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Abstract

Arctic regions are expected to experience far-reaching changes in environmental conditions induced by climate change. The rise of temperature is predicted to increase microbial activity and subsequently accelerate the decomposition of organic matter and other biogeochemical processes. Since permafrost-affected soils store large fractions of organic carbon, the enhanced decomposition of such vulnerable pools can lead to a positive feedback to global climate change through increased greenhouse gas emissions.

The decomposition processes in Arctic regions are thought to be generally nitrogen-limited, but little is known about the microbiota involved in nitrogen cycling in these environments and about their reactions to changing environmental conditions. This study aimed at investigating ammonia oxidizing archaea and bacteria populations that play an important role in nitrification and thus in regulating the balance between major nitrogen sources for both microorganisms and plants. Furthermore, nitrifiers are directly and indirectly linked to the production of the potent greenhouse gas nitrous oxide.

The main goal of this study was to investigate the size and nature of ammonia oxidizer populations in a cryoturbated soil from the Taymyr peninsula and their changes upon changing temperature and moisture regimes. The measurements were done on samples from two horizons of a cryoturbated soil, including the organic top soil layer and cryoturbation pockets, i.e. subducted topsoil material. The soil material had been incubated under different combinations of temperatures (4, 12, 20 °C) and moisture contents (50 , 80, 100 % WHC, water holding capacity) in an incubation experiment performed by the international CryoCarb consortium.

Abundances of ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) were determined by quantitative polymerase chain reaction targeting the functional marker gene *amo*A, which encodes a subunit of the ammonia monooxygenase. While AOA populations were with 9.6×10^5 copies per g soil initially larger than AOB populations in the upper horizon, the latter group increased more markedly upon rising temperature under oxic conditions (50 and 80% WHC) and reached equally large final population sizes as AOA. In contrast, AOB populations dominated in the buried topsoil in which the overall smaller populations (8.4×10^3 copies per g soil) exhibited a positive correlation with temperature at all moisture conditions.

High-throughput *amo*A amplicon sequencing was used on samples from the upper horizon to analyse the AOA community composition in detail. This study showed that

four distinct AOA clades exhibited different, some even contrasting dynamics in response to different environmental conditions.
Gross nitrogen mineralization rates were determined by pool dilution upon addition of ^{15}N-labeled ammonia to investigate potential effects of changing conditions on the processes, leading to changed substrate availability for ammonia oxidizers. They exhibited higher rates in the topsoil compared to organic and mineral horizons originating from lower soil layers, however NH_4^+ production and NH_4^+ consumption could not be directly linked to the dynamics of ammonia oxidizer populations.

Together, this study elucidates dynamics of microbial ammonia oxidizers to changing conditions either introduced by climate change or the process of cryoturbation. Based on the overall results, the most influential factor on both AOA and AOB population responses was temperature, which was positively correlated to abundances. The clear separation of the soil horizons before incubation allowed horizon-specific investigations and revealed differences between topsoil and subducted topsoil material. In addition, the analysis of the AOA community structures revealed distinct responses by different AOA sub-populations indicating that it will be important in future studies to obtain a higher resolution within functional guilds in order to be able to better link population dynamics and activities with processes in the soil.

1 Introduction

Arctic regions represent unique but vast environments which are characterised by low annual temperatures, short growing seasons, low precipitation and nutrient availability as well as great fluctuations in environmental factors (Blaud et al., 2015 and references therein). The prevailing soil types in these ecosystems are commonly classified as Cryosols, which are, in addition to other factors, defined by the presence of permafrost (Jones et al., 2010). Arctic habitats are highly affected by climate change. Permafrost temperatures have risen over the last decades, essentially by the impact of alterations in snow cover and higher air temperatures, which are reported to increase faster in these environments than the global mean (IPCC, 2014). This will promote thawing and consequently increases the thickness of the active layer, which is the upper layer of the soil that is not frozen during summer.

The circumpolar permafrost zone harbours a large fraction of fixed carbon. Approximately half of the global belowground organic carbon is stored in this zone. (Tarnocai et al., 2009). Cryoturbation is one of the major cryopedogenic processes in soils affected by permafrost and can lead to the dislocation of soil material throughout the profile by alternating freezing and thawing events. One of the features displayed in the soil are intrusions of topsoil material in deeper soil layers. (Bockheim & Tarnocai, 1998) It is suggested that these cryoturbation pockets are important in the storage of soil organic carbon (Hugelius et al., 2010). The cryoturbation process has distinct effects on the soil structure. Since material from the topsoil, which is high in organic matter, can be buried into lower soil layers, decomposition processes may be decreased due to unfavourable conditions. On the other side, formerly subducted material, high in soil organic carbon, can also be shifted upwards again and get exposed to more advantageous conditions, which might lead to more rapid decomposition. (Kaiser et al., 2007; Bockheim, 2007)

Predicted climate changes include increasing temperatures, which have a major impact on a broad range of aspects. Primarily, decomposition and biogeochemical processes are thought to be accelerated by increased microbial activity. Elevated temperatures also have a negative effect on the snow cover and the permafrost. The thawing of permafrost enlarges the depth of the active layer and will affect, together with the predicted increase in precipitation, the moisture conditions, prevailing in the soil, which can be either drier or wetter, depending on the drainage. Another aspect includes that plant cover will be positively affected and plant communities will change, which leads to increased nutrient requirements. Amongst others, these factors will greatly influence

the activity, diversity and community structure of microorganisms and furthermore the important geochemical processes mediated by them. (Blaud et al., 2015 and references therein)

It is predicted that the decomposition processes of soil organic matter which are mediated by microorganisms will expand in the circumpolar permafrost zone under the influence of climate change, introducing big impacts on global C balance (Schuur et al., 2008) and on emissions of greenhouse gasses like CO_2, CH_4 and N_2O from permafrost-affected soils (IPCC, 2014), implying a positive feedback on global temperatures. However, the effects and their extent are tightly linked to other element cycles and their responses (Shaver et al., 1992). Since microorganisms are important drivers of biogeochemical processes, it is of major interest how changing conditions, either induced by climate change or by cryoturbation, will affect the microbial activity and community structure in the soil.

Nitrogen cycling in permafrost soils

One of the most important element cycles is the nitrogen cycle, which is tightly connected to the carbon cycle. It is supposed that in Arctic soils, a large fraction of the total nitrogen (N) is bound to organic compounds (Jonasson et al., 1999). However, most Arctic habitats are considered to be N-limited, mainly due to the low amount of soluble N compounds (Schimel et al., 1996, Schimel & Bennett, 2004). The low N availability is the result of decreased decomposition of organic matter and slow N cycling (Schimel & Bennett, 2004), including low N mineralization rates (Nadelhoffer et al., 1991), which are influenced by low temperatures and partially high soil water content (Blaud et al., 2015). The decomposition of soil organic matter by heterotrophic organisms, provides energy and carbon, as well as nutrients to the decomposers. If the soil organic matter contains enough nutrients (e.g. N), the excess is released (mineralized) otherwise additional N has to be taken up from the soil solution (immobilization). Mineralization is one of the processes in the N cycle, that determines the availability of N forms accessible for plants and microorganisms. The organic N compounds are converted to inorganic forms, which can be assimilated, volatilized or be further transformed by biological processes like nitrification. (Robertson & Groffman, 2007)

Nitrification represents an essential component in the N cycle by connecting reduced N forms such as ammonia (NH_3) with oxidized N forms like nitrite (NO_2^-) and nitrate (NO_3^-) and supplying substrate for denitrification (Ward, 2011). The nitrification

process is divided into two major steps, the oxidation of NH_3 to NO_2^- and the oxidation of NO_2^- to NO_3^-, of which the first conversion is thought to be the rate limiting step. (Sayavedra-Soto & Arp, 2011). In soils, nitrification controls the availability of ammonium (NH_4^+) and NO_3^-, respectively, which are the major sources of inorganic N for plants. Whereas NH_4^+ retains in the soil associated to the negatively charged colloids, NO_3^- is highly mobile and can be leached or denitrified under sufficient soil moisture content. Increased nitrification activity can further lead to acidification of the soil (Prosser, 2011).

Additionally, it has been shown that a pathway of nitrifiers, called nitrifier denitrification in which after the oxidation of NH_3, the product NO_2^- is again reduced, can (like denitrification) contribute to the production of the potent greenhouse gas N_2O (Wrage et al., 2001).

Microbial ammonia oxidizer communities in soils

It is suggested that autotrophic bacteria and archaea are the primary ammonia oxidizers In most soils (Robertson & Groffman, 2007). Energy for their growth is derived from the aerobic oxidation of NH_3, catalyzed by the membrane bound enzyme ammonia monooxygenase, which consists of three subunits (AmoA, AmoB and AmoC) (Arp et al., 2002). The *amo*A gene, which encodes the AmoA subunit, can be used as a functional marker for both groups, as well as for detection and quantification of ammonia oxidizers in environmental studies (Rotthauwe et al., 1997; Leininger et al., 2006; Francis et al., 2005). Further, it provides a higher resolution in phylogenetic analysis compared to the highly conserved 16S rRNA gene (Kowalchuk and Stephen, 2001; Oton et al., 2015). Because the archaeal and the bacterial *amo*A genes are only very distantly related homologs (Venter et al., 2004; Treusch et al., 2005) this marker gene can solely be used for each of the two groups separately.

Bacterial ammonia oxidizers (AOB) have been known for more than a century (Winogradsky, 1890) and it is generally accepted that all AOB are associated with the phylum Proteobacteria. In comparison to their archaeal counterparts, they are better characterized and so far it is suggested that lineages affiliated with Betaproteobacteria are prevalent within the terrestrial AOB (Norton, 2011). In contrast, it was only recently discovered that members of the domain Archaea are capable to carry out the first step of nitrification in moderate environments (Könneke et al., 2005; Treusch et al., 2005), which had a major impact on the persisting view of the N cycle (Francis et al., 2007) but their contribution in particular in soils still has to be elucidated (Schleper, 2010). Due to numerous unique characteristics, all ammonia oxidizing archaea (AOA) are

placed within the new phylum Thaumarchaeota (Brochier-Armanet et al., 2008; Spang et al., 2010). However, this phylum is highly diverse and the knowledge about the physiology, ecology and phylogeny of Thaumarchaeota is under constant progress, driven by a fast growing amount of information gathered from genomic, cultivation and environmental studies (e.g. Prosser & Nicol, 2012; Stahl & de la Torre, 2012; Stieglmeier et al., 2014). The rapid increase of sequences affiliated to this phylum, brings along the necessity of a phylogenetic system to elucidate relationships and ecological co-occurrences (e.g. Gubry-Rangin et al., 2011; Pester et al., 2012).
In 2012 Pester and colleagues distinguished five major clusters based on *amo*A gene phylogeny. Organisms associated with these clusters were not restricted to certain habitats, but some clusters seemed to be higher represented in particular environments (Pester et al., 2012). For instance, the *Nitrosopumilus* cluster, also referred to as lineage 1.1a, is highly distributed in marine and freshwater systems, whereas the *Nitrososphaera* and the *Nitrososphaera*-sister clusters, are commonly found in a wide range of soils, the latter are combined in this work as group 1.1b or soil group (Pester et al., 2012; Stieglmeier et al., 2014 and references therein).

Functional differences between archaeal and bacterial ammonia oxidizers

In many ecosystems a co-occurrence of AOA and AOB is found, hence the obvious question of niche specialisation (i.e. different adaptation to environmental soil properties) and niche differentiation (i.e. differences in the utilization of resources) within the ammonia oxidizer guild emerges (Erguder et al., 2009; Prosser & Nicol, 2012; Zhalnina et al., 2012 and references therein).
Various adaptations and capabilities of ammonia oxidizing archaea and bacteria determine the responses of these organisms to changing conditions and consequently affect the functional inventory of soils. Abundances and community structure of ammonia oxidizers depend on a wide set of factors, including temperature, NH_4^+ availability, and moisture, respectively (e.g. Leininger et al., 2006; Eruder et al., 2009; Zhalnina et al., 2012).

The adaptation to nutrient deficiency has been attributed to AOA rather than to AOB and might be an important criterion in the N-limited Arctic soils. The archaeal marine strain *Nitrosopumilus maritimus* exhibited a higher affinity to its substrate than bacterial ammonia oxidizers (Martens-Habbena et al., 2009). In addition, a preference for growth under low NH_3 concentrations was observed for several members of AOA including a moderate thermophilic member of Thaumarchaeota from a hot spring (Hatzenpichler et al., 2008) and an archaeal ammonia oxidizer inhabiting an acidic agricultural soil

(Lehtovirta-Morley et al., 2011). Additionally, low concentrations of NH_4^+ in the environment seem to select for AOA (Hatzenpichler, 2012). Complementary, different studies provide evidence that AOA are sensitive towards increased nutrient concentrations whereas AOB show a high tolerance (Prosser & Nicol, 2012 and references therein). In combination with their abundances in several oligotrophic environments and unfertilized, pristine soils (e.g. Auget et al., 2011; Leininger et al., 2006) a more efficient substrate utilization is proposed for AOA than AOB (Hatzenpichler, 2012). However, Prosser and Nicol (2012) state, that no clear evidence for this hypothesis is present, which is indicated by studies who found a preference towards higher NH_3 levels in AOA originating from soil (e.g. Tourna et al., 2011; Verhamme et al., 2011).

Temperature and moisture are key determinants for nitrification (Robertson & Groffman, 2007). The temperature optimum for nitrification is highly dependent on the environment (Norton & Stark, 2011). Archaeal *amo*A genes have been found abundantly in cold Arctic ecosystems (Kalanetra et al., 2009; Yergeau et al., 2010) and also in high temperature habitats up to 97 °C (Reigstad et al., 2008). Therefore AOA seem to be adapted to a wide temperature range, whereas bacterial ammonia oxidizers have not been found in habitats with temperatures constantly higher than 40 °C (Hatzenpichler, 2012). However, these observations do not allow conclusions of the actual response of certain members of AOA and AOB, respectively. Studies investigating the implication of temperature on ammonia oxidizers are mostly focussing on several parameters, creating crossover effects which cannot be clearly separated. So far, microcosm studies revealed different observations. For example Tourna and colleagues (2008) present changes based on phylogenetic and functional genes and transcripts within AOA with increasing incubation temperatures but not within AOB. Whereas Avrahami and Bohannan (2007) showed, in a seven weeks incubation experiment focussed on the impact of temperature and fertilization, that total abundances of betaproteobacterial ammonia oxidizers increased with decreasing temperatures, however the lowest selected temperature was 20 °C, which represents the highest in this work. Additionally they observed an increase of AOB with rising soil moisture over a gradient from 30, 45 and 60 % WHC. A study investigating the effect of water and nitrogen amendment on ammonia oxidizers in forest soils noticed a preference of AOA to lower water content in one area whereas no effect on AOA and AOB was present in another soil with different characteristics (Szukics et al., 2011).

Besides controlling the availability of oxygen, soil moisture is also a determinant of substrate concentrations in soils, while in dry soils NH_3 is more likely to diminish by diffusion, this physical process is slower in wetter soils (Prosser, 2011).

Generally AOA and AOB are considered to be chemolithoautotrophs and therefore use NH_3 for energy production and CO_2 as C source (Sayavedra-Sota & Arp, 2011). Despite the fact that a study conducted by Schmidt (2009) showed that two members of AOB grew on several organic compounds under anoxic conditions, it is presumed that under the presence of oxygen AOB are not exclusively using organic compounds as source of energy (Sayavedra-Sota & Arp, 2011). Since not many representatives of AOA have been successfully brought to pure culture, information on thaumarchaeal growth is limited and much of the knowledge about archaeal ammonia oxidizers is based on genome analyses. However, in a pure culture of *Nitrososphaera viennensis,* a soil-borne member of AOA*,* enhanced growth was observed upon the addition of small amounts of pyruvate (Tourna et al., 2011). Therefore the possible assimilation of organic compounds and genetically encoded indications for several pathways do not exclude hetero- or mixotrophic growth of AOA (Prosser & Nicol, 2008).

Based on current observations from a broad field of studies, Prosser and Nicol (2012) suggest that both, AOA and AOB, are, with the exception of the prevalence of AOA in acidic soils, highly diverse regarding their physiologies and potential niches and they cannot be distinguished without further investigations. One step towards elucidating the complex interaction of ammonia oxidizers and environmental factors is the more careful identification of populations dominating certain habitats, instead of considering AOA and AOB as two uniform groups.

Through the analysis of different Arctic soils from Greenland, Svalbard and Siberia, Alves and colleagues (2013), found that within the high diversity of archaeal ammonia oxidizers detected in these habitats, distinct phylotypes dominated differently characterised soil types. Phylogenetic analysis placed the observed phylotypes into five major clades which were mainly affiliated within the soil-associated group 1.1b. The combination of quantitative and qualitative marker gene analysis and measurement of gross nitrification allowed them to relate habitat-specific nitrification rates to the different clades. Furthermore, the maintenance of enrichment cultures supported their hypothesis, that the distinct clades have different nitrifying capabilities. Altogether their comprehensive approach lead to the assumption that nitrification rates are highly dependent on the population structure of archaeal ammonia oxidizers. (Alves et al., 2013) These results emphasize, that for a better understanding of potential effects of changing conditions on biogeochemical processes a more detailed investigation of the microbial community is crucial.

Experimental setup and goal of this study

The major aim of this work was to investigate and characterize the ammonia oxidizer communities in cryoturbated, permafrost-affected soils of the North Siberian Lowland. Due to the vulnerability of this ecosystem to changing conditions, it is of high interest to elucidate the responses of this functional guild and its distinct groups to different temperatures and moisture levels.

The incubation experiments were carried out in the frame of the international project CryoCarb at the University of South Bohemia, Czech Republic by the following researchers: Hana Šantrůčková[1], Petr Čapek[1], Katka Diáková[1], Jiří Bárta[1], Iva Lacmanová-Kohoutová[1], Tomáš Picek[1], Jan-Erick Dickopp[2], Saida Azimova[1] with additional help from Ricardo J. Eloy Alves[3], Birgit Wild[3], Jörg Schnecker[3], Veronica Kongevold[4], Markéta Applová[1] from the [1]University of South Bohemia, [2]University of Ulm, [3]University of Vienna and [4] University of Bergen, respectively.

