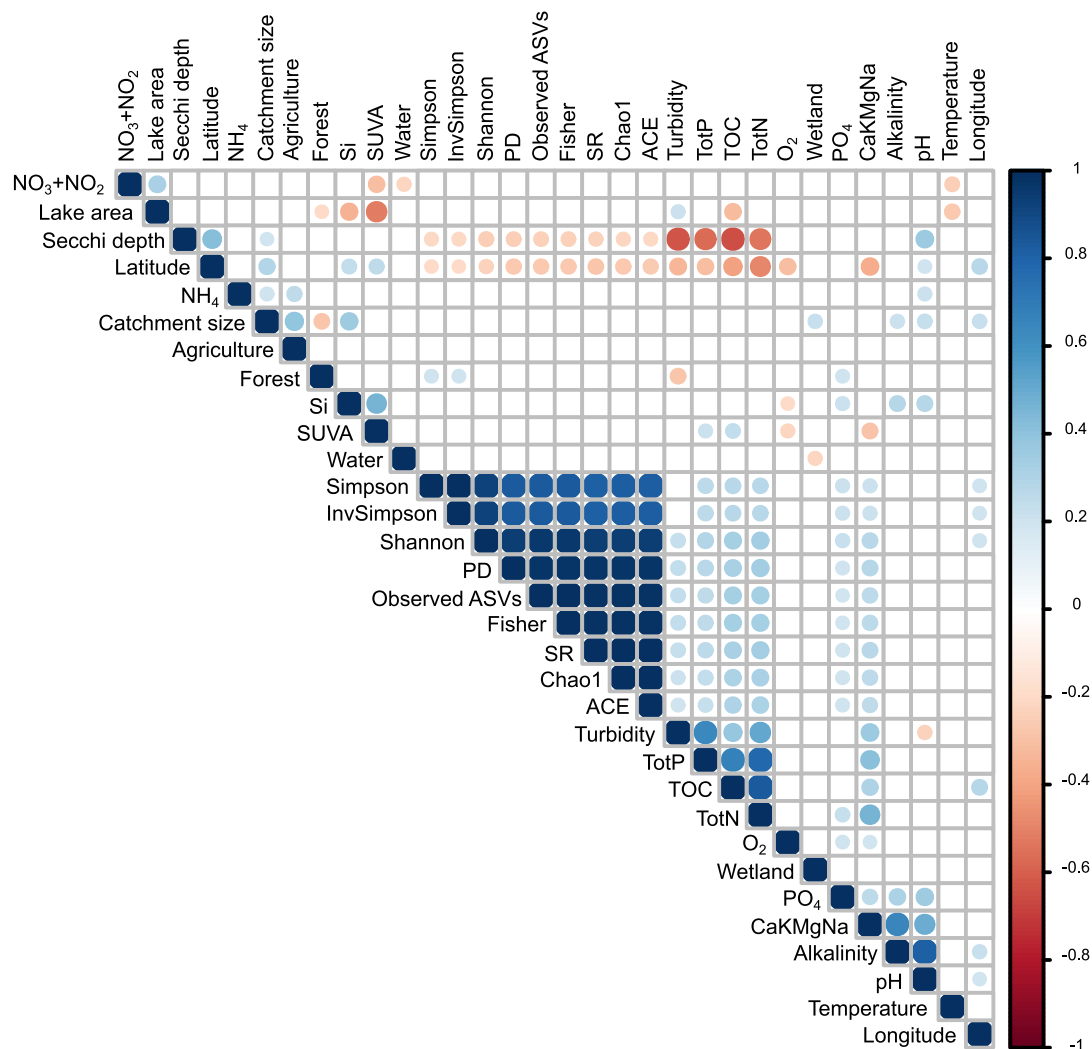


Fig 1. Correlation matrix between alpha diversity measures of archaea and environmental variables of the study lakes. The size of the symbol is inversely related to the *P*-value (correlations with *P* values >0.05 are not shown), while colour coding indicates the correlation coefficient ($r > 0$ in blue, $r < 0$ in red). Abbreviations: nitrite and nitrate nitrogen (NO₂ + NO₃), silicate (Si), ammonium nitrogen (NH₄), total phosphorous (TP), total organic carbon (TOC), total nitrogen (TN), phosphate phosphorus (PO₄-P); specific ultraviolet absorbance (SUVA); diversity measures: Simpson diversity index (Simpson, InvSimpson), Shannon index (Shannon), phylogenetic diversity (PD), species richness (SR), Chao 1 richness estimator (Chao1), abundance based coverage estimator (ACE); oxygen concentration (O₂); catchment characteristics: percentage of Water, Agriculture, and Wetland. [Color figure can be viewed at wileyonlinelibrary.com]

et al., 2003; Auguet and Casamayor, 2008; Ortiz-Alvarez and Casamayor, 2016). Partial least squares modelling revealed that the relative abundance of archaea in relation to bacterial and eukaryotic SSU rRNA genes increased with catchment land cover classified as forests and wetlands (Fig. 2). The highest relative abundances were found in dystrophic lakes. Many dystrophic lakes are characterized by anoxic bottom waters (hypolimnion) where electron acceptors for respiration are highly depleted. Accordingly, the genomes of hypolimnion microbes show the potential for fermentation and methanogenesis (Peura *et al.* 2015; Peura *et al.* 2018). A high prevalence of archaea also in the surface waters (epilimnion) of these net-heterotrophic, greenhouse-gas-emitting systems can be speculated to be the result of anoxic microenvironments (Grossart *et al.*, 2011) and their transitory occurrence in oxygenated waters.

