Niche Differentiation of Ammonia Oxidizing Bacteria and Archaea in Managed Soils

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Abstract

Soils offer humanity a multitude of ecosystem services, including production of food and clean drinking water, and many such services are driven by soil microbial communities. However, human activities are constantly affecting soil ecosystems through altered land use or various management strategies, and thereby influence microbial communities and their functions. Human activities also result in increasing amounts of nitrogen entering terrestrial ecosystems, which modifies the global nitrogen cycle. This can lead to a number of negative environmental effects such as increased amounts of nitrous oxide (N_2O) being emitted to the atmosphere or nitrate (NO_3^-) being leached from soils to surrounding water bodies, causing eutrophication.

This thesis explores how indigenous soil bacterial and archaeal communities are affected in managed soils, with emphasis on ammonia oxidizing bacteria (AOB) and archaea (AOA). The objectives were to identify specific environmental drivers for AOB and AOA and to evaluate how potential changes might affect their activity.

Long-term application of various fertilizer regimes on an agricultural soil was found to alter the total abundance of targeted bacterial and archaeal phyla and classes in similar ways, although different taxa-specific responses were observed for the relative abundance of certain phyla/classes. Long-term organic and fertilizer amendments also affected the ammonia-oxidizing community. The AOA appeared to be functionally more important in the nitrification process than the AOB at the studied field site. Also, the AOA dominated in abundance except under certain conditions, when the AOB dominated, suggesting niche differentiation between the two groups. Studies on a drained forested peat soil revealed that the AOB were numerically superior to the AOA, and that AOB and AOA community structures and AOB abundance exhibited temporal variation. Furthermore, a spatially focused study on an agricultural soil revealed contrasting spatial patterns between the AOB and AOA with regard to both abundance and structure, which correlated differently to soil properties. This too indicates niche differentiation between the two ammonia oxidizing groups and there were also indications that the AOA were the drivers of nitrate leaching from the studied agro-ecosystem.

In conclusion, the AOB and AOA communities clearly respond to different management strategies. Based on current knowledge, it is suggested that the size of the AOB and AOA communities could serve as a good bioindicator when monitoring soil status.

Keywords: soil, phylum, ammonia oxidation, *amoA* gene, AOB, AOA, qPCR

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Nischdifferentiering mellan ammoniak-oxiderande bakterier och arkéer i brukad mark

Sammanfattning

Jordens markekosystem förser mänskligheten med en mängd viktiga tjänster genom att t.ex. producera mat och rent dricksvatten. Många av dessa tjänster är beroende av markens mikroorganismer. Markekosystem påverkas idag till stor del av mänskliga aktiviteter som jord- och skogsbruk vilket även påverkar markmikroorganismerna och deras aktivitet. Grundämnet kväve är essentiellt för alla levande organismer, dock är det ofta brist på biologiskt tillgängligt kväve i många markekosystem. Detta har resulterat i att mänskligheten över tid på olika sätt har ökat kvävetillförseln till mark på konstgjord väg vilket har lett till en modifiering av kvävets naturliga kretslopp. Detta kan i sin tur resultera i en mängd olika negativa miljöeffekter såsom att ökade mängder av lustgas (N_2O) avges till atmosfären eller läckage av nitrat (NO_3^-) från mark till omgivande vattendrag vilket kan leda till övergödning.

Syftet med detta avhandlingsarbete var att studera hur markens mikroorganismer påverkas när den brukas på olika sätt, med fokus på en specifik grupp av markmikroorganismer, de ammoniak-oxiderande bakterierna (AOB) och arkeérna (AOA). Målet var att belysa parametrar som kan påverka förekomst, sammansättning och aktivitet av AOB och AOA i olika markekosystem som påverkats av mänsklig aktivitet.