Soil samples were collected on the Taymyr peninsula, Russia in summer 2011. The sampling area was classified as a shrubby moss tundra with a fine to coarse loamy underground affected by continuous permafrost. The depth of the active layer varied between 50 to 70 cm. The soil was carefully separated into three different layers: the organic topsoil, which was derived from an OA horizon (O horizon), the mineral subsoil (BCg horizon) and the subducted topsoil (Ajj horizon), also referred to as cryoturbation pockets. The soil material was stored at 4 °C and homogenized before the start of the incubation experiment. The soil samples of the three horizons were incubated separately in specially modified 1000 ml flasks.. 50 g of the organic horizons and 75 g of the mineral horizons, respectively, were placed into polyamide nets on top of a layer of moist sand to ensure stable moisture conditions of the headspace and soil. Modifications included e.g. valves for gas sampling and oxygen sensors to monitor O_2 concentrations of the airtight bottles. Subsampling was facilitated by three additional polyamide net constructions, filled with soil, within the sample. Each horizon was incubated with a different combination of three temperatures (4 °, 12 ° and 20 °C) and three moisture levels (50 %, 80 % and 100 % WHC) for 18 weeks. Samples incubated with 50 % and 80 % WHC represented oxic treatments with ambient air in the headspace and samples incubated with 100 % WHC were considered as anoxic treatments with a mixture of He and CO_2 in the headspace.

Each variation of horizon, temperature and moisture content was replicated four times. In total a number of 108 samples were incubated. Additionally, soil from the three horizons was processed and analysed without a preceding incubation to get information about the initial soils. In the frame of the incubation experiment a broad

range of measurements and experiments were performed including the determination of physical and chemical soil properties, gas measurements (CO_2, CH_4, N_2O), the determination of microbial biomass by fumigation-extraction and enzyme activities as well as the extraction of DNA for analysis of bacteria, archaea and fungi and functional groups. For further details on the experiment and additional data see Čapek et al., 2015.

The theoretical background of this incubation experiment and the selection of different treatments relies on changes in environmental conditions introduced either by the impact of climate change or by the process of cryoturbation. The topsoil (O horizon) is usually dry or slightly moist and well aerated with maximum temperatures of 10 to 15 °C during growing season. In contrast, the subducted topsoil (Ajj) and the mineral subsoil (BCg) are located in deeper soil layers where conditions are dominated by low temperatures in the range of 4 to 5 °C and high soil water content up to water-saturation. If air temperature increases, the surface soil gets heated up and increased precipitation leads to elevated water content. However, the topsoil might also get buried and is then exposed to a colder and wetter environment. Additionally, temperature, moisture content and oxygen availability might change in the lower horizons due to the thaw of the permafrost and will thus affect the subsoil. Another transaction could shift deeper soil layers upwards, where they experience common surface conditions.

The following questions were tackled in this study:
What are the absolute and relative abundances of AOA and AOB in the different soil horizons and how do these vary with respect to changes in temperature and moisture?
Can different phylogenetic clades of AOA be dissected and do they respond specifically to environmental changes?
And furthermore, can ammonia oxidizer populations be related to gross N mineralization rates, in the context of changing conditions?

In order to answer these questions quantitative polymerase chain reaction, archaeal *amo*A amplicon sequencing and determination of gross N mineralization rates from the incubation experiment were performed and analysed.

2 Materials and Methods

All experiments in this work are based on an incubation experiment carried out by researchers of the Cryocarb consortium and co-helpers (see introduction).
Soil material from three different soil horizons, organic topsoil (O horizon), subducted topsoil (Ajj horizon, cryoturbation pockets) and mineral subsoil (BCg) were incubated with different treatments to simulate a change of environmental conditions. Each treatment was represented by a different combination of temperature (4 ,12 or 20 °C) and moisture content (50, 80 or 100 % WHC), leading to a total of 27 different endpoint samples and three initial soil samples, which were replicated four times. Nucleic acid extracts from the soil before incubation (startpoint), the endpoint samples of all three horizons and all treatments including intermediate timepoint subsamples from the O horizon and the Ajj horizon incubated at 4 °C and 12 °C, were performed at the University of South Bohemia in České Budějovice according to an optimized phenol:chloroform-based protocol (Urich et al., 2008; Tveit et al., 2012). In this work, extracts from two replicates of the initial soil and each treatment were used for further experiments.

2.1 Nucleic acid extraction

Additionally to the extracts of two replicates, which were obtained from the University of South Bohemia, a third replicate extraction from the topsoil horizon (O horizon) samples was performed in the frame of this study at the University of Vienna following the same protocol.

Nucleic acids were extracted from 0.28 to 0.31 g soil. The frozen soil, stored at -80 °C, was transferred to Lysing Matrix E tubes (MP Biomedicals, USA). After the addition of 0.5 ml 5% CTAB/phosphate extraction buffer (120 mM, pH 8), and 0.5 ml phenol/chloroform/isoamyl alcohol (25:24:1, pH 8) the tubes were placed into the FastPrep®-24 machine (MP Biomedicals, USA) for 30 seconds at 5.5 m s^{-1}. Subsequently samples were centrifuged for 10 min at 4 °C and 13.2 rpm. The supernatant (SN) was transferred into a new tube and an equal volume of chloroform/isoamyl alcohol (24:1) was added, followed by another centrifugation step and the transferral of the SN into a new tube. 1 µl of glycogen was added to improve precipitation followed by two volumes of PEG 6000 Solution. After 1 h of incubation on ice, the samples were centrifuged at 4 °C, 13.2 rpm for 70 min. The SN was removed and the pellet was washed with 0.5 ml cold EtOH (70 %). After 10 min centrifugation,

the EtOH was removed and the pellet was dried in the vacuum concentrator (vaccuum concentrator plus, Eppendorf AG, Germany) at 30 °C for 5 min. The pellet was then resuspended in 50 µl RNase-free H_2O. Samples were stored at -80°C.

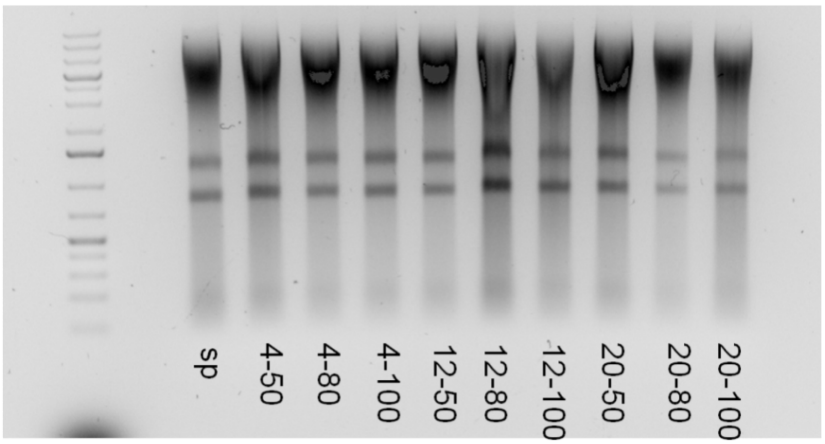

Fig. 1: Results of the nucleic acid extraction of the third replicates of the start- and endpoint of the O-horizon samples checked on an 1.5 % agarose gel. Input: 3 µl of extract, GeneRuler™ 1 kb Plus DNA ladder (Thermo Scientific, USA) as reference. sp: initial soil sample (startpoint), endpoint samples of the incubation sorted by temperature (4, 12, 20 °C) and WHC (50, 80,100 %).

The extraction quality was assessed by gel electrophoresis (Fig.1). Nucleic acid concentrations, determined spectrophotometrically (NanoDrop® ND-1000, Peqlab Biotechnologie GmbH, Germany), were within the range of 302.7 to 472.0 ng μl^{-1}. For all concentrations see (Tab. 1)

Working dilutions with a nucleic acid content of 20 ng μl^{-1} were prepared from all duplicated and triplicated extracts from the O horizon samples. Ajj horizon samples had a low yield of nucleic acids, the nucleic acid input in the quantitative PCR reactions varied and were later considered during calculations (Tab.1).

Tab. 1: Results of the nucleic acid extraction from the initial soil (startpoint) and the different treatments after incubation (°C, % WHC). Replicate 1-2 were extracted at the University of South Bohemia, extraction of replicate 3 was performed at the University of Vienna.

Horizon	Treatment		Replicate	DNA content [ng μl^{-1}]	Total amount of nucleic acids [µg]
	Temperature [°C]	Water content [% WHC]			
O	Startpoint		1	218.3	11.92
			2	218.5	10.92
			3	362.4	18.12
O	4	50	1	478.6	23.93
			2	294.4	14.72
			3	412.5	20.62
O	12	50	1	571.3	28.57
			2	383.4	19.17
			3	362.1	18.10
O	20	50	1	209.8	10.49
			2	349.8	17.49
			3	472.0	23.60
O	4	80	1	508.4	25.42
			2	346.2	17.31
			3	356.6	17.83
O	12	80	1	362.0	18.10
			2	250.0	12.50
			3	439.8	21.99
O	20	80	1	224.0	11.20
			2	293.8	14.69
			3	325.7	16.29
O	4	100	1	616.7	30.83
			2	510.7	25.54
			3	350.7	17.54
O	12	100	1	430.3	21.52
			2	736.1	36.81
			3	382.6	19.13
O	20	100	1	222.1	11.10
			2	300.3	15.02
			3	325.7	16.29
Ajj	Startpoint		1	0.6	0.03
			2	0.6	0.01
Ajj	4	50	1	0.6	0.03
			2	0.5	0.03
Ajj	12	50	1	1.5	0.08
			2	1.8	0.09
Ajj	20	50	1	4.2	0.21
			2	5.1	0.25
Ajj	4	80	1	0.6	0.03
			2	2.0	0.10
Ajj	12	80	1	0.7	0.05
			2	1.2	0.06
Ajj	20	80	1	25.1	1.26
			2	4.3	0.21
Ajj	4	100	1	2.3	0.12
			2	3.2	0.16
Ajj	12	100	1	6.3	0.32
			2	1.5	0.07
Ajj	20	100	1	10.7	0.53
			2	11.10	0.56

2.2 Primer testing and screening of the soil samples

A first screening for the presence of AOA in the incubation samples was performed. The amplification had to be optimized primarily for low DNA concentrations and inhibiting substances in the Ajj horizon and secondly to sustain the diversity of *amo*A genes in the samples. Therefore, different compositions of reaction mixtures and cycling conditions, as well as a variety of primers were tested (Tab. 2).

Tab. 2: List of primers tested for amplification of archaeal *amo*A genes.

	Sequence (5'-3')	Reference
Forward		
TamoA-1F-3	ATGGTCTGGBTWAGAMG	Alves et al., unpublished
TamoA-1F-4	ATGGTCTGGYTWAGAMG	Alves et al., unpublished
Arch-amoA-7F1	GGG GTTTCTACTGGTGGT	Alves et al., 2013
Arch-amoA-7F	ATGGTCTGGBTDAGAMG	Alves et al., 2013
amoA-104Fmod	CAGGAGACTAYATHTTCTA	Alves et al., 2013
Cren-amoA-19F	ATGGTCTGGYTWAGACG	Modified from Leininger et al., 2006
Reverse		
TamoA-632R-4	GCKGCCATCCATCKRTANGTCCA	Alves et al., unpublished
Cren-amoA-616R	GCCATCCABCKRTANGTCCA	Nicol et al., 2008
Arch-amoA-638R	GCRGCCATCCATCTRTA	Alves et al., 2013

A first screening of the O horizon start- and endpoint samples for archaeal *amo*A genes was carried out with TamoA-1F-4 (Alves et al.; unpublished) and TamoA-632R-4 primers (Alves et al.; unpublished). 25 µl reactions were prepared with 40 ng of DNA, 0.125 µl DreamTaq DNA polymerase (5 u μl^{-1}, Promega, USA), 5 µl Green GoTaq® Flexi Reaction buffer (5 X, Promega), 2 µl $MgCl_2$ (25 mM, Promega, USA), 0.5 µl BSA (10 mg ml^{-1}, Thermo Scientific, USA), 0.2 µl dNTPS (25 mM, Thermo Scientific, USA), 2.5 µl forward and reverse primers (final conc. 1 µM) and nuclease free water under the following cycling conditions:

initial denaturing	5	min	95 °C
35 cycles			
denaturing	30	sec	95 °C
annealing	45	sec	55 °C
extension	45	sec	72 °C
final extension	10	min	72 °C

2.3 Quantitative Polymerase Chain Reaction (qPCR)

Archaeal and bacterial *amo*A genes were quantified in the O and Ajj horizon samples. The main focus was on the initial samples and endpoint samples of every treatment. Subsamples of intermediate sampling points were only analysed from the organic topsoil samples. The mineral subsoils were excluded due to the low DNA content. The quantification was carried out on a Thermocycler (Mastercycler® RealPlex^2, Eppendorf AG, Germany).

2.3.1 Preparation of standards

Standards for qPCR were prepared with primers that amplified the full *amo*A region in the genomes of *Nitrososphaera viennensis* for AOA and *Nitrospira multiformis* for AOB respectively. (Tab. 3)

Tab. 3: List of primers used for qPCR standard preparation.

	Sequence (5'-3')	Reference
N. viennensis		
NV-LamoA-F	CGCATGATCGGCCGCAGAGT	Alves et al., unpublished
NV-LamoA-R	GCCTAGTAGCGACCCGCCCT	Alves et al., unpublished
N. multiformis		
NM-amoAB-F	GAGGGAGGGTGCGATGAGCAGA	Alves et al., unpublished
NM-amoAB-R	ACGTTGAAGAAGGCGCGTCCAG	Alves et al., unpublished

Genomic DNA extracted from *N. viennensis* and *N. multiformis* was used as a template in 50 µl reactions consisting of 0.25 µl DreamTaq Polymerase (5 u µl^{-1}), 10 µl Green GoTaq® Flexi Reaction buffer (5 X), 4 µl $MgCl_2$ (25 mM), 0.4 µl dNTPs (25 mM), 0.5 µl BSA (20 mg ml^{-1}), 2.5 µl forward and reverse primers (final conc. 1 µM) and nuclease free water under following conditions:

initial denaturing	5	min	95 °C
30 cycles			
denaturing	45	sec	94 °C
annealing	45	sec	56 °C
extension	2	min	72 °C
final extension	10	min	72 °C

PCR products were purified with the MinElute PCR Purification Kit (Qiagen, Netherlands). A final nucleic acid concentration of 299.41 ng μl^{-1} for AOA and 358.7 ng μl^{-1} for AOB were determined spectrophotometrically (NanoDrop® ND-1000), working stocks with the concentration of 0.5 x 10^9 copies μl^{-1} were prepared.

2.3.2 Quantitative PCR

For quantification Cren-amoA-19F and TamoA-632R-4 primers were used for AOA, and amoA-1F and amoA-2R for AOB, respectively (references see Tab. 4). Archaeal primers were selected based on the results of the previous primer tests and the targeting of a quite conserved region. 20 µl reactions with 2 µl DNA template with 40 ng DNA from the O horizon samples and a varying amount of DNA concentration of the Ajj horizon samples, 10 µl qPCR Mastermix (2 X, Promega, USA), 0.2 µl BSA (20 mg ml^{-1}), 2 µl forward and reverse primers (final conc. 1 µM) and nuclease free water were prepared. A dilution series of eight standards was used to cover a range of 10^1 to 10^8 copies μl^{-1}. Quantification was carried out under the following cycling conditions:

AOA				AOB			
10	min	95 °C		10	min	95 °C	
15	sec	95 °C		15	sec	95 °C	
45	sec	58 °C		45	sec	60 °C	40 cycles
45	sec	60 °C	40 cycles	10	sec	82 °C	
10	sec	78 °C		15	sec	95 °C	
15	sec	95 °C		15	sec	60 °C	↓20 min
15	sec	60 °C	↓20 min	15	sec	95 °C	
15	sec	95 °C		10	min	60 °C	
10	min	60 °C					

Tab. 4: List of primers used for quantification of AOA and AOB.

	Sequence (5'-3')	Reference
AOA		
Cren-amoA-19F	ATGGTCTGGYTWAGACG	Modified from Leininger et al., 2006
TamoA-632R-4	GCKGCCATCCATCKRTANGTCCA	Alves et al., unpublished
AOB		
AmoA-1F	GGG GTT TCT ACT GGT GGT	Rotthauwe et al., 1997
AmoA-2R	CCC CTC KGS AAA GCC TTC TTC	Rotthauwe et al., 1997

For qPCR analysis and standard curve parameters see Tab. 55. All PCR products were checked for specific gene amplification on a 1.5 % agarose gel run at 160 V for 30 min.

Tab. 5: Analysis and standard curve parameters from the different quantification runs on the Mastercycler® RealPlex^2 (Eppendorf AG, Germany).

group	horizon	qPCR run	Threshold level	Standard curve parameters			
				slope	Y-Intercept	Efficiency	R^2
AOA	O	Start- and Endpoint	100	-3.514	36.10	0.93	0.993
AOA	O	Intermediate sampling point 4 °C	100	-3.468	37.18	0.94	0.993
AOA	O	Intermediate sampling point 12 °C	100	-3.678	39.02	0.87	0.993
AOA	O	Triplicate extraction Start- and Endpoint	100	-3.375	36.11	0.98	0.996
AOA	Ajj	Start- and Endpoint	100	-3.511	37.16	0.93	0.989
AOB	O	Start- and Endpoint	100	-3.673	36.44	0.87	0.998
AOB	O	Intermediate sampling point 4 °C	100	-3.734	36.99	0.85	0.997
AOB	O	Intermediate sampling point 12 °C	100	-3.712	36.87	0.86	0.994
AOB	O	Triplicate extraction Start- and Endpoint	100	-3.620	35.94	0.89	0.998
AOB	Ajj	Start- and Endpoint	100	-3.802	35.94	0.93	0.993

2.4 Sequencing

The diversity of AOA in the topsoil samples was analysed with an Ion Torrent Personal Genome Machine™ (Life Technologies, USA) aiming for archaeal *amo*A genes in the start- and endpoint samples of every treatment. A first test run indicated that the amplicon length exceeded the optimum, consequently shorter fragments had to be generated by shearing. Library preparation and sequencing was carried out with the help of Romana Bittner and helpful advice from Clarissa Schwab.