In silico analysis of the selected PCR primers against the SILVA 16S rRNA database predicted 86.8% coverage of archaea for the archaea-specific primers and 71.5% coverage of archaea for the universal primers. The archaeal primers had only minor cross-domain amplification predicted which was confirmed in the sequencing: on average, 95.2% (range 72.2%–100% per sample) of the archaeal sequencing reads mapped to sequences of the target domain. While our approach targeted most of the archaeal diversity deposited to databases at the time of analysis, recently discovered archaeal groups emerging through random shotgun

metagenomic sequencing were potentially missed by our amplicon sequencing approach. Only 4.9% of Asgardaeota in SILVA matched the archaea-specific primers, and we determined an incomplete matching to Altiaarchaeota (40.7%), Diapherotrites (52.5%) and Nanoarchaeota (58.5%). The selection against the unknown diversity is a well-known limitation of primer-based sequencing approaches (Karst *et al.*, 2018), and thus uncertainty in the taxonomic coverage needs to be accounted for in data interpretation.

The archaea-specific amplification detected in total 119 483 archaeal amplicon sequence variants (ASVs) with an average of 1309 ASVs per sample (range 107–4024). Rarefaction analysis suggested that a large part of the diversity in individual lakes was recovered (Fig. 3A). Most of the archaeal ASVs occurred in single lakes (90.6%) with another 7.1% occurring in two or three lakes. The remaining 2.0% of the ASVs ($n = 2488$) were found in more than three lakes. In these lakes, these ASVs were the dominant ASVs with a combined relative abundance ranging from 22.7% to 82.4% per lake and an average of 50.1% across the lakes (Fig. S3A). There were 346 ASVs that occurred in more than 10 lakes, and six ASVs (including a *Woesearchaeia*, a *Methanobacterium*, two *Methanosaeta* and two *Methanoregula* ASVs) that occurred in more than 50 lakes. The most prevalent ASV (a *Woesearchaeia*) occurred in 76 lakes (prevalence distribution of the ASVs is shown in Fig. S3B). The high number of ASVs that were restricted to a single lake together with the low prevalence of most ASVs (Fig. S3C) indicates high among-lake richness. High richness was confirmed using species accumulation curves (Fig. 3B). Unsaturation strongly suggests that the ASV pool is widely undersampled along the broad environmental gradients sampled, despite sequencing effort with almost 120 000 unique ASVs detected. However, although these results are based on denoised data, which aim to remove artificial diversity introduced by PCR amplification and sequencing, outputs likely include incorrect sequences that can inflate richness and distort community composition.

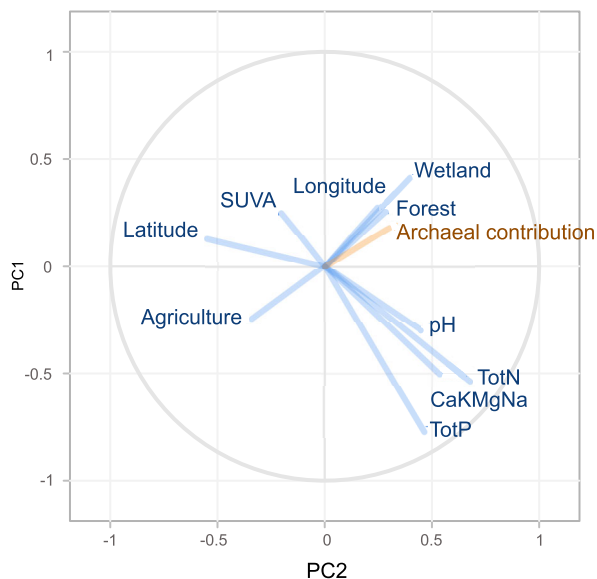


Fig 2. Plot representing the results from a partial least squares regression model that predicts archaeal contribution (in orange) to the microbial community from environmental data (in blue). Environmental variable arrows with the smallest angle (i.e. closest) to the archaeal arrow have the highest correlation with archaeal contribution. The model is based on data from 103 Swedish lakes. [Color figure can be viewed at wileyonlinelibrary.com]

Community-level biogeography corresponds with lake characteristics

We examined trends in archaeal diversity and richness in relation to lake physicochemical variables, latitude and catchment land cover. Archaeal diversity measures (phylogenetic PD, inverse Simpson, Shannon, Fisher indices, ACE, Chao1, observed ASVs) were highly intercorrelated ($r > 0.7$, $P > 0.001$) and positively correlated with TOC, TP, TN, chlorophyll a, conductivity (ion concentrations) and turbidity (Fig. 1). All of these environmental variables are indicative of productivity, suggesting that high productivity enhances