Långsiktig användning av olika organiska och oorganiska gödningsmedel i en jordbruksmark visade sig påverka förekomsten av flera fylogenetiska grupper av bakterier och arkeér på samma sätt. Dock ändrades den relativa mängden av grupperna på olika sätt beroende på vilket gödningsmedel som tillförts marken. Denna långsiktiga användning av olika gödningsmedel visade sig även påverka AOB och AOA samhällena. Resultat indikerade att AOA var de mest aktiva ammoniak-oxiderarna i den studerade jordbruksmarken. AOA var även fler till antalet jämfört med AOB, förutom under vissa förhållanden, vilket indikerar en nischdifferentiering mellan de två ammoniak-oxiderande grupperna. Resultat från en annan marktyp, en dränerad och därefter skogsbevuxen torvjord, visade att AOB var dominerande till antal jämfört med AOA. Torvjordens AOB och AOA samhällsstruktur förändrades över tid, vilket även antalet AOB gjorde. En kartläggning av den rumsliga fördelningen av AOB och AOA i ytterligare en jordbruksmark påvisade kontrasterande mönster mellan både antalet och strukturen av de båda samhällena. Markparametrar som korrelerade till dessa förändringar överlappade aldrig utan korrelerade bara till ett av samhällena, vilket indikerar en nischdifferentiering mellan AOB och AOA även i denna mark. Dessutom indikerade resultat att det var AOA-samhället som drev nitratutlakningen från den studerade jordbruksmarken.

AOB och AOA samhället i mark påverkas markant av olika brukningsmetoder och baserat på nuvarande kunskap föreslås det att storleken på både AOB och AOA samhället skulle kunna användas som en bioindikator för markstatus.

Till pappa

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List of Publications

I Wessén, E., Hallin, S. and Philippot, L. (2010). Differential responses of bacterial and archaeal groups at high taxonomical ranks to soil management. *Soil Biology and Biochemistry* 42, 1749-1765.

II Wessén, E., Nyberg, K., Jansson, J.K. and Hallin, S. (2010). Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management. *Applied Soil Ecology* 45(3), 193-200.

III Andert, J., Wessén, E., Börjesson, G. and Hallin, S. Temporal changes in abundance and composition of ammonia oxidizing bacterial and archaeal communities in a drained peat soil in relation to N_2O emissions (manuscript).

IV Wessén, E., Söderström, M., Stenberg, M., Bru, D., Hellman, M., Welsh, A., Thomsen, F., Klemedtson, L., Philippot, L. and Hallin, S. Spatial distribution of ammonia oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning (*ISME J*; e-pub ahead of print 13 January 2011; doi: 10.1038/ismej.2010.206).

V Wessén, E. and Hallin, S. Abundance of archaeal and bacterial ammonia oxidizers – possible bioindicator for soil monitoring (In revision with Ecological Indicators).

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The contribution of the author to the papers included in this thesis was as follows:

I Performed the majority of the laboratory work, data analysis and writing of the manuscript

II Participated in planning the project, performed the majority of the laboratory work, data analysis and writing of the manuscript

III Performed parts of the laboratory work, and participated in minor parts of writing the manuscript

IV Performed parts of the laboratory work and data analysis, and a major part of writing the manuscript

V Participated in planning the project, and of writing the manuscript

In addition to Papers I-V listed above, the author performed the majority of the work in the following paper within the timeframe of this thesis:

Wessén, E., Philippot, L., Enwall, K., Jansson, J.K. and Hallin, S. Type of soil organic matter and nitrogen fertilization impact soil bacterial communities and their function at a long-term field site. Manuscript.

Abbreviations

amoA	gene encoding the ammonia monooxygenase enzyme
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
NH_3	ammonia
NH_4^+	ammonium
NO	nitric oxide
N_2O	nitrous oxide
N_2	dinitrogen gas
NO_2^-	nitrite
NO_3^-	nitrate
NMS	non-metric multidimensional scaling
qPCR	quantitative real-time PCR
RDA	redundancy analysis
T-RFLP	terminal-restriction fragment length polymorphism

1 Introduction

Microorganisms play a fundamental role in upholding the Earth's ecosystems. They are important drivers of major biogeochemical processes such as the cycling of carbon and nitrogen, and a variety of important habitats for these microorganisms are found in soils. However, anthropogenic activities such as agricultural production or changes in land use affect soils and their microbes in many different ways. As a result, environmental conditions are altered for the microorganisms and this may also alter the capacity of the soil to provide important ecosystem services and functions. Essential services provided by soils include production of food and clean drinking water and examples of important functions are cycling and processing of nutrients and support for terrestrial vegetation (Haygarth & Ritz, 2009). An understanding of how changing soil conditions affect the soil microbial community is important in identifying management strategies that will result in sustainable use of soil ecosystems in the long-term.