2.4.1 Preparation of amplicons

Archaeal *amo*A genes amplicons were generated from one replicate of the endpoint sample of every treatment and the initial organic topsoil sample. 50 µl reactions were prepared, containing 3 µl with a total of 60 ng of template DNA, 0.25 µl DreamTag® DNA Polymerase (5 u μl^{-1}), 10 µl of Green GoTaq® Flexi Reaction Buffer (5X), 4 µl $MgCl_2$ (25 mM), 0.4 µl dNTPs (25 mM), 0.5 µl BSA (20 mg ml^{-1}), 2.5 µl of primers TamoA-1F-4 and TamoA-632-R4 (final conc. 1 µM, references see Tab. 2) and nuclease free water. Every reaction was quadruplicated. Amplification was carried out under the following cycling conditions:

initial denaturing	5	min	95 °C
30 cycles			
denaturing	30	sec	95 °C
annealing	45	sec	55 °C
extension	45	sec	72 °C
final extension	10	min	72 °C

The PCR products were checked on an 1.5 % Agarose gel with 5 µl PCR product input, run at 120 V for 60 min.

The PCR products of the four replicates were pooled to get a total of ten samples. Subsequently the pools were purified with the MinElute PCR purification kit (Qiagen, USA), according to the manufacturers protocol. Samples were eluted with 11 µl of 70 °C warm nuclease free water, incubated for 2 min. DNA concentrations were determined on the NanoDrop® ND-1000 (Peqlab Biotechnologie GmbH, Germany) (Tab. 6). Working solutions of 10 µl with a total nucleic acid content of 100 ng were prepared.

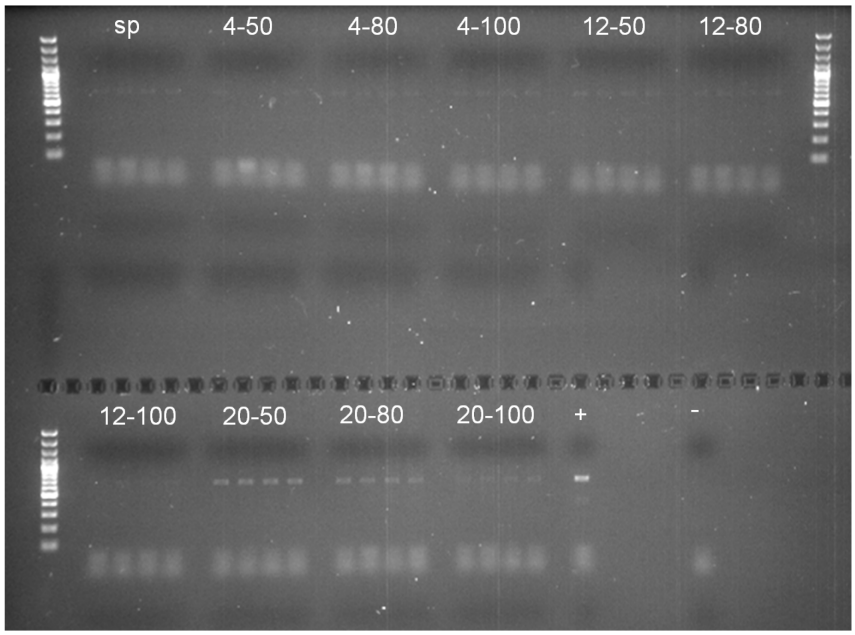

Fig. 2: Results of the archaeal *amo*A gene amplification for generating amplicons for sequencing. Each sample was quadruplicated and later combined for sequencing. sp: startpoint, endpoint samples are indicated by their different treatments (°C, % WHC) + : positive control, - : negative control, GeneRuler™ 100 bp Plus DNA ladder for reference.

Tab. 6: Final concentrations of archaeal *amo*A gene amplicons determined spectrophotometrically after purification on the NanoDrop® ND-1000.

Horizon	Treatment		concentration	260/280	260/230
	Temperature [°C]	Water content [% WHC]	[ng µl^{-1}]		
O	Startpoint		15.61	1.42	1.47
O	4	50	14.61	1.52	1.35
O	4	80	17.62	1.54	1.37
O	4	100	13.61	1.34	1.55
O	12	50	19.93	1.49	1.44
O	12	80	19.09	1.39	1.32
O	12	100	17.26	1.36	1.33
O	20	50	25.05	1.68	1.74
O	20	80	24.17	1.53	1.76
O	20	100	17.04	1.46	1.59

2.4.2 Shearing tests

The length of the *amo*A gene amplicons had to be optimized, do decrease the original fragment size down to approximately 200 to 250 bp. Therefore different methods of

enzymatic and mechanical shearing were tested. For all tests *amo*A genes were amplified from the same soil samples, containing different representatives of AOA. Amplicons were prepared as already described in section 2.4.1 with a modified extension time of 2 min and 35 cycles.

Mechanical shearing

Amplified *amo*A genes were fragmented on the Covaris® S2 (Covaris Inc, USA). Several tests were carried out with the settings according to the manufacturers protocol to reach a target size of 250 bp, but different variations of sonication duration (100 - 180 sec) and DNA input (100 - 800 ng) were tested. The results of mechanical shearing were assessed with an 1.5 % Agarose gel (Fig. 3). This method did not seem to be suitable for processing amplicons from the soil samples due to the broad range of obtained fragment sizes and the low amount of template available for sequencing.

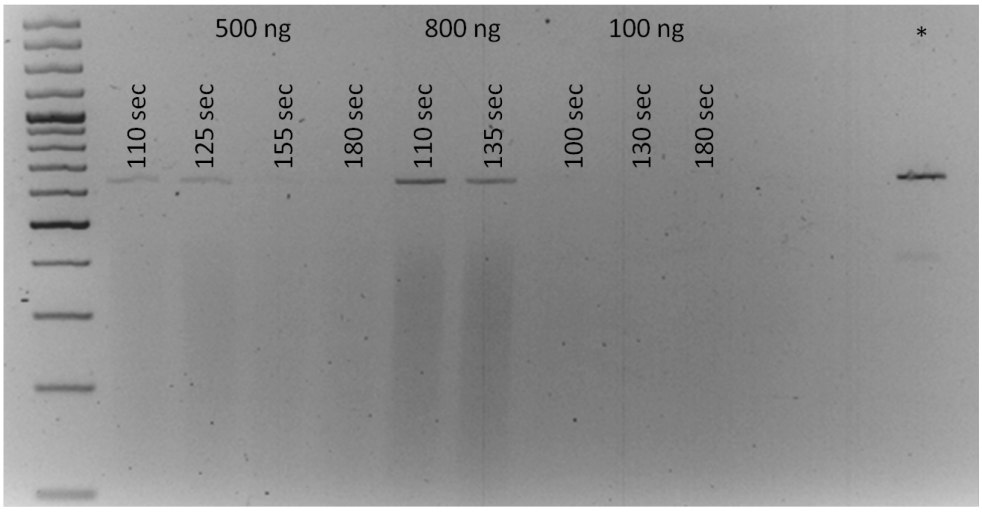

Fig. 3: Results of the *amo*A amplicon shearing test through mechanical shearing. Different quantities of DNA (100 - 800 ng) and different shearing durations (100 - 180 sec) were applied. GeneRuler™ 100 bp Plus DNA ladder for reference, * indicates 200 ng of unsheared PCR product

Enzymatic shearing

Two different approaches of enzymatic fragmentation were tested using the specific enzyme HpyF10 VI (Thermo Scientific, USA), and a mixture of enzymes (Ion shear™ plus enzyme mix, Life Technologies, USA), respectively.

Restriction digest

The restriction digest was performed with the enzyme HpyF10 VI (Thermo Scientific, USA). The selection of this enzyme was based on preceding *in silico* analysis which included simulations in TRiFLe (Junier et al, 2008) to cover the high diversity of archaeal *amo*A genes.

Two different restriction reactions were tested. Half of the PCR products were purified with the QiaQuick Kit (Qiagen, Netherlands) according to the manufacturers protocol before further processing. A second reaction was set up with unpurified PCR products. Both restriction digests were carried out as described in the provided, enzyme specific protocol. Gel electrophoresis showed similar results of the different set ups. *Amo*A gene amplicons were much higher concentrated in the purified sample. Besides the requested fragment size, many smaller fragments appeared. (Fig. 4) Therefore this method was excluded.

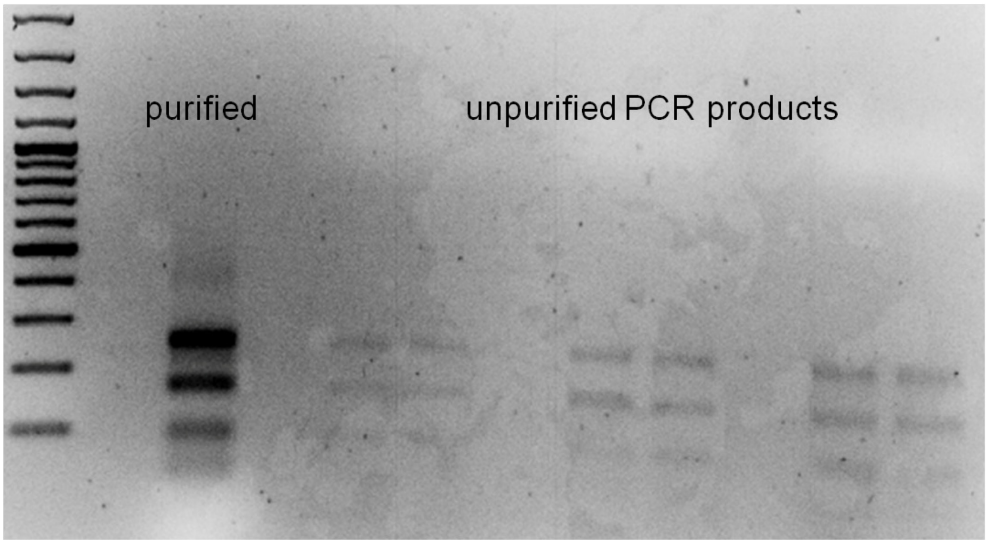

Fig. 4: Results of the *amo*A amplicon restriction digest using the specific enzyme HpyF10 VI. Results from restriction of purified PCR products (left) and restriction of unpurified samples (right) run on an 2 % agarose gel, for 20 min at 160 V followed by 1 h 30 min at 100 V. GeneRuler™ 100 bp Plus DNA ladder for reference.

Ion shear kit

Before shearing with the Ion shear™ plus enzyme mix, PCR products were purified with the QiaQuick Kit. 20 µl reactions were prepared according to the Ion Xpress™ gDNA and Amplicon Library Preparation User Guide, consisting of 4 µl Ion shear™ plus enzyme mix, 2 µl Ion Shear™ Plus 10 X Reaction Buffer, 2 µl template DNA (100 ng), 12 µl nuclease free water were prepared. Different incubation durations were tested in the range from 5 min and 50 min. After incubation at 37 °C, 2 µl Ion shear™ Plus Stop Buffer was added, subsequently the samples were purified with the MinElute kit and eluted in 25 µl water. Shearing results were asses on a 2 % agarose gel using a 50 bp marker (E-Gel 50 bp DNA Ladder, Invitrogen, USA) (Fig. 5). This method was chosen for the fragmentation of the amplicons prior to the library preparation.

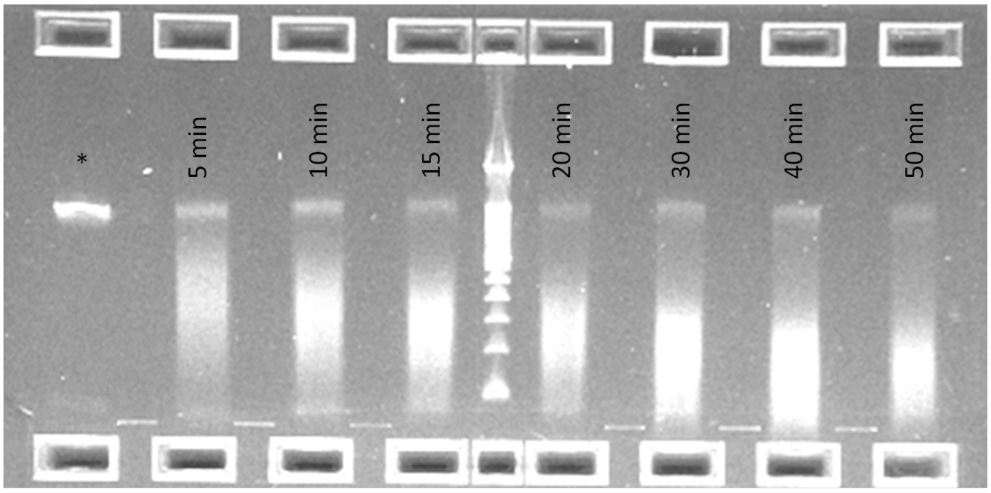

Fig. 5: Results of the *amo*A amplicon shearing test using the Ion shear™ plus enzyme mix. Incubation times in the range of 5 to 50 min were tested, products were checked on an 2 % agarose gel. * indicates unsheared PCR products. E-Gel 50 bp DNA Ladder *(*Invitrogen, USA) as reference.

2.4.3 Library preparation and Sequencing

In total ten barcoded archaeal *amo*A gene amplicon libraries were prepared from incubation samples according to the Ion Xpress Plus™ gDNA and Amplicon Library Preparation protocol for 200 base-read libraries.

Subsequently to amplicon preparation (see section 2.4.1) samples were sheared enzymatically with the Ion shear™ plus enzyme mix to obtain fragments with an average size of 200 to 300 bp as described in the manufacturers protocol. An incubation time of 11 minutes was selected based on previous tests. (see section 2.4.2) After a purification step with the MinElute Kit, samples were eluted in 25 µl H_2O. Adapter ligation and nick repair was carried out as described for short amplicons with Ion Xpress™ Barcodes followed by another purification step with the MinElute Kit. Samples were then stored at -20 °C over night. Size selection was carried out with an E-Gel® SizeSelect™ 2% Agarose Gel (Invitrogen, USA).

A 1:100 dilution of every library was prepared and then quantified with qPCR according to the KAPA Library Quantification Kits for Ion Torrent ™ platform (KAPA Biosystems, USA). The dilution factor was calculated as described in the manufacturer's protocol and samples were pooled to get an equal amount of template from each sample. Template-Positive Ion One Touch™ 200 Ion Sphere™ Particles were produced exactly following the Ion OneTouch™ 200 Template Kit v2 DL User Guide (Pub. no. MAN0007112 Rev. 5.0) and subsequently sequenced according to the Ion PGM™ Sequencing 200 Kit v2 User Guide (Pub. no. MAN0007273) with the Ion 314™ Chip for

200-base reads. Another run was performed with the same libraries following the Ion PGM™ Sequencing 300 Kit User Guide (Pub. no. MAN0007062).

2.4.4 *In silico* analysis

The phylogenetic assignment of archaeal *amo*A gene fragments, with different length, was evaluated *in silico*. Several subdatasets with 200 and 250 bp long fragments, respectively, were created from an aligned dataset of archaeal *amo*A gene sequences. Subsequently the datasets were clustered with different parameter settings with CD-HIT est (Weizhong & Godzik, 2006) and the structure as well as the assignment were compared to the original aligned dataset. Phylogenetic trees were visualized in Mega5 (Tamura et al., 2011).

2.4.5 Sequence processing

The reads obtained from the Ion Torrent PGM™ were analysed using the CREST pipeline (Lanzén et al., 2012). A database was constructed, consisting out of an alignment of 51 selected thaumarchaeal *amo*A gene sequences to cover the diversity of ammonia oxidizing archaea. Based on this database the nucleotide BLAST algorithm (Altschul et al., 1990) was used to compare the obtained reads of every sample individually to known AOA sequences (parameters –e 0.1 –v 50 –b 50 &). The data was further analysed with MEGAN 4 (Huson et al., 2011) using an alternative taxonomy obtained by Tim Urich. The alternative taxonomy included 590 nt long archaeal *amo*A gene sequences and covered members of group 1.1a, group 1.1a-associated, group 1.1b and group ThAOA. Several clades and subclades were defined for members of this groups, clades A-D and clade Nitrososphaera are based on results published in Alves et al., 2013 (Fig. 6).