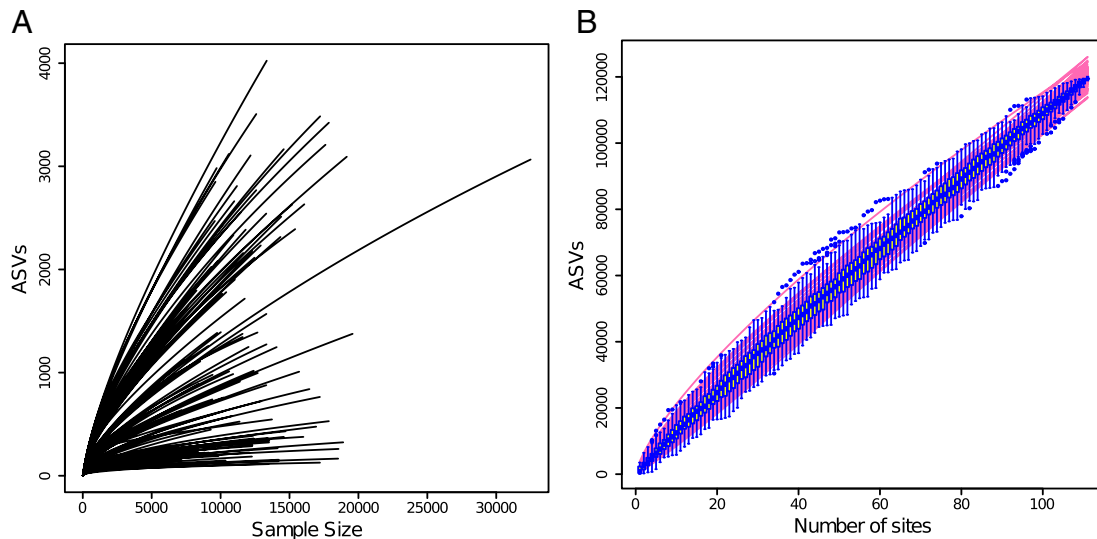


Fig 3. Sample (lake) specific (A) and region wide (B, Sweden) accumulation curves of archaeal sequence variants (ASVs). [Color figure can be viewed at wileyonlinelibrary.com]

Table 1. Environmental variables co-varying with archaeal beta diversity in freshwater lakes based on Bray–Curtis and UniFrac distance measures as provided by dbRDA.

Variable	Bray–Curtis pseudo- <i>F</i>	Bray–Curtis <i>P</i>	UniFrac pseudo- <i>F</i>	UniFrac <i>P</i>
CaKMGNa	2.01	0.005**	2.31	0.005**
Alkalinity	1.98	0.005**	2.29	0.005**
Conductivity	1.96	0.005**	2.27	0.005**
pH	1.68	0.005**	1.87	0.005**
Total nitrogen	1.66	0.005**	1.88	0.005**
Turbidity	1.62	0.005**	1.96	0.005**
Total phosphorus	1.58	0.005**	1.69	0.005**
Total organic carbon	1.45	0.005**	1.53	0.010**
Aluminium	1.42	0.005**	1.28	0.010**
Chlorophyll	1.42	0.005**	1.53	0.005**
Secchi depth	1.40	0.005**	1.59	0.005**
Lake area	1.28	0.010**	1.50	0.010**
Catchment-to-lake-ratio	1.21	0.015*	–	NS
Latitude	–	NS	1.24	0.045*

NS, not significant.

archaeal richness. Increased richness with increased productivity, as predicted by ecological theory (Cardinale *et al.*, 2009; Duffy *et al.*, 2017), was also corroborated by positive correlations with the combined bacterial, eukaryotic and archaeal diversity in the universal primer dataset.

Archaeal beta diversity across the lakes showed a similar pattern of community composition when assessed either by Bray–Curtis distance or by UniFrac distance (Procrustes superimposition; $R = 0.261$, $P = 0.001$). Among-lake community dissimilarity based on either of the two distance measures was poorly explained by the spatial distance between the lakes (Partial Mantel test controlling for environmental

variables, $R_{\text{UniFrac}} = 0.03$, $P_{\text{UniFrac}} = 0.19$, $R_{\text{Bray}} = 0.08$, $P_{\text{Bray}} = 0.011$). This result does not support a distance–decay relationship of the archaeal species distribution, similar to what has been described for bacteria (Bell, 2010). Controlling for the effects of between-lake geographic distance revealed that archaeal community structure was weakly correlated with the measured environmental variables (Partial Mantel test, $R_{\text{UniFrac}} = 0.18$, $P_{\text{UniFrac}} = 0.04$, $R_{\text{Bray}} = 0.17$, $P_{\text{Bray}} = 0.001$). Correlation showed that archaeal phylogenetic composition was associated with lake productivity (nutrients, TN and TP and chlorophyll concentrations), as well as with pH, conductivity and other lake physicochemical variables (Table 1).

As observed in other large-scale spatial studies on bacteria and protists (e.g. Lima-Mendez *et al.*, 2015; Thompson *et al.*, 2017), archaeal beta diversity was significantly related to many potential explanatory variables, indicating that environmental sorting contributes to structuring archaeal communities over large regional scales. However, ecological drift, a stochastic process, should be the driving force of archaeal community assembly when the basic entities of diversity are assessed at the ASV level. Populations of ASVs are expected to be small and inhabit geographically isolated habitats (i.e. lake systems), a pattern that is supported by the low abundance and narrow distribution of most archaeal ASVs in our study lakes. Regarding the low abundant ASVs, demographic stochasticity is expected to play a strong role. In addition, drift processes may dictate the likelihood of population detection when dormant life stages, such as a seed bank, dominate the community (Lennon and Jones, 2011). The consequences of ecological drift are that ASV abundances fluctuate randomly, increasing the differences among otherwise equivalent communities (Gilbert and Levine, 2017). Such fluctuations potentially explain the high number of ASVs with low prevalence, often occurring only in single lakes.