Nitrogen is a crucial element for plants, and indeed for all living organisms. A major issue within modern agricultural management is the increasing amount of nitrogen fertilizer that needs to be added to the soil in order to sustain food production. These growing amounts of nitrogenous compounds entering terrestrial ecosystems can lead to modifications of the nitrogen cycle (Galloway *et al.*, 2003), resulting in a number of negative environmental effects. These include acidification of soils, streams and lakes, eutrophication, increased production of the potent greenhouse gas nitrous oxide (N_2O) or even decreased microbial growth (Treseder, 2008; Vitousek *et al.*, 1997). Thus, increased knowledge of microorganisms involved in the cycling of nitrogen is important in order to improve nitrogen use efficiency and thereby reduce negative environmental effects. The cycling of nitrogen is regulated by several groups of microorganisms and one of the key processes involved is nitrification.

Nitrification is a two-step reaction. The first step, considered to be rate limiting, is conducted by the ammonia oxidizing bacteria (AOB) and archaea (AOA), while the second step, nitrite oxidation, is carried out by the nitrite oxidizing bacteria. The ammonia oxidizing organisms are both agriculturally and environmentally important due to their role in the cycling of nitrogen. In addition, ammonia oxidation is an important part of the nitrogen removal process in wastewater treatment plants. However, it can also lead to nitrate (NO_3^-) leaching from soil, and thereby to contamination of groundwater and freshwater, and can contribute directly or indirectly to emissions of N_2O (Kowalchuk & Stephen, 2001; Schuster & Conrad, 1992).

1.1 Aims and outline of the thesis

The aims of this thesis were: to explore how indigenous soil bacterial and archaeal communities are affected in managed soils, with emphasis on the AOB and AOA; to identify specific environmental drivers for the AOB and AOA; and to evaluate how potential changes might affect their activity. These were addressed in the following papers:

Paper I: Effects of fertilization on the abundance of major bacterial and archaeal taxa are poorly understood. Hence, a model system comprising different long-term fertilization treatments in agricultural soil was sampled to explore the response of bacterial and archaeal communities to varying fertilization regimes. The overall aim was to identify possible drivers of soil bacterial and archaeal groups, targeted at high taxonomic ranks.

Paper II: Impacts of changing soil nutrient and soil organic matter content on the AOB and AOA communities needs further elucidation. Soil was sampled from plots in a long-term field trial amended with either labile (straw) or more recalcitrant (peat) organic matter, with or without nitrogen fertilization. The overall aim was to assess the response of the AOB and AOA communities to soil organic matter quality and soil organic carbon and nitrogen content.

Paper III: Peat soils play an important role in global climate change due to their potential as sinks and sources of greenhouse gases, but the role of the AOB and AOA in these soils is relatively unknown. The overall aim was to explore relationships between temporal differences in the abundance and community structure of the AOB and AOA in a drained forested peat soil and *in situ* emissions of N_2O and ammonia oxidation capacity.

Paper IV: Understanding spatial patterns of the AOB and AOA in soil might generate information regarding processes that shape the size and structure of these communities and their activity. Soil was sampled from a 44-hectare large farm consisting of two farming systems subjected to different fertilization regimes. Spatial distributions of the AOB and AOA community activity, abundance and structure were mapped and related to nitrate leaching from the soil. The overall aim was to explore spatial distributions of the AOB and AOA at field scale related to different soil parameters and ecosystem functioning.

Paper V: The AOB have frequently been proposed as good ecological indicators due to their sensitivity to changes in environmental conditions (Kowalchuk & Stephen, 2001). Indeed, the community structure of the AOB was recently given top ranking as a biological indicator for soil monitoring (Ritz *et al.*, 2009). The overall aim was to advocate a re-evaluation to include the AOA with the AOB to be regarded as good bioindicators and to propose quantitative real-time PCR (qPCR) to be the choice of method when monitoring status of soil.