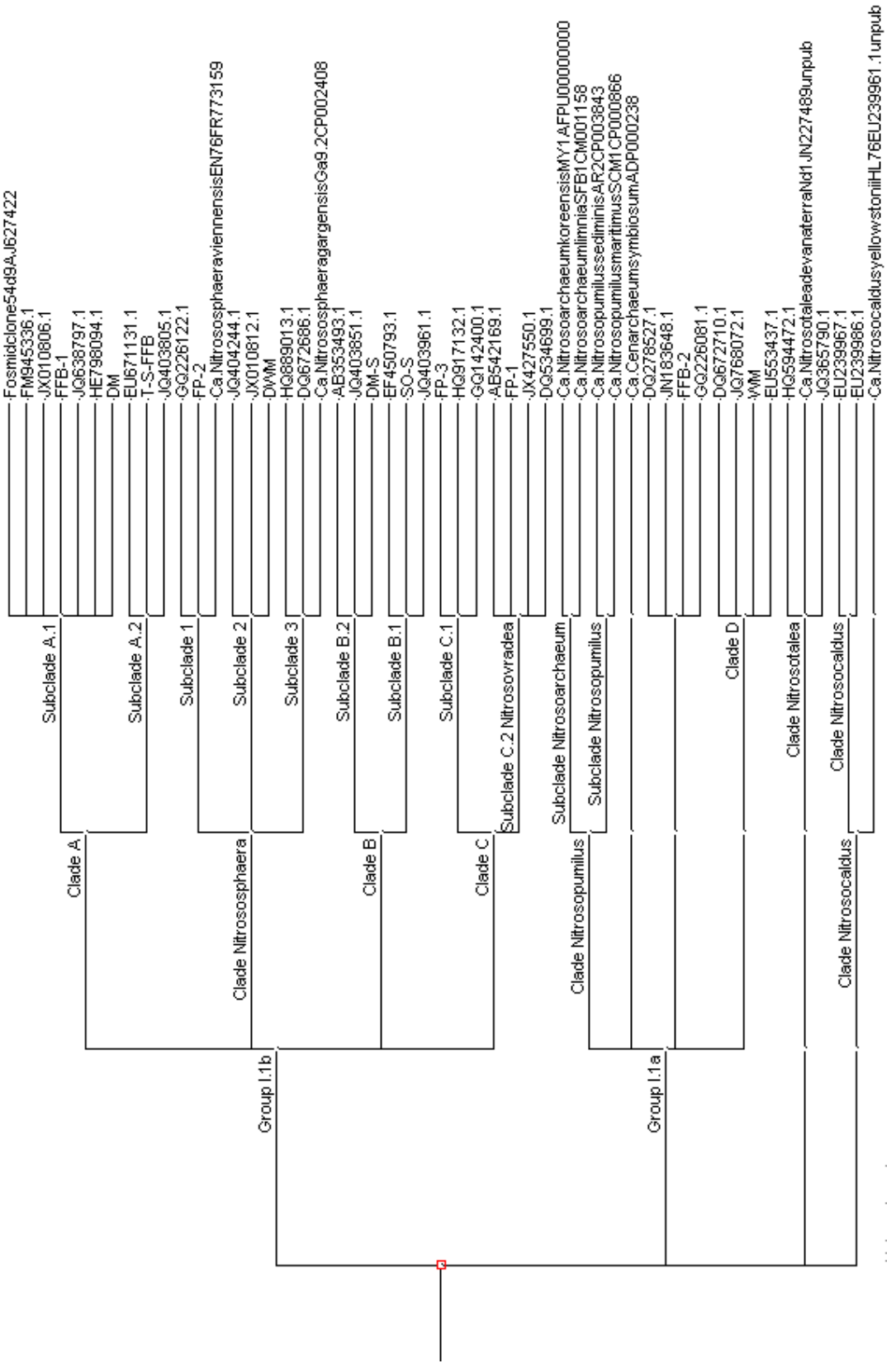

Fig. 6: Schematic overview of sequences used for the in-house taxonomy for sequence data analysis. Classification of the sequences to clade A-D and clade *Nitrososphaera* are based on Alves et al., 2013.

2.5 Nitrogen mineralization

Gross nitrogen mineralization and NH_4^+ consumption rates were determined with a $^{15}NH_4^+$ pool dilution assay, as described in Kaiser et al., 2007. The assay was performed with soils collected at the end of the main incubation experiment, for all treatments and soil horizons, all samples were four times replicated. For the mineralization approach, 500 µl of $(^{15}NH_4)_2SO_4$ (0.125 mM, 10 atm%) was added to 2 g of each soil sample. These soils were incubated for 4 h to determine the start point and for 24 h to determine the end point, respectively. The incubation was stopped by adding 15 ml of KCl (2 M). After shaking the samples for 1 h at RT, samples were filtered. After the addition of 20 µl of phenylmercuric acetate (5 mM) the samples were stored at -20°C. These steps were carried out by Birgit Wild and Jörg Schnecker.

Thawed extracts were centrifuged for 5 min at 5.000 rpm. The supernatants were transferred to scintillations vials, followed by the addition of 100 mg MgO in order to convert the NH_4^+ pool to NH_3 gas which was subsequently collected by diffusion in acid traps placed inside the vials during incubation on a shaker (75 rpm) at RT for 5 days. The acid traps consisted of two discs of ash-free filter paper, each containing 5 µl of $KHSO_4$ (2.5 M), which were then enclosed in Teflon tape in orderto avoid contact between liquid sample and the discs.
Subsequently, the acid traps were collected and dried in a glass desiccator with H_2SO_4. (conc.). The dried filter discs were removed from the Teflon tape, placed inside zinc capsules and closed tightly for analysis with an Elemental Analyzer coupled to an Isotope Ratio Mass Spectrometer (EA-IRMS; DeltaPLUS, Finnigan MAT, Germany).

Gross N mineralization, gross NH_4^+ consumption and net N mineralization rates were calculated as described in Kaiser et al., 2011 using the following equations:

$$gm = (A_t - A_0) / t \times (\ln [APE_0 / APE_t] / \ln [A_t / A_0])$$
$$gcm = gm - (A_t - A_0) / t$$
$$nm = (A_t - A_0) / t$$

The gross N mineralization, or gross NH_4^+ production rates (gm), and the gross NH_4^+ consumption were determined based on the dilution of the $^{15}NH_4^+$ pool and on the difference between the N-NH_4^+pool size after 24 h (A_t) and after 4 h of incubation (A_0).

The dilution of the ^{15}N pool is determined by differences between the atom percent excess (APE) of ^{15}N at the start- and endpoint. APE_0 and APE_t are calculated by subtracting the natural abundance of ^{15}N from an unlabelled control from the abundance of ^{15}N in the sample after 4 h incubation and the abundance of ^{15}N after 24 h incubation, respectively. Variations in the APE_0:APE_t ratio determine the balance of NH_4^+ mineralization and NH_4^+ consumption, since both processes occur simultaneously. The actual rates of gross NH_4^+ consumption were then calculated by subtracting the difference between the N-NH_4^+ pools at the start- and endpoints from the gross mineralization rate.

The net N mineralization rate (nm) is the difference between gross N mineralization and NH_4^+ consumption rates, and therefore positive net mineralization occurs when NH_4^+ release exceeds the NH_4^+consumption.

In contrast to net N mineralization rates, gross N mineralization rates and gross NH_4^+ consumption rates cannot be negative. Negative gross rate values result from an unsuccessful pool dilution assay, which can be due to insufficient dilution of the ^{15}N-labelled NH_4^+ pool. Therefore, all negative gross rate values were excluded from further data analysis.

3 Results

3.1 Presence of archaeal *amo*A genes in the incubated samples

A first screening of the topsoil samples was performed using primers TamoA-1F-4 (Alves et al., unpublished) and TamoA-632R-4 (Alves et al., unpublished) in PCR for amplification of archaeal *amo*A genes. This confirmed the purity of the isolated DNA and the presence of ammonia oxidizing archaea (AOA) in the initial sample (sp) and at the end of the incubation of all treatments. Different quantities of archaeal *amo*A amplicons of the expected size of 630 bp (incl. primers) were obtained in this non-quantitative PCR using the same amount of template. (Fig. 7)

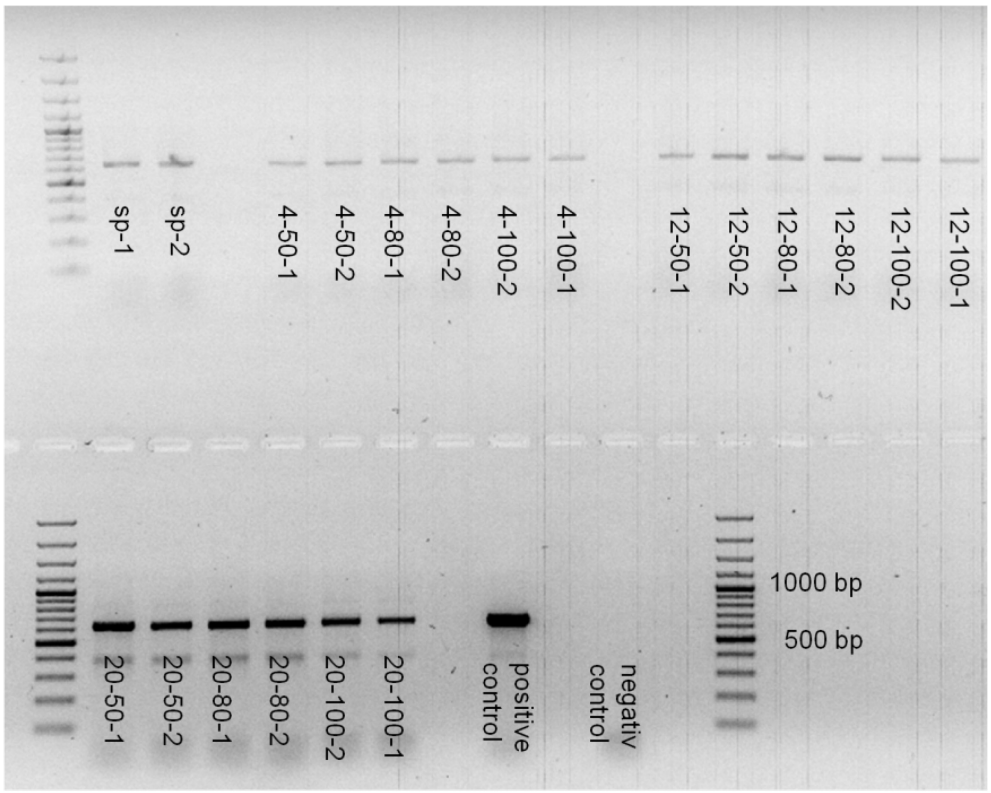

Fig. 7: Archaeal *amo*A gene PCR products obtained from the topsoil samples (O horizon). sp: start point, sample code of endpoint samples: temperature [4, 12, 20 °C], moisture content [50, 80, 100 % WHC], replicate number [1, 2]. 40 ng DNA input, 35 cycles, 5 µl PCR product loaded, GeneRuler™ 100 bp Plus DNA ladder (Thermo Scientific, USA) for reference.

3.2 Abundances of archaeal and bacterial *amo*A genes

Archaeal and bacterial *amo*A genes were quantified from the soil samples of the organic topsoil (O horizon) and the subducted topsoil (Ajj horizon, cryoturbation pockets) using quantitative PCR (qPCR) and primers Cren-amoA-19F and TamoA-632R-4 (modified from Leininger et al., 2006, Alves et al., unpublished) for

AOA and primers amoA-1F and amoA-2R (Rotthauwe et al., 1997) for AOB. A total of 40 samples were analysed, including duplicates for each incubation treatment. *Amo*A gene amplicons of *N. viennensis* and *N. multiformis* were used as standards for the archaeal and bacterial gene, respectively. AOA and AOB were detected in both soil horizons, however, in some samples archaeal *amo*A gene copy numbers were below the lowest amount of standard DNA used and therefore their quantification remained ambiguous and was not taken into account.

The qPCR analysis of start- and endpoint samples of the incubation revealed a dominance of AOA throughout all treatments in the organic topsoil, with abundances within the range of $4.8x10^5$ to $6.7x10^6$ copies g^{-1} dw soil. Bacterial *amo*A gene copy numbers varied from $3.0x10^4$ to $4.2x10^6$ copies g^{-1} dw. Regarding relative numbers, AOA were 24.5 times more abundant than AOB in the initial organic topsoil, whereas this ratio decreased during the incubation and in several endpoint samples archaeal and bacterial copy numbers were detected in similar amounts. Lowest AOA:AOB ratios were observed at 20 °C in the oxic treatments (50 % and 80 % WHC), emphasizing that bacterial *amo*A gene copy numbers increased to a great extent under these conditions. (Fig. 8, Tab. 7)

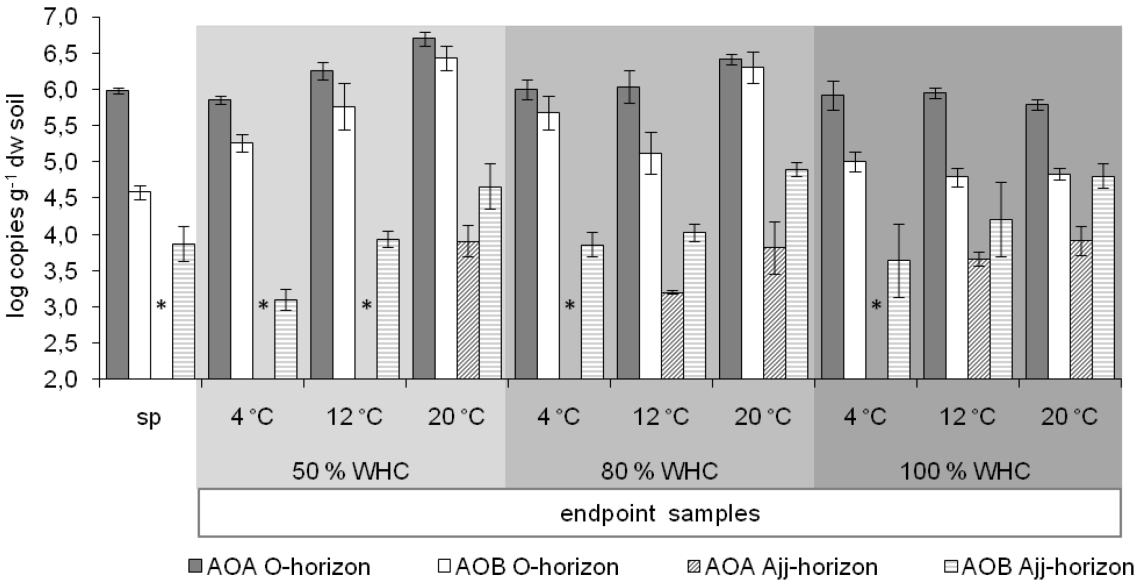

Fig. 8: General overview of archaeal and bacterial *amo*A gene copy numbers g^{-1} dw soil before and after incubation in the O (plain) and Ajj horizon (patterned). X-axis shows different combinations of temperature [°C] and WHC [%]. sp: startpoint of incubation, values are plotted as the arithmetic mean of six replicates with SD, * indicates values below detection limit of qPCR.

A significant shift of AOA and AOB abundances could be observed in the subducted topsoil (Ajj). Whereas bacterial *amo*A genes could be quantified in all samples, archaeal *amo*A genes could only be amplified successfully in samples which were

incubated at 12 °C and 20 °C. AOB outnumbered AOA throughout all treatments in the Ajj horizon, with copy numbers in the range of $8.7x10^2$ to $1.2x10^5$ copies g^{-1} dw soil, compared to $1.5x10^3$ to $1.5x10^4$ of archaeal *amo*A gene copies g^{-1} dw soil. The overall comparison of the two horizons revealed that *amo*A genes of both groups, AOA and AOB, were orders of magnitude less abundant in the Ajj horizon than in the O horizon. (Fig. 8, Tab. 7)

3.3 Response to changing temperature and moisture regimes

The soil samples had been incubated under different combinations of temperature and moisture content, the latter expressed as percent water holding capacity (% WHC). Changes in conditions revealed distinct responses of the ammonia oxidizer populations.

Pronounced trends of temperature affecting ammonia oxidizer populations were observed. In particular, AOA and AOB abundances increased with rising temperatures in the O horizon under oxic conditions. Copy numbers ascended continuously under dryer conditions (50 % WHC), with maximal abundances detected in the 20°C samples, where archaeal *amo*A gene copy numbers were 7-fold higher and bacterial *amo*A gene copy numbers were 15-fold higher compared to their corresponding abundances observed in the 4 °C treatment. Throughout the 80 % WHC incubations abundances were only considerably higher at 20 °C. No temperature dependent responses were perceived under anoxic conditions (100 % WHC). (Fig. 9 a., b.)

These trends were similarly reflected in the Ajj horizon within the AOB populations. Compared to the startpoint 9 to 12-fold increases were observed in the treatments incubated at 20 °C. Whereas at 12 °C no major changes could be detected, compared to the initial soil sample. AOB abundances dropped considerably at 4 °C under dry conditions (50 %), resulting in 7-fold lower copy numbers detected in this treatment than at the startpoint. Additionally, the existing AOA data also indicate increased occurrence at highest temperatures, at all three different water contents and at 12 °C under intermediate and high water contents. Different from the O horizon, a slight effect of temperature could also be observed under anoxic conditions, with 2-fold differences between the 12 and the 20 °C samples. (Fig. 9 c., d.). A considerable effect of moisture content on the ammonia oxidizer populations was exclusively observed in the O horizon. No major changes of archaeal and bacterial *amo*A gene copy numbers were observed in the 100 % WHC treatments, implying little growth under anoxic conditions in the organic topsoil. Whereas bacterial *amo*A gene copy numbers were on average 10-fold higher at 20°C than in the initial Ajj horizon sample and 24-fold higher than in

the sample incubated at 4°C, 100 % WHC. (Fig. 9) Overall, similar changes in AOA and AOB abundances in response to the different treatments were revealed, however the responses of the bacterial ammonia oxidizer population were more pronounced. Meaning that, in the topsoil highest AOA abundances were 2 to 5-fold higher than in the initial soil, whereas highest observed AOB abundances were 56 to 73-fold higher than before incubation.