To assess the importance of random versus deterministic factors shaping the archaeal communities, we performed Monte Carlo simulations on the co-occurrence patterns of the ASVs in the study lakes. We observed a positive Standard Effective Size (5.34) significantly different from random ($P < 0.001$) indicative of low co-occurrence and deterministic processes being important in community assembly. Although the random ecological drift is suggested to be ever present, important deterministic processes that determine archaeal community composition can be environmental filtering and species interactions (Diamond, 1975; Gotelli and McCabe, 2002), as well as biogeographic and evolutionary history (Vuilleumier and Simberloff, 1980; Cracraft, 1988). According to the size-dispersal hypothesis, small organisms such as archaea are more likely affected by species sorting than dispersal limitation, because organisms with a size on the micrometer-scale can widely disperse (Cottenie, 2005; Beisner *et al.*, 2006; Shurin *et al.*, 2009). Thus, the distribution is mainly a reflection of the environmental properties (Farjalla *et al.*, 2012). Lack of distance decay in the archaeal community composition along our geographic gradient, as suggested by the partial Mantel tests, further emphasizes that environmental sorting plays a more prominent role than deterministic dispersal processes such as mass effects or biogeographic history in community assembly of archaea. Our finding corroborates results from the study on bacteria (Lindström and Langenheder, 2011).

Taxonomical distribution of archaea across freshwater lakes

The most abundant archaeal classes across the successfully sequenced 109 lakes were Woesearchaeia, Methanomicrobia and Nitrososphaeria (Fig. 4A). As there is a current revolution in archaeal taxonomy, we also linked SILVA taxonomy (v. 132) with other current taxonomic hierarchies (Castelle *et al.*, 2015). The Woesearchaeia (previously DHVEG-6 and Parvarchaea, also termed Woesearchaeota phylum), which are recently discovered members of the DPANN superphylum (Castelle *et al.*, 2015), were the most dominant class in our dataset in the total number of reads and the number of unique ASVs ($n > 58\,000$). While most lakes were dominated by Woesearchaeia, in a fifth of the lakes the dominant archaeal class was Methanomicrobia or Nitrososphaeria (Fig. 4A). Redundancy analysis indicated that nutrient status and the aromatic character of the dissolved organic matter are important explanatory variables underpinning the taxonomic shift at the class level (Fig. 4B). However, if this shift reflects the metabolic predictions from the available Woesearchaeota genomes such as potentially fermentative metabolism (Castelle *et al.*, 2015; Lazar *et al.*, 2017) or symbiotic lifestyle (Castelle *et al.*, 2015) is still unknown.

Methane-cycling archaea and their distribution

Lakes are sources of GHGs such as CH₄, CO₂ and N₂O, with archaea playing a particularly important role in CH₄ cycling. Consistent with other freshwater systems such as wetlands (Borrel *et al.*, 2011; Bridgman *et al.*, 2013; Narrowe *et al.*, 2017), the second most dominant archaeal class was methanogenic Methanomicrobia. We identified multiple methanogen groups including hydrogenotrophic methanogens (*Methanoregula*, *Methanobacterium*), acetoclastic methanogens (*Methanoseta*) and as minor groups methylotrophic methanogens that use methylated compounds such as methylamines and methanol to produce CH₄ (Methanomassiliococcales, *Candidatus* Methanofastidiosa; Nobu *et al.*, 2016). The most abundant methanogen genera in our dataset were hydrogenotrophic *Methanoregula* and acetoclastic *Methanoseta* (Fig. 5A). As in previous studies of freshwater lakes and wetlands, these two genera were frequently identified together. While their relative dominance has been suggested to depend on factors such as pH, season and carbon availability (Kotsyurbenko *et al.*, 2007; Sun *et al.*, 2012; He *et al.*, 2016), our study points to the importance of nutrient status and catchment land cover as important determinants. For example, *Methanoregula* showed the highest relative abundances in