2 Soils and their microbes

Soils, defined as the top layer of the Earth´s crust, are a mixture of inorganic and organic constituents. They are highly complex and can differ in composition regarding texture, amount of roots, amount of organic and inorganic materials, moisture content, salinity and pH both over short and long distances. These factors and others lead to soils offering a variety of heterogeneous habitat ‘niches’ for microorganisms that can be highly variable, both at macroscopic and microscopic scale. In the past, *Bacteria* and *Archaea* were classified in the same group, but this changed when Woese *et al.*, (1990) proposed a new tree of life containing the three domains *Bacteria*, *Archaea* and *Eukarya*. The *Bacteria* and *Archaea* domains have been shown to be highly diverse and include microorganisms that inhabit the soils of every terrestrial ecosystem (Sylvia *et al.*, 1997). In a single gram of soil there are probably thousands or even millions of different bacterial and archaeal species and their total number would be in the order of 10^7-10^{10} (Torsvik *et al.*, 2002; Whitman *et al.*, 1998; Torsvik *et al.*, 1990). It was long believed that the *Bacteria* domain included microorganisms found in all types of environments and that the *Archaea* only contained microorganisms that grew in extreme ecosystems. However, since the late 1990s, representatives of the *Archaea* domain have also been found in a wide variety of non-extreme environments (DeLong, 1998).

Soils provide ecosystems and the human population with a number of services and functions, and are a rich source of biological productivity and biodiversity on Earth (Bone *et al.*, 2010). Many of these services and functions are largely driven by soil microorganisms, which can be regarded as the engines of the major biogeochemical processes whereby chemical elements are transformed and moved within and between different ecosystems. Examples of more specific processes that are determined by microbial activities are decomposition of organic matter and cycling of nitrogen (Griffiths *et al.*, 2000; Wardle & Giller, 1996). Awareness of the importance of soil ecosystems and their functions is increasing (Bone *et al.*, 2010; Ritz *et al.*, 2009) and this is leading to increasing demand for knowledge regarding soil microbial communities and their drivers. There are many factors, including human activities, that can influence soil microorganisms and their functions and thus generate significant changes in soil characteristics. This results in managed soils being interesting study objects when seeking to identify significant drivers of microbial communities and possibly also their functions.

2.1 Human activities affect bacterial and archaeal communities and their functions in soil

Ecosystem properties are said to depend greatly on the organisms present, as well as their functional characteristics, distribution and abundance (Hooper *et al.*, 2005). It is known that human activities such as agricultural management and deforestation affect soil ecosystems in a multitude of ways, including impacts on the bacterial and archaeal communities (Roesch *et al.*, 2007; Borneman & Triplett, 1997). However, the degree to which these human activities affect soil bacterial and archaeal diversity and how much this ultimately affects functions and services provided by soil ecosystems is still not fully understood.

Agricultural systems represent a type of soil that is heavily affected by human activities, and intensive management can lead to a decrease in soil organic carbon content and total nitrogen content (Hamer *et al.*, 2008). Changes in the carbon and nitrogen cycles will unavoidably affect the soil bacterial and archaeal community, since there is a strong connection between microbial processes and these cycles (Taylor & Townsend, 2010). Loss of soil nutrients can result in decreased productivity, which could generate problems in meeting future global food demands. Furthermore, when nutrients are not incorporated into plant biomass, they are likely to end up as mobile elements or gaseous compounds causing negative environmental effects. One commonly used method to counteract problems with nutrient losses from soils is to apply organic or inorganic fertilizers that boost primary production. However, agricultural fertilization is known to also affect soil bacterial and archaeal communities in terms of size, structure and activity (Shen *et al.*, 2010; Birkhofer *et al.*, 2008; Jangid *et al.*, 2008; Enwall *et al.*, 2007).

As well as agricultural soils that are more or less continuously being affected by human activities, there are some soils that are currently undisturbed, but which were previously subjected to anthropogenic activities. An example of such a soil ecosystem is drained peat. Undisturbed peatlands are a natural sink for carbon and nitrogen, but in drained peats these sinks can be changed into sources of greenhouse gases, since drainage can lead to major changes in many soil parameters. The lowering of the groundwater level brought about by drainage increases the aeration of the peat and thus also its rate of decomposition. In addition, temperature, pH and litter quality are altered post-drainage, as well as carbon and nitrogen dynamics (Minkkinen *et al.*, 2002; Kasimir-Klemedtsson *et al.*, 1997). These changes in environmental conditions will have an unavoidable impact on the indigenous soil bacterial and archaeal communities and their activities.