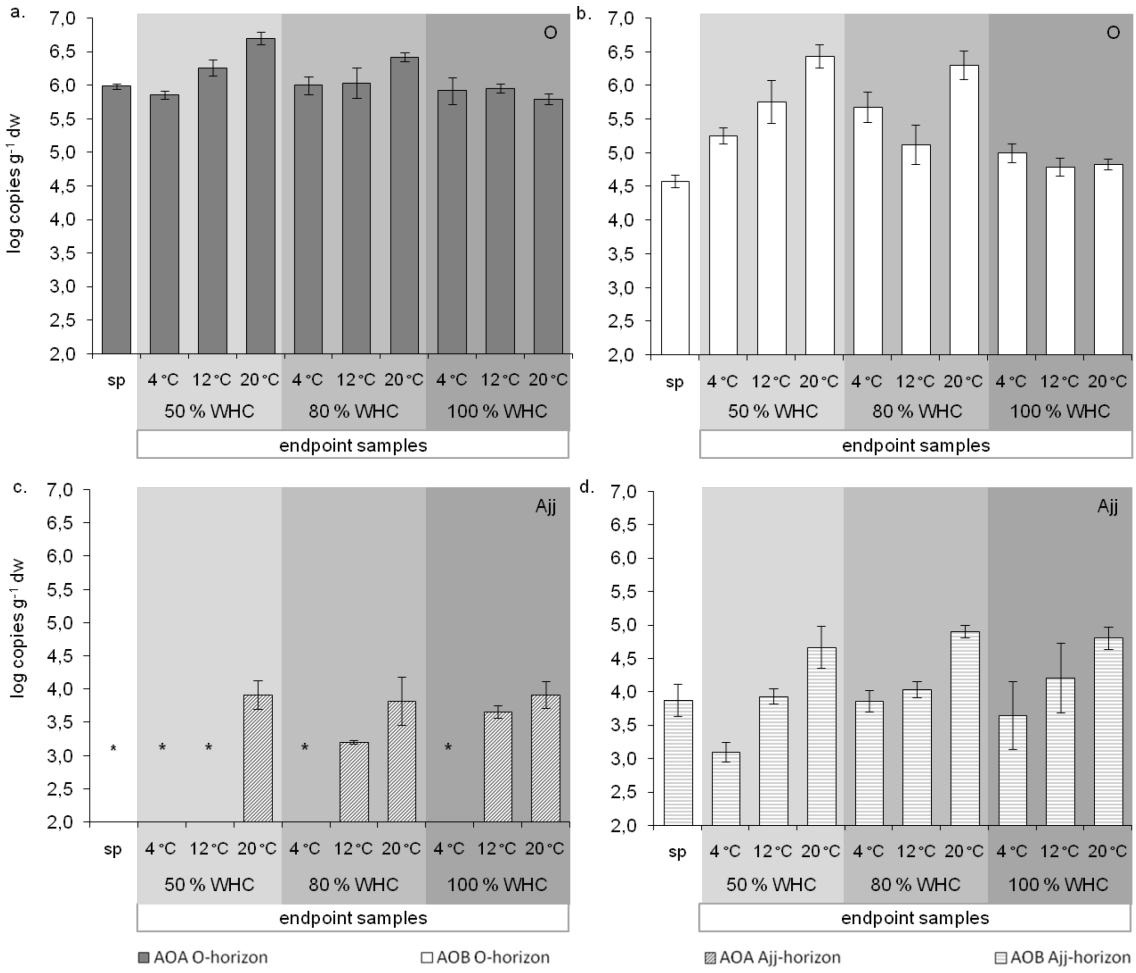

Fig. 9: Abundance changes of archaeal (panels a. and c.) and bacterial (panels b. and d.) *amo*A gene copy numbers under different temperature and moisture conditions. Startpoint (sp) and endpoints of incubation of all treatments of O and Ajj horizon. X-axis shows different combinations of temperatures [°C] and WHC [%]. sp: startpoint of incubation, values are plotted as the arithmetic mean of six replicates with SD, * indicates values below detection limit of qPCR.

3.4 Analyses of the intermediate time points

During the incubation three subsamples were taken in four week intervals from the 4 °C and 12 °C treatments of the O horizon, to follow changes in the ammonia oxidizer populations by qPCR analysis.

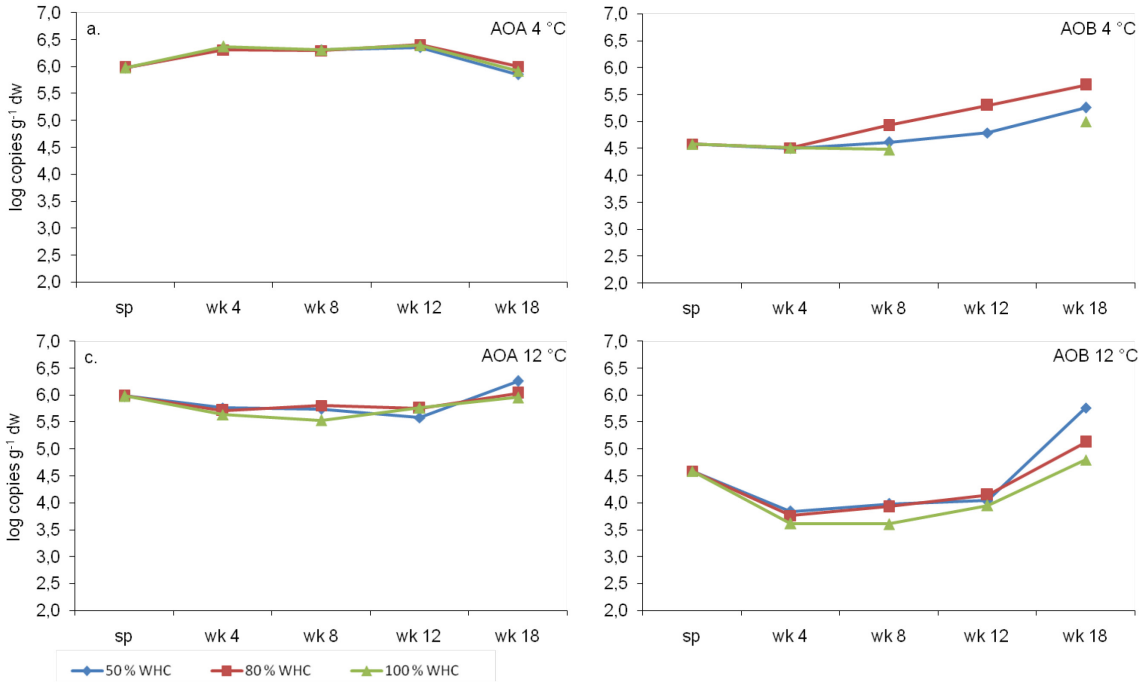

Fig. 10: Abundance changes of *amo*A gene copy numbers in the O horizon during the incubations at 4 °C (panels a and b) and 12°C (panels c and d). AOA (panels a. and c.), AOB (panels b. and d.). sp: initial soil before incubation, wk 18 = endpoint. Values are the arithmetic mean of six replicates. Value for AOB, 4 °C, 100 % WHC, wk 12 could not be determined.

Each of the two ammonia oxidizer groups showed distinct patterns in their population sizes over time. Abundances of AOA in the 12 °C samples and AOB in general, dropped after the start of incubation, leading to 2-fold lower abundances of AOA and 5 to 7-fold lower abundances of AOB after four weeks of incubation at 12 °C. The archaeal *amo*A gene abundances developed differently at 4 °C and 12 °C. Their abundances were around 2 to 3-fold higher during the incubation in the 4 °C samples and dropped at the end reaching their initial abundances. Bacterial *amo*A gene copy numbers increased along incubation time at both temperatures. The continuous increase of bacterial *amo*A gene copy numbers from week four to week 18 in the 4 °C happened in steps of 2 to 4-fold increases. At the end of incubation growth of AOA was only indicated in the oxic treatments at 12 °C, in contrast to AOB, where the abundances at the end of the incubation were continuously higher compared to the initial soil sample. (Fig. 10)

3.5 Third DNA extraction

An additional nucleic acid extraction was carried out at a later date, from a third set of replica of the start- and endpoint samples of the organic topsoil. Fig. 11 shows archaeal and bacterial *amo*A gene copy numbers after qPCR analysis. The first and second extraction are displayed combined, in contrast to the results of the third extraction.

Overall, a higher amount of archaeal *amo*A gene copies was detected, however the trends already described in section 3.3 regarding responses to different treatments were identical (Fig. 11 a.). Archaeal *amo*A gene copy numbers obtained from the third extraction were 3 to 6-fold higher than those detected in samples from the first two extractions, highest ratios were observed in the samples incubated under oxic conditions at 20 °C. The comparison of the bacterial *amo*A gene abundances quantified from the first two extractions with the third extraction revealed discrepancies. Only in three treatments *amo*A gene copy numbers of the additional extraction exceeded considerably the copy numbers obtained from the previous extractions. Bacterial *amo*A gene copy numbers in the 4 °C 50 % WHC sample and the 20 °C 50 % WHC sample were 3-fold higher in the third extraction, whereas the 12 °C 80 % WHC sample was even 5.5-fold higher. In a single sample (12 °C, 50 % WHC) considerably more bacterial *amo*A genes could be quantified in the first two extractions than in the third. (Fig. 11 b.)

Aside from these deviations, the general assumptions about the responses of ammonia oxidizers to changes in temperature and oxygen availability were also reflected in the new dataset. The results of the third extraction were not included in the overall abundance analysis to ensure a better comparability of the data from the O horizon and Ajj horizon, where no additional extraction was carried out.

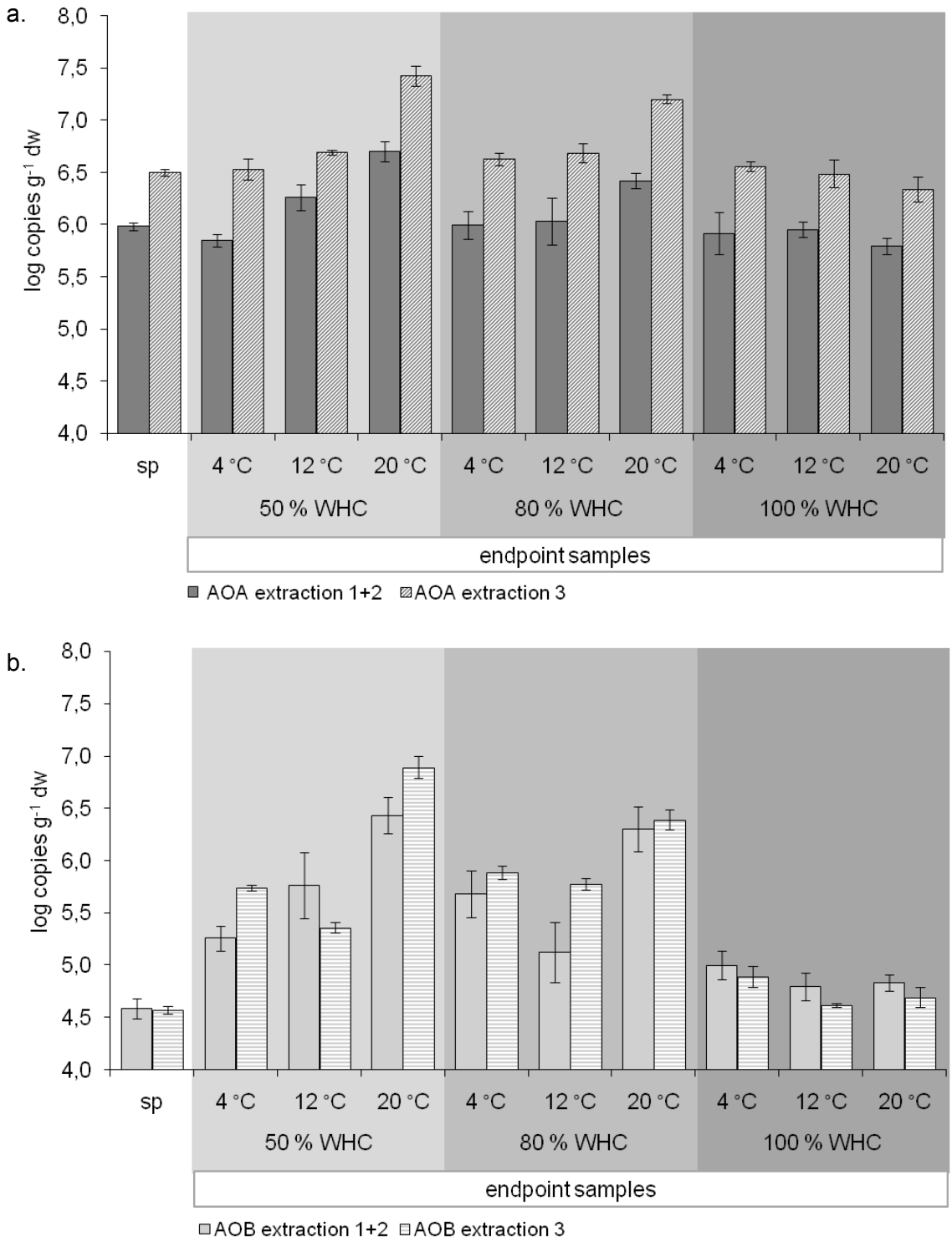

Fig. 11: Comparison of archaeal (panel a.) and bacterial (panel b.) *amo*A gene abundances in the different extractions of the O horizon. Values of the first and second extraction (plain bars) and third extraction (patterned bars) are shown as the arithmetic mean of six and three (technical) replicates, respectively, with SD. X-axis shows different combinations of temperature [°C] and WHC [%]. sp: startpoint of incubation.

Tab. 7: Absolute numbers of *amo*A gene copies of all treatments at the start (SP) and end of the incubation (EP). Standard deviation is shown in brackets. n.d. – not determined. b.d. – below detection

				*amo*A gene copies µg^{-1} DNA			*amo*A gene copies g^{-1} soil DW		
horizon	**temperature [°C]**	**WHC [%]**	**sampling point**	**AOA**	**AOB**	**AOA:AOB**	**AOA**	**AOB**	**AOA:AOB**
O			SP	1.1 x 10^4 *(9.5 x 10^2)*	4.7 x 10^2 *(9.6 x 10^1)*	24.5 *(1.6)*	9.6 x 10^5 *(8.1 x 10^4)*	3.9 x 10^4 *(8.0 x 10^3)*	24.5 *(1.6)*
O	4	50	EP	7.9 x 10^3 *(3.3 x 10^3)*	2.2 x 10^3 *(1.3 x 10^3)*	4.7 *(3.4)*	7.1 x 10^5 *(9.4 x 10^4)*	1.9 x 10^5 *(4.6 x 10^4)*	3.8 *(0.4)*
O	4	80	EP	8.0 x 10^3 *(1.8 x 10^3)*	3.9 x 10^3 *(1.3 x 10^3)*	2.2 *(0.08)*	1.0 x 10^6 *(3.3 x 10^5)*	5.3 x 10^5 *(2.6 x 10^5)*	2.4 *(1.3)*
O	4	100	EP	4.9 x 10^3 *(1.8 x10^3)*	4.0 x 10^2 *(2.0 x 10^2)*	12.9 *(6.1)*	9.0 x 10^5 *(4.0 x 10^5)*	1.0 x 10^5 *(3.5 x 10^4)*	9.5 *(4.9)*
O	12	50	EP	1.4 x 10^4 *(2.8 x 10^3)*	5.7 x 10^3 *(4.2 x 10^3)*	4.2 *(3.2)*	1.9 x 10^6 *(5.0 x 10^5)*	7.1 x 10^5 *(4.6 x 10^5)*	4.0 *(2.8)*
O	12	80	EP	1.2 x 10^4 *(4.4 x 10^3)*	1.9 x 10^3 *(1.2 x 10^3)*	10.4 *(8.1)*	1.2 x 10^6 *(5.9 x 10^5)*	1.6 x 10^5 *(8.5 x 10^4)*	10.1 *(7.8)*
O	12	100	EP	5.4 x 10^3 *(1.6 x 10^3)*	3.8 x 10^2 *(6.8 x 10^1)*	14.2 *(3.9)*	9.1 x 10^5 *(1.4 x 10^5)*	6.4 x 10^4 *(1.9 x 10^4)*	14.1 *(3.5)*
O	20	50	EP	6.8 x 10^4 *(3.1 x 10^4)*	3.9 x 10^4 *(2.3 x 10^4)*	2.3 *(1.8)*	5.1 x 10^6 *(1.1 x 10^6)*	2.9 x 10^6 *(1.0 x 10^6)*	2.0 *(0.08)*
O	20	80	EP	4.7 x 10^4 *(9.8 x 10^3)*	3.7 x 10^4 *(1.0 x 10^4*	1.3 *(0.3)*	2.7 x 10^6 *(4.0 x 10^5)*	2.2 x 10^6 *(9.3 x 10^5)*	1.4 *(0.06)*
O	20	100	EP	6.8 x 10^3 *(1.7 x 10^3)*	7.4 x 10^2 *(9.8 x 10^1)*	9.2 *(1.6)*	6.3 x 10^5 *(1.2 x 10^5)*	6.9 x 10^4 *(1.1 x 10^4)*	9.2 *(1.2)*
Ajj			SP	b.d	1.1 x 10^5 *(6.0 x 10^4)*	n.d	b.d	8.4 x 10^3 *(4.4 x 10^3)*	n.d
Ajj	4	50	EP	b.d	2.0 x 10^4 *(6.2 x 10^3)*	n.d	b.d	1.3 x 10^3 *(4.3 x 10^2)*	n.d
Ajj	4	80	EP	b.d	5.8 x 10^4 *(2.6 x 10^4)*	n.d	b.d	7.8 x 10^3 *(2.9 x 10^3)*	n.d
Ajj	4	100	EP	b.d	1.9 x 10^4 *(1.4 x 10^4)*	n.d	b.d	7.1 x 10^3 *(6.1 x 10^3)*	n.d
Ajj	12	50	EP	b.d	2.5 x 10^4 *(9.3 x 10^3)*	n.d	b.d	8.8 x 10^3 *(2.1 x 10^3)*	n.d
Ajj	12	80	EP	1.4 x 10^4 *(6.7 x 10^3)*	8.6 x 10^4 *(2.1 x 10^4)*	0.1 *(0.1)*	2.7 x 10^3 *(1.9 x 10^3)*	1.1 x 10^4 *(3.3 x 10^3)*	0.1 *(0.1)*
Ajj	12	100	EP	5.9 x 10^3 *(1.2 x 10^3)*	4.7 x 10^4 *(2.5 x 10^4)*	0.1 *(0.1)*	4.6 x 10^3 *(9.7 x 10^2)*	2.8 x 10^4 *(2.7 x 10^4)*	0.2 *(0.4)*
Ajj	20	50	EP	1.0 x 10^4 *(1.3 x 10^3)*	5.9 x 10^4 *(1.5 x 10^4)*	0.2 *(0.0)*	9.0 x 10^3 *(4.3 x 10^3)*	5.7 x 10^4 *(3.9 x 10^4)*	0.2 *(0.2)*
Ajj	20	80	EP	5.9 x 10^3 *(5.1 x 10^3)*	3.7 x 10^4 *(2.1 x 10^4)*	0.2 *(0.2)*	8.4 x 10^3 *(5.5 x 10^3)*	8.1 x 10^4 *(1.7 x 10^4)*	0.1 *(0.1)*
Ajj	20	100	EP	6.4 x 10^3 *(2.7 x 10^3)*	4.9 x 10^4 *(1.5 x 10^4)*	0.1 *(0.1)*	9.0 x 10^3 *(4.1 x 10^3)*	6.8 x 10^4 *(2.3 x 10^4)*	0.1 *(0.1)*

3.6 Sequencing of archaeal *amo*A gene amplicons

Sequencing of archaeal *amo*A gene amplicons was carried out on the in house Ion torrent Personal Genome Machine™ using a 314R chip. Based on primer test, described section 2.2 amplicons were generated with primers TamoA-1F-4 (Alves et al., unpublished) and TamoA-632R-4 (Alves et al., unpublished) with 30 cycles of the amplification steps. This forward primer covers a broad range of thaumarchaeal diversity in comparison to the primer used for qPCR which targets a more conserved region of the *amo*A gene. Subsequently amplicons were sheared enzymatically, with an enzyme mix, exhibiting best results in the shearing tests (see section 2.4.2). In total ten libraries were created, one from a single replicate of the startpoint sample and one each of the endpoint samples of all treatments of the organic topsoil. Two separate runs were performed, using 200 bp and 300 bp sequencing chemistry.