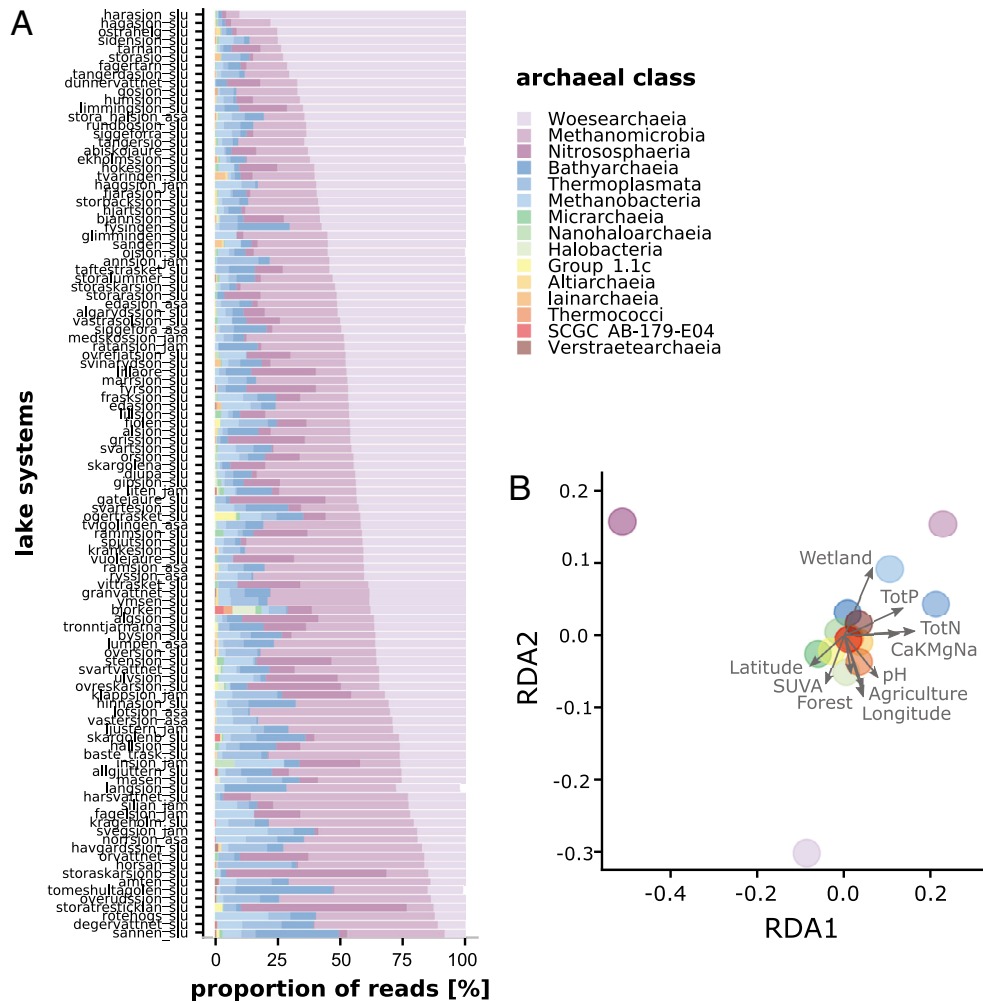


Fig 4. Taxonomic composition of the archaeal classes (following SILVA 132 taxonomy) (A) and their number of reads (relative abundance in percentage) in relation to environmental parameters as inferred by redundancy analysis (B) with the 15 most abundant classes represented. In lake system names, 'slu' refers to the lakes of the national sampling campaign and 'jam' and 'asa' to separate sampling campaigns. [Color figure can be viewed at wileyonlinelibrary.com]

lakes with a forested catchment, while in eutrophic (phosphorus and nitrogen rich) lakes *Methanobacterium* and *Methanolinea* had their highest relative abundances (Fig. 5B). In two lakes, the dominant methanogen genus was *Ca. Methanoperedens*. Members of this taxon have been shown to conduct anaerobic oxidation of CH_4 using nitrate (Raghoebarsing *et al.*, 2006; Haroon *et al.*, 2013; Arshad *et al.*, 2015) or Fe(III) and Mn(IV) (Ettwig *et al.*, 2016) as electron acceptors. Related sequences have also been linked to anaerobic oxidation of CH_4 coupled to sulfate reduction in freshwaters (Schubert *et al.*, 2011; Timmers *et al.*, 2015). As mentioned earlier, it can be speculated that the detected methanogens may either represent transient members originating from anoxic environments or inhabit anoxic microenvironments (Grossart *et al.*, 2011) in the oxygenic part of the water column. Either way, their presence and taxonomic composition

suggest an active role in CH_4 cycling of freshwater lakes.

Ammonia-oxidizing archaea

Thaumarchaeota classes detected in the lakes included Group 1.1c, SCGC_AB-179-E04 and most prominently Nitrososphaeria including *Ca. Nitrosotalea* and *Ca. Nitrosoarchaeum*, which are described as AOA. In freshwater ecosystems, especially those with high allochthonous inputs such as dystrophic lakes, increased nitrogen supply could promote the occurrence of ammonium oxidizers. AOA, however, are favoured at low levels of nitrate, low light and low pH (French *et al.*, 2012; Hatzenpichler, 2012). Accordingly, Nitrososphaeria showed the highest relative numbers in lakes with low pH and TN (Fig. 5B). Recent genome analysis of a *Ca. Nitrosotalea devanaterre* revealed genes encoding both