Both datasets contained a comparable amount of reads. Around 3.5 % of the obtained reads were not barcoded and could not be assigned to a sample, therefore those reads were excluded from further analysis. The average read length was in the range of 101 to 156 nt. Unexpectedly, reads from the 200 bp dataset were on average 21 (±7) nt longer than the reads from the 300 bp dataset. (Tab. 8).

Tab. 8: Comparison of the two datasets obtained from sequencing. The table shows the amount of barcoded reads and the absolute, relative amount of assigned reads and the average read length of the two separate sequencing runs.

chemistry	obtained reads with barcode	assigned reads [abs. abundance]	assigned reads [rel. abundance]	avg. read length [nt]
200	367321	55756	15 %	146 (±*14*)
300	355800	175087	49 %	124 (±*12*)

Tab. 9: Results from the *amo*A gene amplicon sequencing of all samples from the startpoint (sp) and the different treatments. The table shows all obtained reads, the absolute and relative numbers of assigned reads and the average read length for each sample.

Temp. [°C]	WHC [%]	chemistry	obtained reads	ass. reads [abs. abundance]	ass. reads [rel. abundance]	av. read length [nt]
sp		300	39411	13709	34.8 %	101
4	50	300	31675	16106	50.8 %	129
4	80	300	39266	16554	42.2 %	127
4	100	300	45975	26043	56.6 %	130
12	50	300	48173	26700	55.4 %	132
12	80	300	29355	13112	44.7 %	133
12	100	300	23591	8732	37.0 %	122
20	50	300	41456	26827	64.7 %	121
20	80	300	27210	15618	57.4 %	122
20	100	300	29688	11686	39.4 %	127

The obtained reads for the individual samples were in a range of 23,591 to 48,173 with an average of 35,580 received barcoded reads. From these obtained reads 26,827 could be related to thaumarchaeal sequences from the database (assigned) in the 20 °C 50 % WHC sample, whereas the lowest amount (8,732) were assigned in the 12 °C 100 % WHC sample. In five samples less than 50 %, with a minimum of 34.8 %, of the obtained reads could be assigned. (Tab. 9)

Analysis of the obtained archaeal *amo*A gene amplicon fragments was performed with MEGAN 4 using an in-house taxonomy database, comprising 51 selected thaumarchaeal *amo*A sequences, which was provided by Tim Urich (for further details see Material and Methods) (Fig. 12). Similar trends regarding the relative abundances of the different AOA taxa could be observed for both datasets (200bp and 300bp chemistry). The detailed data description below is exclusively based on the results from the 300 bp dataset, due to the improved assignment of the reads (Tab. 8, Tab. 9).

The in-house taxonomy database covered taxa of four major groups of Thaumarchaeota. In the soil samples only reads assigned to group 1.1b could be identified. No reads affiliated to members of group 1.1a, group 1.1a-associated or group ThAOA were detected. Within group 1.1b four different major clades were found: clade A, clade B, clade C and clade *Nitrososphaera* (Fig. 12). This clades, including clade D, are defined based on phylogenetic analyses and apparent different nitrification capabilities (Alves et al., 2013).

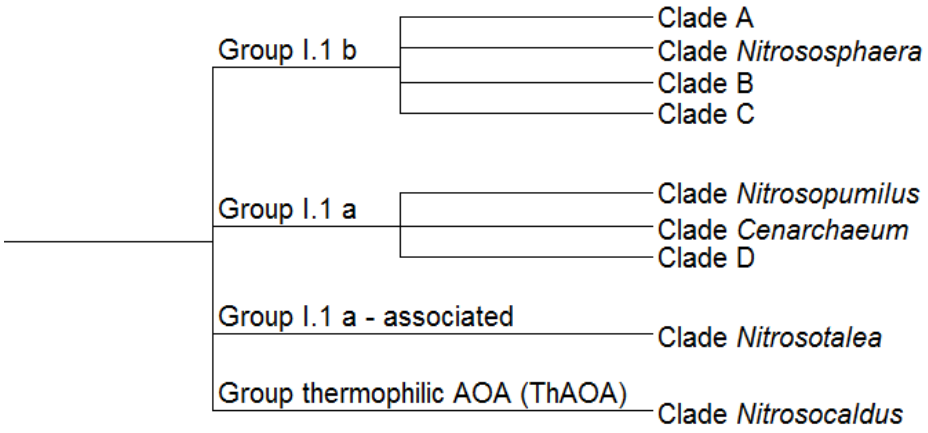

Fig. 12: Schematic overview of the different thaumarchaeal groups and clades used for the classification of the reads.

Clade A was the overall dominant one of the four different clades, reaching relative abundances of 59.5 to 87.2 %. Abundances of clade C were in the range of 8.1 to 35.5 % followed by clade B with 0.5 to 29.8 %. Clade *Nitrososphaera* was the least frequent group. In four endpoint samples clade *Nitrososphaera* was not detected or below 0.5 %, whereas the highest percentage observed was 9.4 %. (Fig. 13)

The analysis of the relative abundances of clades in the endpoint samples revealed distinct patterns within the different incubations. Therefore their responses to alternating moisture and temperature regimes were analysed further. (Fig. 14, Fig. 15, Fig. 16)

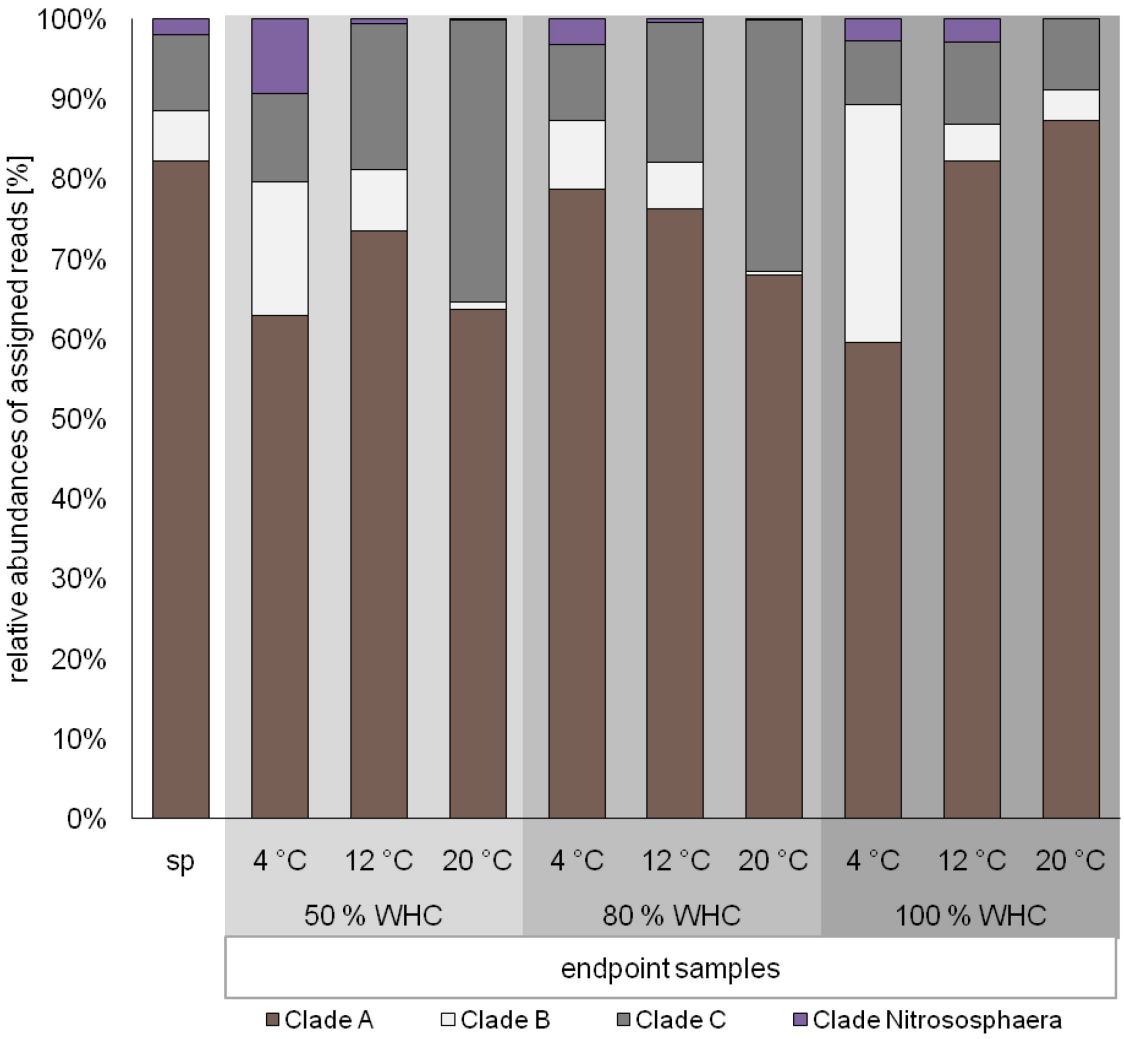

Fig. 13: Relative abundances of the different clades of ammonia oxidizing archaea at the start- and endpoint of the incubation in the O horizon. Archaeal *amo*A gene amplicons were sequenced on the Ion torrent PGM™ and analysed using the CREST pipeline (Lanzén et al., 2012).

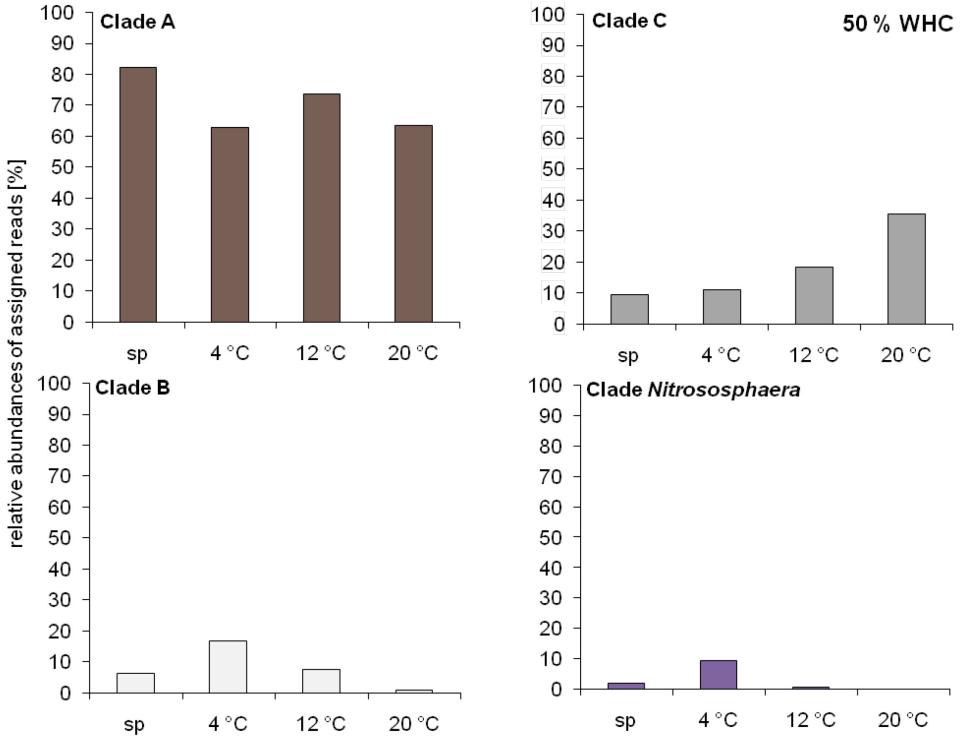

Fig. 14: Relative abundances of the different clades of ammonia oxidizing archaea detected in the O horizon at the start- (sp) and endpoint of the 50 % WHC incubations. X-axis shows the different incubation temperatures, sp: startpoint..

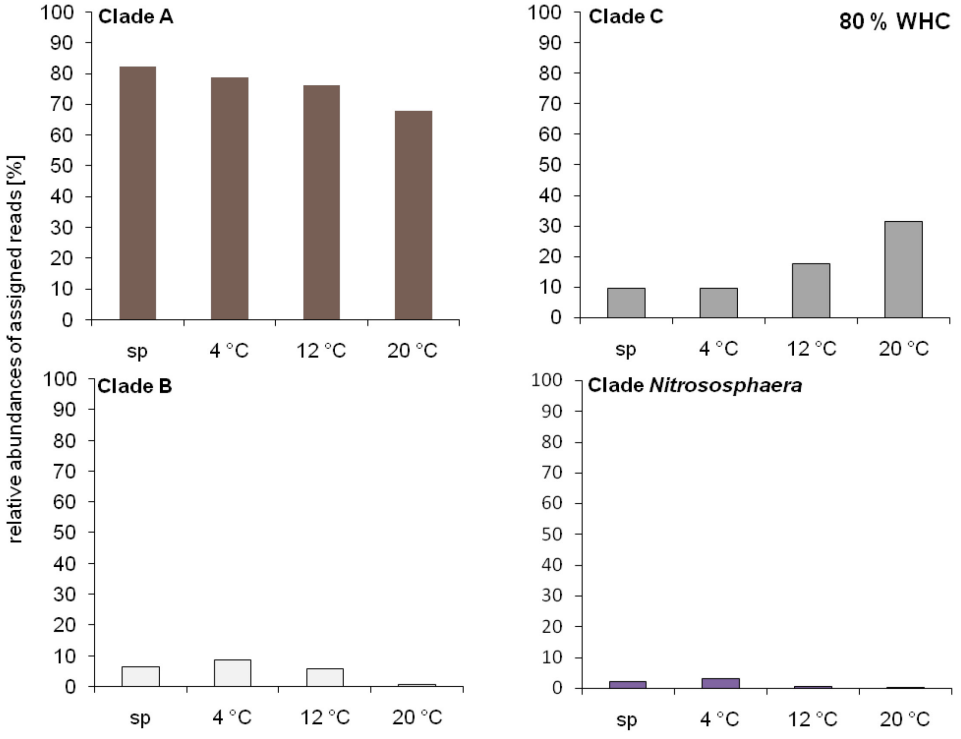

Fig. 15: Relative abundances of the different clades of ammonia oxidizing archaea detected in the O horizon at the start- (sp) and endpoint of the 80 % WHC incubations. X-axis shows the different incubation temperatures, sp: startpoint**.**

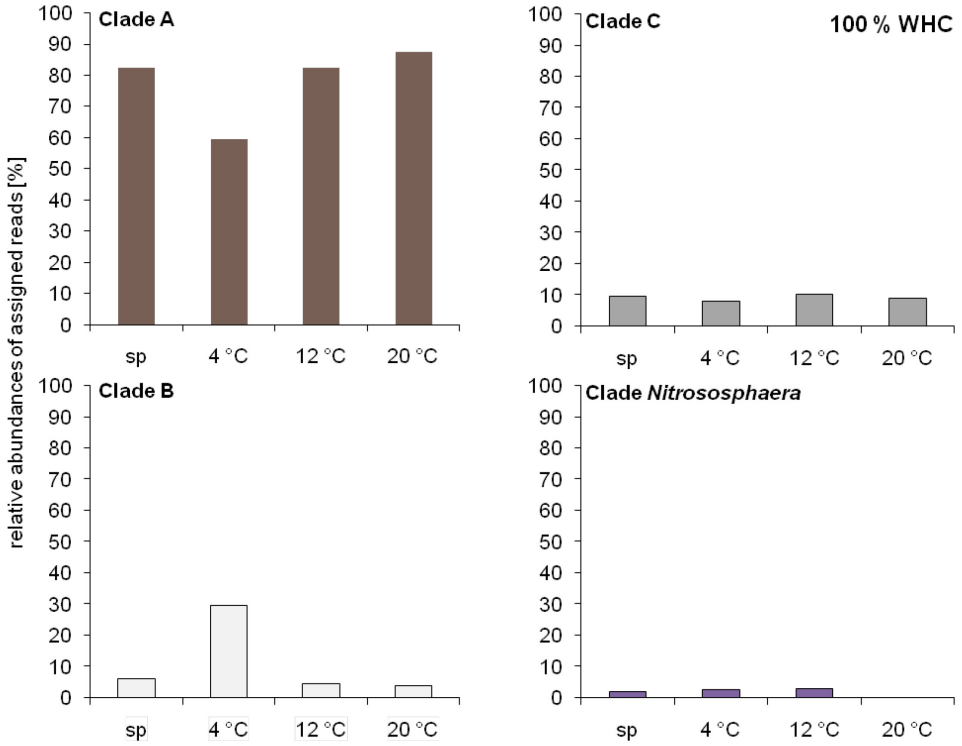

Fig. 16: Relative abundances of the different clades of ammonia oxidizing archaea detected in the O horizon at the start- (sp) and endpoint of the 100 % WHC incubations. X-axis shows the different incubation temperatures, sp: startpoint.