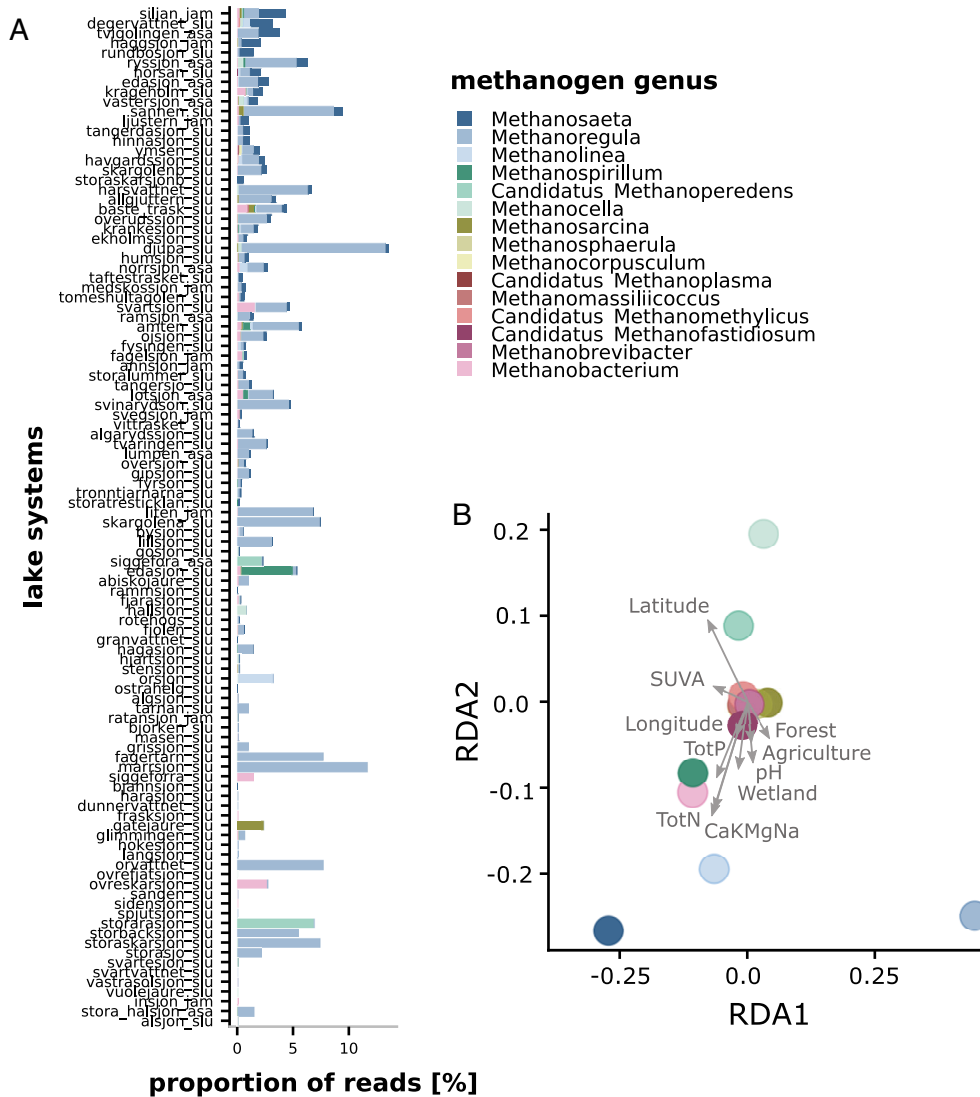


Fig 5. Taxonomic composition of the archaeal methanogenic genera (following SILVA 132 taxonomy) (A) and their number of reads (relative abundance in percentage) in relation to environmental variables as inferred by redundancy analysis (B) with the 15 most abundant genera represented. In lake system names, 'slu' refers to the lakes of the national sampling campaign and 'jam' and 'asa' to separate sampling campaigns. [Color figure can be viewed at wileyonlinelibrary.com]

a predicted high-affinity substrate acquisition system and potential pH homeostasis mechanisms, which were expressed during acidophilic growth (Lehtovirta-Morley *et al.*, 2016). While *Ca. Nitrosotalea* were abundant in dystrophic lakes, *Ca. Nitrosoarchaeum*, previously found in low-salinity habitats worldwide (Blainey *et al.*, 2011), were the abundant members of Nitrososphaeria in most other lake types.

Novel taxa with potentially versatile metabolic roles

In addition to canonical methanogens, we detected Bathyarchaeota and Verstraetearchaeota (*Ca. Methanomethylicus*; Vanwonterghem *et al.*, 2016), which are hypothesized to represent methylotrophic methanogens.

The class Bathyarchaeia contributed highly to the archaeal community in lakes with agricultural land use in their catchments, as indicated by redundancy analysis (Fig. 4B). The detected Bathyarchaeia were highly diverse, represented by more than 7761 ASVs that were collectively abundant across the lakes. Bathyarchaeia are among the most abundant organisms reported in marine and freshwater sediments globally (Biddle *et al.*, 2006; Borrel *et al.*, 2011; Lloyd *et al.*, 2013; Fillol *et al.*, 2015; Lazar *et al.*, 2015; Wurzbacher *et al.*, 2017). Verstraetearchaeia, occurring as a minor archaeal class in our dataset, appear to be capable of fermentation utilizing sugars as a carbon source and generating acetyl-CoA via the Embden–Meyerhof–Parnas pathway and pyruvate-ferredoxin oxidoreductase (Vanwonterghem

et al., 2016). Combined with the taxonomic results discussed above, our findings support the diverse functional roles of archaea in freshwater systems, beyond CH₄ and ammonium metabolism.

Conclusion

Monte Carlo simulations on archaeal ASV patterns suggested that freshwater archaeal communities were shaped by deterministic processes such as environmental sorting as well as biogeographic and evolutionary history, overriding stochastic processes such as ecological drift. Large among-lake variability was reflected in the cumulative rarefaction curves of archaeal ASVs that did not saturate over the lake gradient, although many rarefaction curves of archaeal ASVs in individual lakes did reach an asymptote. Furthermore, our observations of a coupling between productivity indicators and archaeal richness provide evidence for the generality of the productivity–diversity relationship across all organismal kingdoms.

We revealed a marked phylogenetic diversity of archaea in freshwater lakes, not restricted to functionally characterized groups such as canonical methanogens or AOA. As such our study expands the knowledge of archaeal diversity inhabiting freshwater environments and shaping carbon and nitrogen cycling and CH₄ emissions of lakes. Future studies should aim to estimate the mass and energy fluxes through the archaeal compartment in these aquatic environments of regional (water quality) and global (GHG emissions) significance.

Experimental procedures

Sampling. Samples of freshwater microbes were collected from 95 Swedish lakes in August 2014 as part of a national lake monitoring programme (Fig. S1). In addition, 24 additional lakes were sampled in two separate campaigns during the same time period (Fig. S1). Water samples for analysis of water chemistry were collected from the depth of 0.5 m with a 0.5 m long tube sampler (Ruttner-type). Water samples for analysis of microbes were sampled from the depth of the whole epilimnion (usually down to between 2 and 8 m) with a 2-m long tube sampler. The epilimnion samples were pooled in a large bucket and a subsample of 100 and 500 ml was filtered with a peristaltic pump on 142 mm Millipore (Billerica, MA, USA) polycarbonate filters with 0.2-µm pore size until the filters clogged. Filters were immediately stored at –80 °C until further processing.

Environmental data. Geographical information, such as lake area and catchment area, was derived from the database Svenskt Vattenarkiv (Swedish Water Archive,

SMHI, 2012). Catchment land use/cover for each lake (e.g. % forest, % agriculture, % urban) were downloaded from the Swedish Land Cover Data database (Naturvårdsverket, 2014), part of the CORINE Land Cover data (European Environment Agency, 2014).

Water physicochemical data from the Swedish national lake monitoring program at the time of sampling are publicly available from the national data host (<https://www.slu.se/en/departments/aquatic-sciences-assessment/data-host/>). The physicochemical variables were measured according to international (ISO) or European (EN) standards.

Large surveys, like in our case, result in datasets containing missing values for various reasons, often encoded as NaNs, blanks, or any other placeholders. One way to handle this problem is to get rid of the observations that have missing data. However, such an approach will risk losing overall statistical power and data points with valuable information, as well as introducing systematic biases. In addition, different subsets of the metadata for different analysis results in different values, which can lead to biased comparisons among analysis results and most importantly do not allow the application of multivariate statistical analyses. Thus, we used a strategy where we factored in the missing values ($n = 425$; 16% of total metadata entries) and learned the best imputation values for the missing data (see below for further description). Prior to analyses, two samples were entirely removed as they contained highly incomplete metadata. In addition, 10 variables were removed because they were redundant or contained high numbers of NAs.

DNA extraction, amplification of SSU rRNA genes and Illumina MiSeq sequencing. Filters were cut into small pieces and DNA was extracted following MoBio Power Soil kit protocol. For both PCR assays (i.e. archaeal and universal) used in this study, a two-step PCR protocol was applied to minimize PCR biases, such as chimera and heteroduplex formation (Thompson *et al.*, 2002) and to add barcodes, Illumina handles and adapters to the amplicons of individual samples. In the archaeal protocol, we added 1 µl of DNA extract to duplicate PCR tubes containing dNTPs (0.2 mM), the archaeal primers 340f (5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNN-CCCTAYGGGGYGCASCAG-3') and 1000r (5'-AGACGTGTGCTCTTCCGATCT-GGCCATGCACYWCYTCTC-3') [modified from Gantner *et al.* (2011) with added Illumina adapters] at 0.25 µM, as well as Q5 Polymerase (0.4 unit), Q5 enhancer (4 µl) and PCR buffer (1 ×) in a final volume of 20 µl. Amplicon size was 650–750 bp. The first PCR consisted of an initial denaturation step of 98 °C for 30 s and then 30 cycles of 10 s at 98 °C, 30 s at 63 °C, 30 s at 72 °C and a final elongation of 2 min at 72 °C.

Using universal primers 926f (5'-ACACTCTTTC CCTACACGACGCTCTTCCGATCT-AC-AACTYRAAG RAATWGRGCGG-3') and 1392r (5'-AGACGTGTGCTCT TCCGATCT-CA-GACGGGCGGTGWGTRC-3') at 0.4 μ M, we added 1 μ l of DNA extract to duplicate PCR tubes containing dNTPs (0.25 mM), as well as Herculase II Fusion Polymerase (Agilent Technologies, 0.6 μ l) and PCR buffer (1 \times) in a final volume of 40 μ l. Amplicon size was around 500 bp. The first PCR consisted of an initial denaturation step of 95 °C for 3 min and then 30 cycles of 30 s at 95 °C, 45 s at 50 °C, 90 s at 70 °C and a final elongation of 5 min at 72 °C.

In both cases, duplicate amplicons were pooled and purified using Agencourt AMPure XP purification system. Then, we did a second PCR step (using Q5 polymerase) of 15 cycles (archaea) or 12 cycles (universal) (initial denaturation 30 s at 98 °C; 10 s at 98 °C, 30 s at 66 °C, 30 s at 72 °C; with a final step of 2 min at 72 °C) using 1 μ l of the previous PCR product as template. This second PCR added barcodes and complete ThruPLEX adapters for Illumina sequencing (forward primer 5'-AATGATACGGCGACCACCGAGATCTACAC-[i5 index]-ACACTCTTTCCTACACGACG-3' and reverse primer 5'-CAAGCAGAAGACGGCATACGAGAT-[i7 index]-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3').