Clade A represented the majority of the detected AOA. Highest percentages of this clade were observed in the samples incubated at a saturated water level at 20 °C and 12 °C. Solely in these two treatments, the relative abundances exceeded those from the initial soil sample before incubation. The lowest quantities were determined under the same moisture conditions at 4 °C. (Fig. 10)

The abundances of clade A followed different patterns, depending on the water content. At moderate moisture conditions (80 % WHC) the number of clade A associated sequences decreased with rising temperature, whereas in the anoxic samples (100 % WHC) a continuous increase of relative abundances from 4 °C to 20 °C was observed. Elevated moisture levels seemed to positively affect members of clade A in the 12 °C and 20 °C incubations, depicted as a steady increase of relative abundances from 50 to 100 % WHC. (Fig 8. - 10.)

A distinct response related to temperature was observed within clade B. Highest relative abundances of this clade were detected in the 4 °C samples, reaching a peak under anoxic conditions. Independent of the moisture content clade B-associated reads decreased towards warmer conditions, reaching lowest abundances at 20 °C.

Abundances of members of clade C increased in the oxic treatments with rising temperatures, comprising abundances around three times higher at the end of incubation compared to the initial soil. Clade C showed a pronounced negative response towards increased moisture content. A decline from 50 % to 100 % WHC was observed at all temperatures. This effect got more pronounced at higher temperatures.

Clade *Nitrososphaera*, the least abundant group, showed peaks of abundance at 4 °C, exceeding numbers before incubation. Lower abundances were detected under oxic conditions at 12 °C. In the samples incubated at 20 °C, assigned reads dropped to < 0.5 %. A negative response to rising water content was observed at 4 °C.

The distinct clades displayed specific responses. Within clade A a moisture dependence seemed to be more pronounced than a temperature preference, whereas the populations of clade B and clade *Nitrososphaera* are more likely driven by temperature. Clear trends regarding the influence of both environmental variables, were observed within clade C.

Tab. 10: Overview of the relative abundances [%] of the different AOA clades in the O horizon samples. Sequences were assigned according to an in-house taxonomy targeting Thaumarchaeota. sp: startpoint, endpoint samples are indicated by the different treatments. Clade A-C and clade *Nitrososphaera* are defined according to Alves et al., 2013.

Treatment		**Relative abundances of the different clades [% of assigned reads]**			
Temperature [°C]	Moisture [%WHC]	Clade A	Clade B	Clade C	Clade N.
sp		82.2	6.3	9.5	2
4	50	62.9	16.6	11.0	9.4
4	80	78.7	8.5	9.5	3.2
4	100	59.5	29.8	8.1	2.7
12	50	73.5	7.6	18.2	0.7
12	80	76.2	5.8	17.5	0.5
12	100	82.2	4.7	10.2	3.0
20	50	63.6	0.9	35.5	0.0
20	80	67.9	0.5	31.5	0.0
20	100	87.2	3.8	9.0	0.0

3.7 Gross N mineralization and NH_4^+ consumption

Gross N mineralization and NH_4^+ consumption rates were determined from soils collected at the end of the main incubation for all treatments and soil horizons in four replicates each, using a $^{15}NH_4^+$ pool dilution assay performed over a 20 h incubation (see section 2.5). Additionally, net N mineralization rates were also determined based on the NH_4^+ pool changes over this short incubation. Data of replicates with negative gross rates were excluded. The process rates are displayed here as the arithmetic mean of three to four replicates, unless indicated otherwise.

Gross N mineralization and NH_4^+ consumption rates were overall higher in the incubated topsoil (O horizon) samples, exceeding those observed in both the subducted topsoil (Ajj horizon) and the mineral subsoil (BCg horizon) throughout all treatments. Gross N mineralization were on average 3 to 16-fold and 2 to 248-fold higher in the O horizon compared to those in Ajj and BCg horizons, respectively. In turn, gross NH_4^+consumption rates in the topsoil were 2 to 9-fold higher in comparison to those in both subsoil types. (Fig. 17, Fig. 18, Fig. 19)

In the O horizon, gross N mineralization rates were within the range of 1.83 to 14.81 µg N g^{-1} dw d^{-1}. The highest N mineralization rates were observed in samples incubated at 12 °C and 20 °C under oxic conditions. Within the 12 °C and 80 % WHC treatment, gross N mineralization rates were up to 2-fold higher than those in samples incubated at 4 °C, where the lowest rates were observed. The average rates in anoxic samples were only 1.2-fold higher at 12 °C and 20 °C than those at 4 °C. (Fig. 17)
Gross NH_4^+consumption rates in the O horizon were in the range of 1.93 to 13.83 µg N g^{-1} dw d^{-1}. Average gross NH_4^+ consumption rates were lowest at 4 °C in samples incubated under oxic conditions. In samples incubated under anoxic conditions, 1.2-fold more NH_4^+ was consumed in the 4 and 12 °C incubations than in those incubated at 20 °C. In samples incubated under oxic conditions, the highest NH_4^+ consumption rates were observed at 12 °C and 20 °C, whereas in those from anoxic incubations the consumption of NH_4^+ was higher at 4° and 12 °C. (Fig.17)

Gross N mineralization rates in the Ajj horizon varied from 0.13 to 3.49 µg N g^{-1} dw d^{-1}, whereas rates in BCg horizon were in the range of 0.01 to 3.93 µg N g^{-1} dw d^{-1}. In the Ajj horizon soil, gross N mineralization rates were slightly higher in the samples incubated with 80 % and 100 % WHC, compared to the drier samples (50 % WHC). Gross NH_4^+ consumption rates were in the range of 0.50 to 9.13 µg N g^{-1} dw d^{-1} and

0.29 to 3.54 µg N g^{-1} dw d^{-1} in the Ajj and BCg soils, respectively (Fig. 18. and Fig. 19). Further temperature or moisture-associated trends in gross N mineralization or NH_4^+ consumption rates were not observed in soils from either horizon type.

Average positive net mineralization occurred only in four endpoint samples of the O horizon with rates within 0.07 and 4.29 µg N g^{-1} dw d^{-1} in the following treatments: 4 °C 50 % WHC, 12 °C 50 and 80 % WHC, 20 °C 100 % WHC. In the Ajj and the BCg horizon, no considerable net N mineralization was observed. (Data not shown)

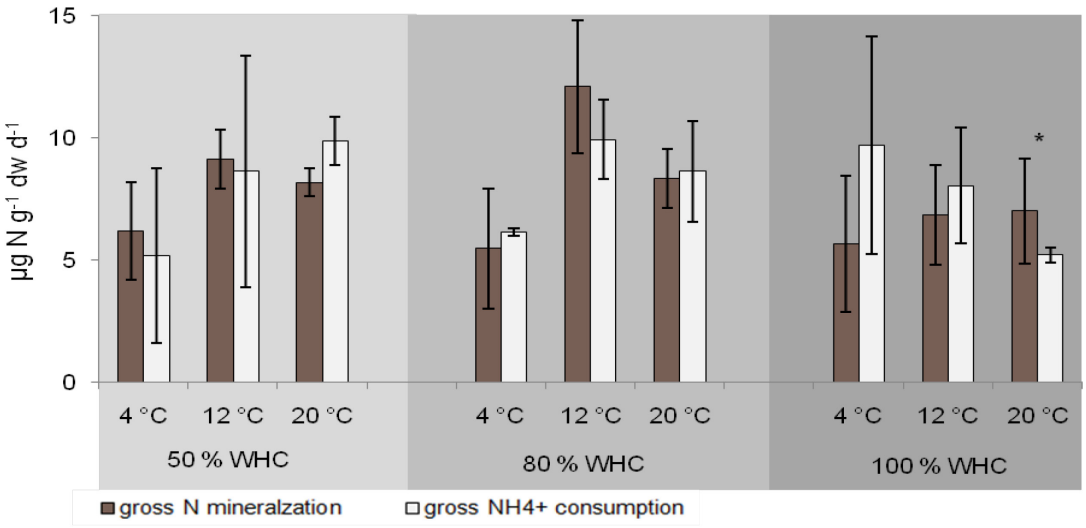

Fig. 17: Gross N mineralization (brown) and gross NH_4^+ consumption (white) rates determined by an ^{15}N pool dilution assay of the different treatments in the O horizon. X-axis shows different combinations of temperatures [°C] and WHC [%]. Values are plotted as the arithmetic mean of three or four replicates with SD, * indicates values calculated from two replicates.

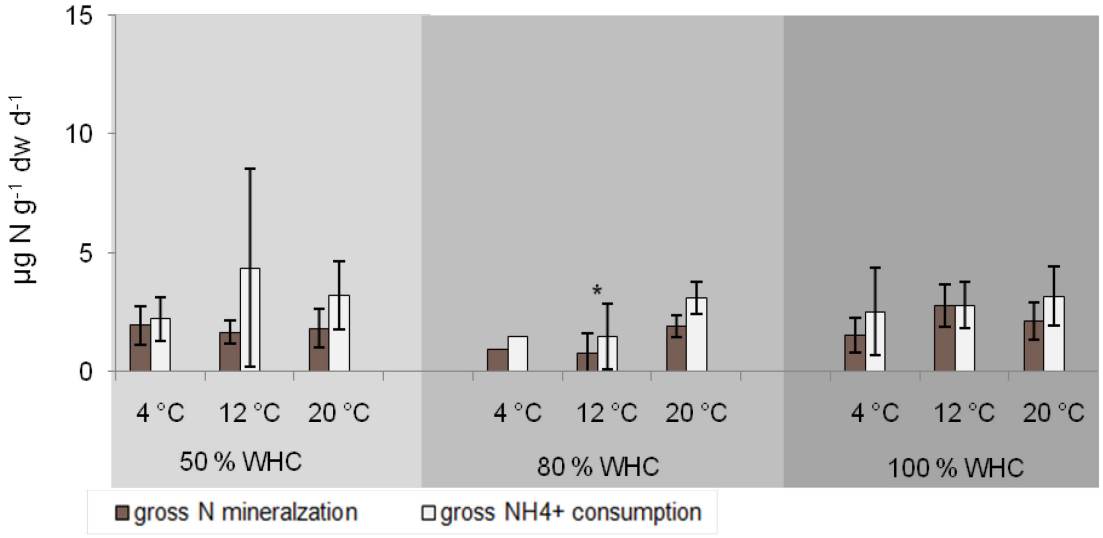

Fig. 18: Gross N mineralization (brown) and gross NH_4^+ consumption (white) rates determined by an ^{15}N pool dilution assay of the different treatments in the Ajj horizon. X-axis shows different combinations of temperatures [°C] and WHC [%]. Values are plotted as the arithmetic mean of three or four replicates with SD, * indicates values calculated from two replicates, only one value was obtained for bars without SD.

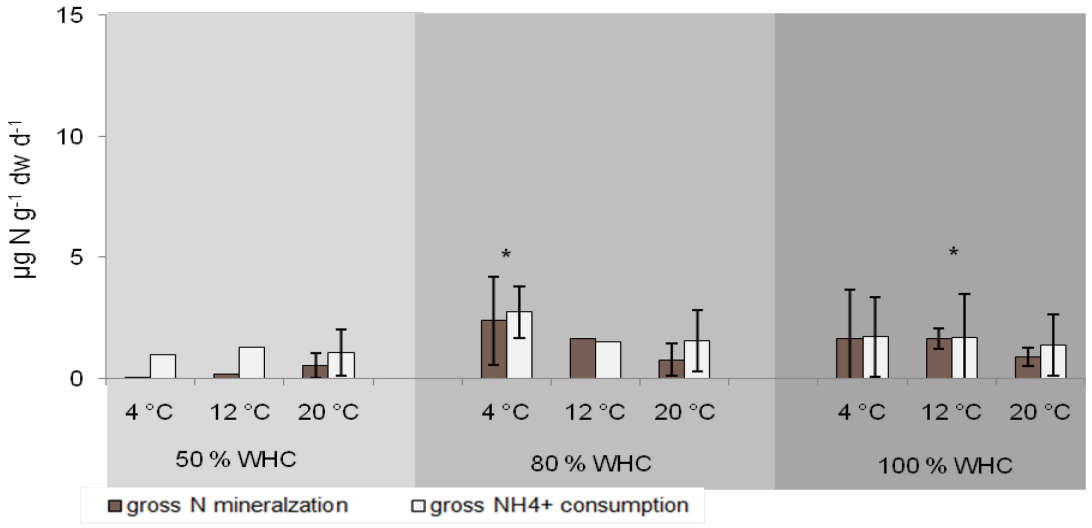

Fig. 19: Gross N mineralization (brown) and gross NH_4^+ consumption (white) rates determined by an ^{15}N pool dilution assay of the different treatments in the BCg horizon. X-axis shows different combinations of temperatures [°C] and WHC [%]. Values are plotted as the arithmetic mean of three or four replicates with SD, * indicates values calculated from two replicates, only one value was obtained for bars without SD.

4 Discussion

In this work we present insights into the ammonia oxidizer community in Arctic soils and its responses to changes in abiotic conditions. The incubation experiment showed that alterations in temperature, soil moisture content and the availability of oxygen have distinct effects on the abundance of archaeal (AOA) and bacterial (AOB) ammonia oxidizers. Those effects were further examined with respect to shifts in the AOA population (Thaumarchaeota) to analyse the heterogeneity within this group. Additionally, the influence of the mentioned environmental factors on processes related to the N cycle were investigated, including gross N mineralization, gross N consumption and net mineralization.

The separation of the soil into three horizons allowed the individual examination of the corresponding material in consideration of their distinct characteristics. Basic soil properties, determined by Čapek and colleagues (2015), had shown that the topsoil (O horizon) exhibited the highest content of organic carbon (OC) and total nitrogen (N_{tot}) compared to the subducted topsoil (Ajj) and the mineral subsoil (BCg). Despite the higher C and nutrient content in the buried topsoil (Ajj) as compared to the mineral subsoil (BCg), significantly lower microbial biomass was detected in both subsurface horizons compared to upper topsoil (Čapek et al., 2015). This was confirmed by the obtained amount of extracted DNA. Hence, extended experiments were restricted to the O horizon samples due to limited amount of isolated DNA from the other two horizons. The extremely low yield of nucleic acids retrieved from the BCg horizon did not allow for molecular analyses, therefore only data of the N mineralization are available for this horizon.

Beside low biomass yields of lower soil layers, another challenge in this project was the sensitivity for sample processing procedures on the obtained DNA yield analysis. The higher archaeal *amo*A gene abundances recovered from the third replicates reflect that additional variance can be introduced by separate processing. However, the deviations observed did not confound the major trends observed in this study.

4.1 Abundances and distribution of ammonia oxidizers

This study confirms the presence of AOA and AOB in the permafrost-affected soil from the Taymyr peninsula, Russia. The population sizes of AOA and AOB were comparable to abundances reported from Arctic soils across the northern hemisphere (Yergeau et al., 2010; Banerjee & Siciliano, 2012; Alves et al., 2013). Remarkably, pristine and agricultural soils from different climatic zones investigated by Leininger and

colleagues (2006) exhibited only slightly higher quantities of AOA and AOB compared to this study. This again shows the broad distribution and high abundances of this functional guild even under extreme environmental conditions.

AOA predominated the topsoil samples, outnumbering their bacterial counterparts considerably in the majority of the treatments. High AOA:AOB ratios have been reported from a diverse selection of soils (e.g. Leininger et al., 2006; Nicol et al., 2008) including Arctic soils (e.g. Siciliano et al., 2009; Yergeau et al., 2010; Lamb et al., 2011; Alves et al., 2013). Habitats of higher latitude or altitude might share common characteristics that benefit AOA (Alves et al., 2013), which could explain the selection towards archaeal ammonia oxidizers in the investigated soils. Generally, the prevalence of AOA might be based on the versatile metabolism within this phylum. As it was reported from Tourna and colleagues (2011), *Nitrosososphaera viennensis*, the first soil-borne AOA in pure culture, increased growth based on the oxidation on ammonia by the addition of pyruvate, indicating a mixotrophic lifestyle. This might suggest a selection towards AOA in the organic topsoil, which usually exhibits a higher amount of simple organic compounds derived by root exudates, compared with the subducted topsoil horizon. This is in line with observations by Karlsson and colleagues (2012) who linked Archaea with C fluxes and the presence of roots and mycorrhizas.

The overall abundances of ammonia oxidizers showed clear differences in the two investigated horizons. Larger population sizes were observed in the organic topsoil samples compared to the cryoturbation pockets which were sampled from a depth of 50 to 70 cm. Soil material which is located deeper in the profile, is frozen for a longer time and exposed to lower temperatures during summer, therefore microbial growth and activity could be influenced by these factors. In cryoturbated soils from Greenland and Siberia higher microbial biomass in the topsoil horizons and decreases with depth were reported by Gittel and colleagues (2014a, 2014b). Interestingly the authors showed that the deeper located cryoturbation pockets harboured much higher abundances of microorganisms than the surrounding subsoil which were in comparable numbers to the topsoil, interrupting the trend of decreasing abundances with depth. This contrasts with the results of this study, which showed up to orders of magnitudes lower ammonia oxidizer abundances and as already mentioned, generally lower microbial biomass (Čapek et al., 2015) in the Ajj horizon. Therefore it is suggested that the microbial community is negatively impacted by insufficient C and nutrient supply due to certain chemical-physical characteristics of the OC in the Ajj (Čapek et al., 2015; Gentsch et al., 2015), which could also affect the ammonia oxidizer populations, e.g. by low mineralization activity. Furthermore, abundances of ammonia oxidizers in soil might

not be directly depending on ammonia concentrations but on the flux and removal rates (Prosser, 2011) which are assumed to be higher in the topsoil.

A remarkable shift towards AOB was observed within the cryoturbated pockets. AOB clearly dominated the Ajj horizon whereas AOA abundances were partially below detection limit. Opposed results of an ammonia oxidizer abundance analysis along a depth gradient in agricultural and pristine soils were reported, which revealed pronounced decreases of AOB with increasing depth whereas abundances of AOA did not change in managed soils and exhibited only minor changes in the pristine soils (Leininger et al., 2006). Unfortunately, it was not possible in this study to quantify AOA and AOB in mineral subsoil, therefore it cannot be revealed whether AOB are generally more abundant than AOA in the lower soil layers or if the unique features of the cryoturbated pockets, such as higher SOM content in combination, selects towards AOB.