After once again purifying the samples using the Agencourt AMPure XP kit and quantification by a PicoGreen assay (Quant-iT PicoGreen, Invitrogen), 16S rRNA gene and universal SSU rRNA gene samples were pooled separately in equimolar amounts. Samples were sequenced on a MiSeq using v3 chemistry and software version 2.6.1.1 (Science for Life Laboratory, Uppsala). Final sequencing results were obtained from 103 samples (94 samples from the national monitoring program and nine from the separate sampling campaign) using the universal primer pair and for 109 samples (88 from the national program and 21 from the separate campaigns) using the archaea-specific primer pair.

Amplicon sequences produced in this study are publicly available at the NCBI-SRA under accession numbers SRR10321796-10321906 (archaea) and SRR10965090 - SRR10965180 (universal).

Amplicon data processing. After raw sequence data had been trimmed of primers with CUTADAPT (Martin, 2011) and sequences without matching primers removed, they were analysed with R package dada2 (Callahan *et al.*, 2016) for de-replicating, denoising and sequence-pair assembly. After manual inspection of quality score plots, the forward and reverse reads of the universal amplicons were trimmed at 280 and 260 bp length respectively and the archaeal forward and reverse reads at 230 and 180 bp length respectively. Additional quality filtering removed any sequences with unassigned base

pairs and reads with a single phred score below 10. The universal SSU rRNA gene amplicons were assembled by merging the read pairs. The archaeal 16S rRNA gene amplicon reads, for which the read pairs did not overlap, were concatenated. After reads were dereplicated, forward and reverse error models were created in dada2 with a subset of the sequences (10^7 reads). Chimeras were removed using 'removeBiomeraDenova' in the dada2 package, which resulted in the final taxon table.

Phylogenetic and taxonomic analysis. MAFFT (Kato and Standley, 2013) version 7.305 was used for sequence alignment. FastTree 2 (Price *et al.*, 2010) was used for generating a phylogenetic tree of the aligned sequences. The function assignTaxonomy of the DADA2 package was used to assign taxonomy using version 132 of the Silva database (Quast *et al.*, 2013). Chloroplast and mitochondrial reads were removed from the universal dataset and non-archaeal reads from the archaeal dataset. Taxonomic coverage of the archaeal primers was tested *in silico* with Silva TestPrime (Klindworth *et al.*, 2013; <https://www.arb-silva.de/search/testprime/>) allowing one mismatch and no mismatches in the 3' end.

Statistical analysis. All statistical analyses and plotting were performed in R version 3.5.2 (R Core Team, 2016) using multiple R packages with R code deposited to https://gitlab.com/eiler_lab/scandinavian-archaea-diversity. Missing values in the metadata were approximated using multiple imputation with Fully Conditional Specification implemented by the MICE algorithm as described in van Buren and Groothuis-Oudshoorn (2011). This approach uses variables as predictors that (i) appear in the complete data model, (ii) are related to the none-response, and (iii) explain a considerable amount of the variance while (iv) removing variables that have too many missing values within the subgroup of incomplete cases. If variables autocorrelated, a subset of the variables was retained for downstream statistical analysis. Partial least squares regression models were used to infer environmental variables that could predict the contribution of archaeal rRNA gene reads to the total read abundance in the dataset obtained with universal rRNA gene primers. Prior to alpha and beta diversity analyses, ASV data were rarefied to the smallest sample size (4649 reads) using the function rrarefy in the vegan package (Oksanen *et al.*, 2019). Alpha diversity measures were obtained using EstimateR on rarefied community data (i.e. ACE, Chao1, Shannon, Fisher and Simpson index) and picante (phylogenetic diversity; PD). Beta diversity matrices were calculated using UniFrac and Bray–Curtis distances on the rarefied and Hellinger transformed community data. We used distance-based

redundancy analysis (dbRDA) with the function `capscale` in `vegan` to identify environmental variables co-varying with archaeal beta diversity.

Model significance of dbRDA was tested by permutational analysis (function `anova.cca`) for the overall model and for the significance of individual factors (marginal effects). Redundancy analysis on the relative proportions of archaeal classes and methanogen genera was carried out with the function `rda` to identify environmental variables co-varying with the classes and genera. The importance of random versus deterministic factors was assessed with `EcoSim` (Gotelli and McGill, 2006). `EcoSim` performs Monte Carlo randomizations to create 'pseudo-communities' (Pianka, 1986), then statistically compares the patterns in these randomized communities with those in the real data matrix.

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Author Contributions

The research was conceptualized by H.J. and A.E. Sample collection was coordinated by S.D. while molecular and data analyses, as well as writing, was coordinated by A.E. Molecular analyses were performed by H.J. and C.W. The main analyses and visualization of the data were performed by H.J. and A.E., with additional analyses performed by L.F. and C.W. The first version of the manuscript was drafted by A.E. with help from H.J. All authors provided comments and were involved in writing the final version of the manuscript. Financial support for the project was acquired by S.D., S.P. and A.E.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Map of the sampled Swedish lakes.

Figure S2 Proportion of domain-specific rRNA gene reads resulting from amplicon sequencing with universal primers.

Figure S3 The contribution of archaeal amplicon sequence variants (ASVs) unique to individual study lakes to the overall community of each lake as well as the contribution of archaeal ASVs occurring in two to three lakes and more to each lake community (A). The prevalence of archaeal ASVs cumulation curve (B) and (C).

Table S1 Summary statistics of metadata including 119 sampled lakes.