However, abundances of *amo*A gene copy numbers and their implications on nitrification have to be considered carefully. The detection of functional genes does not imply a major contribution to the affiliated processes. As commented e.g. by Prosser and Nicol (2008), the occurrence of *amo*A genes does not provide information about the actual expression and activity of the enzyme. And especially regarding limitations in the knowledge of Thaumarchaeota physiology so far, the capability of the organisms to oxidize ammonia cannot be revealed by the detection of the functional gene. However, some insights into the nitrification activity of members of AOA detected in this study, were proposed by Alves and colleagues (2013).

4.2 Thaumarchaeal community composition

Phylogenetic analysis of the archaeal *amo*A genes revealed that only members of group 1.1b were present in the topsoil of the shrubby moss tundra on the Taymyr peninsula. In this work group 1.1b refers to the *Nitrososphaera* and the *Nitrososphaera* sister cluster that have been proposed by Pester and colleagues (2012). Sequences affiliated to this group have been detected in a wide range of soils and were found in high abundances and diversity (e.g. Ochsenreiter et al., 2003; Francis et al., 2005, Leininger et al., 2006; Nicol et al., 2008). It was also suggested that they comprise the majority of Thaumarchaeota in a diverse selection of soils from South Africa, Europe, South America and a greenlandic tundra soil (Pester et al., 2012) which is in line with a

high-throughput sequencing study investigating 146 soils around the world (Bates et al., 2011).
In their comprehensive approach, Alves and colleagues (2013) emphasized the importance of differentiation within AOA and the diverse soil lineages, respectively. They defined five clades upon investigating 11 different arctic soils, based on their phylogenetic separation and their apparent distinct capability of ammonia oxidation.

Members of four out of the five clades were identified in this work, which emphasizes that most of the major clades of soil-affiliated AOA are present in the investigated soil samples. The clades were not homogenously distributed. The most prominent cluster was comprised by clade A, which is generally represented by the fosmid clone 54d9, which was the first indicator of AOA in soils (Treusch et al., 2005). Remarkably, if the relative abundances of this group are linked to the absolute *amo*A copy numbers measured by qPCR, clade A exceeded abundances of bacterial ammonia oxidizers in the initial topsoil and reached abundances comparable to the whole AOA and AOB community in the Ajj horizon, respectively (data not shown). This is in line with Bates and colleagues (2011) who reported high abundances of this ubiquitously distributed organisms. The second most abundant group was clade c, which harbours members affiliated to fosmid clone 29i4 (Quaiser et al., 2002). An enrichment culture from our laboratory confirms ammonia oxidizing activity for a member of this clade, although the organism in these enrichments grows extremely slowly (see Alves et al., 2013 and unpublished). Clade B and clade *Nitrososphaera* comprised only a small part of the AOA community in the organic topsoil.

4.3 Responses to changes in temperature and moisture content

Temperature and moisture content are determinants of ammonia oxidizing communities in soils. This study analysed responses of this functional guild and its archaeal and bacterial populations to changes of these two environmental factors.
The results of the incubation approach suggest that temperature is a major driver of archaeal and bacterial ammonia oxidizer communities in permafrost-affected soils. Increasing soil temperature affected the abundances of both groups positively. Despite differences of the topsoil (O horizon) on the surface and the subducted topsoil (Ajj horizon) already outlined in the previous section, this trend was observed in both horizons.
This finding proposes, that abundances of ammonia oxidizers will increase if the Ajj horizon is shifted back to the surface by cryoturbation and whenever the topsoil horizon

gets increasingly exposed to higher temperatures caused by climate change. This implies, without consideration of the moisture conditions, that if the soil material gets warmer, nitrification rates will be enhanced, independently whether AOA or AOB are the main nitrifier population.

The positive effect of temperature on ammonia oxidizers in the uppermost part of the active layer seems to be depending on the water content. Under dry conditions growth was observed continuously with rising temperature, whereas at slightly increased soil moisture content (80 % WHC) this clear trend could not be observed, and considerably higher population sizes were only found at 20 °C. In the water-saturated samples growth of AOA did not seem to be supported and was very limited for AOB. This leads to the assumption, that the low concentrations of oxygen prevailing under waterlogged conditions, have a negative effect on ammonia oxidizer. This is in line with general assumptions, that nitrification is decreased under conditions of low oxygen availability, since most nitrifiers are obligate aerobes (Robertson & Groffman, 2007) and additionally increased microbial activity can cause anoxia in soils with high water content (Prosser, 2011).
Remarkably, in the cryoturbation pockets both populations exhibited no response to changed water content. This could lead to the presumption, that the ammonia oxidizers in the topsoil horizon are depending on a high availability of oxygen, in contrast to the communities in the subducted topsoil horizon, which might be better adapted to waterlogged conditions caused by poor drainage. Culturing approaches and studies in natural systems showed that nitrifiers tolerate very low oxygen levels, despite the fact that ammonia oxidation is an obligate aerobic process (Ward, 2011). This assumptions would imply, that the topsoil and the cryoturbation pockets harbour distinct AOA and AOB populations, which differ in their response to oxygen deprivation. This could be linked with results from other studies with a focus on cryoturbated soils, where the authors found that microbial community structures differed in the topsoil and the subducted topsoil horizon (Gittel et al., 2014 a.; Gittel et al., 2014 b.; Schnecker et al., 2014).
This findings lead to the assumption, that if the upper layers of the soil are exposed to higher soil water content due to increased precipitation, higher surface temperatures might not considerably affect AOA and AOB populations.

Growth of AOB (i.e. abundances related to the initial soil sample) was much more pronounced than growth of the already highly abundant AOA, this is additionally supported by the results of the intermediate timepoints obtained by subsampling during

incubation, which indicated that AOB grew continuously towards the end of incubation whereas archaeal ammonia oxidizers showed different patterns. This findings proposes, that AOA might be well-adapted to the prevailing conditions in these ecosystems but under changing conditions AOB might dominate based on their high responsiveness.

In all topsoil samples growth of AOB was supported, even under conditions which are less favourable (e.g. 4 °C, 100 %WHC) compared to the prevailing conditions on the surface. This indicates that AOB might have been favoured by the incubation process.

4.3.1 Responses of AOA subpopulations

Major changes regarding the community structure of AOA were observed in the different treatments. The influence of temperature, moisture content and the derived oxygen availability on the structure of the AOA community has been observed in several studies with different approaches (e.g. Tourna et al., 2008; Stres et al., 2008).

This work revealed distinct responses of the clades to changing temperature and moisture content. As already mentioned, members affiliated with clade A showed highest relative abundances, therefore the response of this clade has a major impact on the whole AOA community. According to Alves and colleagues (2013) phylotypes of this cluster are associated with dry soils and dominated cultures incubated at 4 °C and 28 °C. These preferences are only partially displayed in the results of this work, where the largest fraction of clade A was found under water-saturated conditions but at higher temperatures. Whereas warmer conditions did not seem to promote the clade in drier soils. Trends considering responses to different treatments were ambiguous, suggesting that the sensitivity to the combination of different environmental factors is very complex or that the population did not change and differences in their distribution is based on responses of the other clades. Since low nitrification rates are attributed to this clade (Alves et al., 2013), the latter would imply that these organisms do not have a significant impact on ammonia oxidation under changed conditions and their role as ammonia oxidizers remains unclear. This might indicate again, that versatile adaptations and metabolisms are found within group I.1b, as suggested earlier by Schleper (2010).

Clade B was evidently negatively affected by elevated temperatures. Given that, conditions prevailing in the subsoil (i.e. cold and wet) might promote these organisms, members associated to Clade B could be a dominant group in the cryoturbation

pockets. When the organic topsoil, harbouring these Thaumarchaeota, is subducted by cryogenic processes they might obtain an important role in ammonia-oxidation.
According to Alves and colleagues (2013) highest nitrification capability is suggested for members affiliated to Clade *Nitrososphaera* but as the authors already found, affiliated members only comprise a minor fraction of the AOA and might find optimal conditions in dry but cold soils, which is in line with trends observed in this work.
None of the habitats investigated by Alves and colleagues (2013) was dominated by representatives of clade C, which presents the second most abundant group in this work. However, a culturing approach in their study confirms an autotrophic growth as ammonia oxidizer. Remarkably, observed abundance changes of the topsoil AOA populations within the different treatments are reflected by the positive responses of clade C to increased temperatures under dry and intermediate moisture conditions and no increase of abundances of this clade in the water-saturated soils. This proposes that members affiliated to clade C are major players of AOA in the upper layers of the permafrost-affected soils. This is in line with findings by Tourna and colleagues (2008) who showed that increased temperature not only lead to changes of the AOA community structure but also proved that the actively nitrifying members of AOA responded positively to high temperatures.
Together the contrasting findings for all four clades demonstrate the importance to examine functional guilds with high resolution methods.

4.4 Nitrogen mineralization

Gross N mineralization and gross NH_4^+ consumption rates were determined in the organic topsoil (O horizon), subducted topsoil (Ajj horizon) and mineral subsoil (BCg horizon). Nitrification is dependent on substrate availability; therefore, if high N mineralization occurs and N immobilization rates are low, the process of ammonia oxidation could be promoted (Robertson & Groffman, 2007).
Overall interpretation of the results was greatly hindered by the high variability in gross N mineralization and NH_4^+ consumption rates among the replicates within the individual treatments. Furthermore, several measurements had to be excluded due to negative gross rates derived from unsuccessful pool dilution assays, in which, for example, no dilution of the $^{15}NH_4^+$ could be detected. Because of this shortcomings, only limited conclusions could be drawn and thus only general trends are discussed here.

Low gross N mineralization and gross NH_4^+ consumption rates were generally observed in this work, similar to those previously reported in tundra soils from Siberia

and Greenland (Wild et al., 2013). It is suggested, that nitrification has proportionally an increased impact on the NH_4^+ availability under low mineralization rates, whereas at high mineralization rates NH_4^+ assimilation seems to dominate (Booth et al., 2005). This might indicate an important role of nitrification in the permafrost-affected soils.

The current results show that gross rates in organic topsoil are generally much higher than those in buried topsoil and mineral subsoil, regardless of major environmental conditions, such as temperature. This is in agreement with the lower rates of N transformation processes, including N mineralization, observed in cryoturbated soil material compared to the topsoil horizon in studies investigating soils from Greenland and Siberia (Wild et al., 2013; Kaiser et al., 2007). This could lead to the assumption that, based only on N mineralization activity, ammonia oxidizer populations in the topsoil might be positively affected, whereas AOA and AOB abundances in the subducted topsoil might be substrate-limited. Additionally, it has been suggested that substrate availability through N mineralization, in contrast to that available through fertilization, favors AOA growth and activity, possibly due to the slow release of NH_4^+ (Prosser & Nicol, 2012). This could be another explanation for higher abundances of archaeal ammonia oxidizers in the upper soil layer.

Regarding the impact of different incubation treatments to NH_4^+ availability, no constant patterns could be observed and responses of gross NH_4^+ production could not be related to gross NH_4^+ consumption. Positive net N mineralization was hardly observed and the fraction of NH_4^+ consumed by nitrifiers cannot be estimated in this work. Therefore, the changes of gross and net rates under different conditions could not be directly linked to the responses of AOA and AOB populations, or shifts in the AOA community. Nevertheless, gross N mineralization rates in the topsoil exhibit a pattern similar to that observed on the abundance changes in ammonia oxidizer populations (Fig. 9 a., b.). Increased rates were found at moderate and warm temperatures under oxic conditions, but no pronounced temperature dependent effect in the water-saturated samples was demonstrated. This indicates that optimal conditions for mineralization are met at intermediate water content and that activity increases with temperature, in accordance with previous observations (Robertson & Groffman, 2007). This results suggest that if the topsoil is exposed to warmer conditions, mineralization rates will be enhanced, possibly providing greater substrate availability for nitrifiers. However, NH_4^+ availability will be dependent on the interaction between the responses of N mineralization and NH_4^+ immobilization by both plants and microbes to temperature changes.

4.5 Conclusion

High abundances and dynamics of AOA and AOB in this cryoturbated, permafrost-affected soils indicate that both groups have an important role in Arctic ecosystems. Changing environmental conditions will have distinct effects on the ammonia oxidizer populations and community structure. Increasing temperatures could be positively linked to higher abundances of ammonia oxidizers in both investigated horizons and consequently will have an impact on nitrification in this ecosystem. However, if increased precipitation evoke water-saturated conditions in upper soil layers, the positive effect of higher temperatures will diminish. These results help to elucidate possible responses of ammonia oxidizer populations to changing conditions and highlight the importance of a detailed analysis of functional groups at higher resolution.

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Zusammenfassung

Durch den Einfluss von Klimawandel werden weitreichende Veränderungen der Umweltbedingungen in Polarregionen erwartet. Es wird angenommen, dass der Anstieg der Temperaturen die Aktivität von Mikroorganismen steigert und infolgedessen der Abbau von organischer Substanz und biogeochemische Prozesse beschleunigt werden. Da Böden die von Permafrost beeinflusst sind große Anteile an organischem Kohlenstoff speichern, kann der verstärkte Abbau dieser empfindlichen Reservoirs zu erhöhten Treibhausgasemissionen führen, die wiederum den globalen Klimawandel verstärken.
Die Abbauprozesse in arktischen Regionen gelten generell als Stickstoff-limitiert, jedoch gibt es wenig Informationen über die am Stickstoffkreislauf beteiligten Mikroorganismen und deren Reaktionen auf veränderte Umweltbedingungen. Die Zielsetzung dieser Arbeit war die Untersuchung der Ammoniak oxidierenden Archaea- und Bakterienpopulationen, die eine wichtige Rolle in der Nitrifikation spielen und damit das Gleichgewicht zwischen den Hauptstickstoffquellen von Mikroorganismen und Pflanzen regulieren. Darüber hinaus sind Nitrifizierer direkt und indirekt an der Produktion des wirksamen Treibhausgases N_2O beteiligt.

Der Schwerpunkt dieser Arbeit war die Charakterisierung der Ammoniak oxidierenden Populationen in kryoturbierten Böden der Taimyr Halbinsel hinsichtlich ihrer Größen und Beschaffenheit sowie deren Wandlung unter veränderten Umweltbedingungen. Die Messungen wurden in zwei unterschiedlichen Horizonten des kryoturbierten Bodens durchgeführt, dem organischen Oberboden an der Oberfläche (O-Horizont) und aus tieferliegenden Schichten des Oberbodens, die durch Kryoturbations-Prozesse begraben wurden (Ajj Horizont). Das Bodenmaterial wurde unter unterschiedlichen Bedingungen inkubiert, die sich aus Kombinationen von drei verschiedenen Temperaturen (4, 12, 20 °C) und drei verschiedenen Feuchtigkeitsgehalten (50, 80, 100 % WHC) zusammensetzten. Das Inkubationsexperiment wurde vom internationalen CryoCarb Konsortium ausgeführt.
Die Abundanzen von Ammoniak oxidierenden Archaea (AOA) und Ammoniak oxidierenden Bakterien (AOB) wurden mittels quantitativer Polymerase-Kettenreaktion ermittelt, die auf das funktionelle Markergen *amo*A ausgerichtet war, welches eine Untereinheit der Ammoniak-Monooxygenase kodiert.
Obwohl die AOA Populationen mit 9.6 x 10^5 Kopien pro Gramm Boden anfangs im Oberboden entscheidend größer waren als die AOB Populationen, sind Letztere unter

erhöhten Temperaturen und sauerstoffreichen Bedingungen (50 und 80 % WHC) erheblich angestiegen und haben vergleichbare Populationsgrößen erreicht.
Im Gegensatz dazu dominierten AOB Populationen den tieferliegenden begrabenen Oberboden in dem generell kleinere Populationsgrößen (8.4 x 10^3 Kopien pro Gramm Boden) vorgefunden wurden. Eine positive Korrelation dieser Populationen mit steigenden Temperaturen wurde unter allen Feuchtigkeitsbedingungen gefunden.
Hoch-Durchsatz *amo*A Amplikon Sequenzierung wurde zur detaillierten Analyse der Zusammensetzung der AOA Gemeinschaft des Oberbodens durchgeführt. Diese Arbeit zeigt, dass die vier vorgefundenen phylogenetischen Gruppen unterschiedliche und zum Teil gegensätzliche Dynamiken bezüglich ihrer Reaktionen gegenüber veränderten Umweltbedingungen entwickeln.

Brutto-Stickstoff-Mineralisierungsraten wurden mittels Pool-Verdünnung durch die Zugabe von ^{15}N markiertem Ammonium bestimmt, um auf potenzielle Effekte von veränderten Bedingungen auf die Prozesse und somit auf die geänderte Substratverfügbarkeit von Ammoniak Oxidierern zu schließen. Die Raten der Brutto-Stickstoff-Mineralisierung waren wesentlich höher im organischen Oberboden, verglichen mit dem tieferliegenden Mineralboden und dem begrabenen Oberboden, jedoch konnten NH_4^+ Produktion und NH_4^+ Aufnahme nicht direkt mit den Dynamiken der Ammoniak oxidierenden Populationen in Verbindung gebracht werden.

Zusammenfassend gibt diese Arbeit Einblicke in die Dynamiken von mikrobiellen Ammoniak Oxidierern unter dem Einfluss veränderter Umweltbedingungen, bewirkt durch den Klimawandel oder dem Prozess der Kryoturbation. Basierend auf den Resultaten stellt die Temperatur den einflussreichsten Faktor für Populationsveränderungen von AOA und AOB dar, und wirkt sich positiv auf die Abundanzen aus. Die klare Trennung der Bodenhorizonte vor der Inkubation ermöglichte eine Horizont-spezifische Untersuchung und zeigte Unterschiede zwischen dem Oberboden an der Oberfläche und dem begrabenen Oberboden. Zusätzlich wurden durch die Analyse der AOA Gruppe unterschiedliche Reaktionen der verschiedenen AOA Subpopulationen gezeigt. Diese Erkenntnisse demonstrieren, dass es für zukünftige Studien wichtig ist, funktionale Gruppen in einer hochauflösenden Weise zu betrachten, um Verbindungen zwischen Populationsdynamiken, Aktivitäten und Prozessen im Boden herstellen zu können.

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Publications and presentations

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