The practices mentioned above are examples of how human activities can affect bacterial and archaeal communities in soil. Maintaining species diversity in managed systems is becoming increasingly important from both an economic and ecological view, because systems with low diversity are potentially more sensitive to various disturbances, and thus might be less likely to maintain high productivity in the long-term (Hooper *et al.*, 2005). It is therefore important to determine how human activities drive the changes in bacterial and archaeal diversity, and how this is coupled to changes in ecosystem functions. These questions and their links to the overall environmental consequences are of central importance in the field of microbial ecology.

2.2 Effects of agricultural management on bacteria and archaea in soil

Agricultural management regimes often include fertilization in order to attain high productivity and this can greatly alter soil parameters (Jangid *et al.*, 2008). It is generally accepted that agricultural management also affects microbial community structure (Shen *et al.*, 2010; Gu *et al.*, 2009; Stark *et al.*, 2008; Steenwerth *et al.*, 2002; Buckley & Schmidt, 2001). However, the specific effects of individual soil parameters on different groups of bacteria and archaea need further exploration. **Paper I** assessed the influence of different fertilizer regimes on several taxonomic groups of the soil bacterial and archaeal community. Most studies examining similar research questions have focused on targeting the microbial communities at species or sub-species level using different fingerprinting techniques such as terminal-restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE) or phospholipid fatty acid analysis (PLFA) (Hamer *et al.*, 2008; Wakelin *et al.*, 2008; Hartmann *et al.*, 2006). However, it was recently argued by Philippot and colleagues (2010) that it is possible to show ecological coherence between bacterial groups targeted at higher taxonomic ranks, such as phylum or class. If true, this would enable the identification of biological patterns by characterizing bacterial communities at phylum level that can be related to specific soil parameters, and thereby to identify potential environmental drivers for each phylum. It could also aid in identifying the environmental conditions that promote different taxonomic groups. The idea of targeting microbial communities at higher taxonomic levels is not new, and others have previously reported observed effects of different land use practices at these levels (Fierer *et al.*, 2007; Roesch *et al.*, 2007; Ochsenreiter *et al.*, 2003). However, studies addressing these questions often focus on differences between communities in soil sampled from various soil types over regional and global scales. Soils can vary greatly between sites with regard to physical and chemical properties, which can have strong effects on both the presence and activity of the bacterial and archaeal communities. It has previously been shown that soil type is an important determinant of bacterial communities in soil (Girvan *et al.*, 2003), and that land use influences phylum richness, e.g. with increased richness being identified in a forest soil compared with three agricultural soils (Roesch *et al.*, 2007). In order to identify specific treatment effects on individual groups of bacteria and archaea, and possibly also find drivers, it can thus be beneficial to compare soils with the same geological origins, thereby excluding soil type as a potential overriding factor.

Figure 1. The Ultuna long-term soil organic matter field experiment at Ultuna campus, Swedish University of Agricultural Sciences, Uppsala, Sweden (Photo: E. Wessén).

Paper I describes the responses of bacterial and archaeal groups identified at phylum and class level to different long-term fertilization regimes at the same field site (Figure 1). Fertilizer regimes were found to alter soil characteristics, but quantification of seven bacterial and archaeal phyla and one bacterial class revealed that total abundance of the eight groups was affected in similar ways, irrespective of fertilization treatment. However, certain taxa responded differently to the fertilizer treatments in terms of relative abundance of total bacteria. Similarly, in previous studies soils of differing origins have been suggested to be rather stable with regard to higher taxa being represented, but instead vary with regard to relative abundance of the different taxa (Youssef & Elshahed, 2009). Philippot *et al.*, (2009) found similar results to those reported in **Paper I**, with no difference in total abundance between targeted taxa in a grazed field subjected to varying impacts by cattle, but with clear differences between relative abundances of targeted groups in different parts of the field. Our results showed that the Firmicutes increased in relative abundance in plots treated with ammonium sulphate, while the Verrucomicrobia declined in abundance. This confirms findings from a study of an Alpine tundra soil, where nitrogen fertilization had a negative effect on the Verrucomicrobia (Nemergut *et al.*, 2008). Additions of peat and ammonium sulphate fertilization were both found to increase the relative abundance of Acidobacteria in **Paper I**. This was attributed to the low pH observed in the studied soils, which might have provided a habitat preferred by members of these taxa. Soil pH has previously been shown to be a strong driver for the soil microbial community composition (Shen *et al.*, 2010; Lauber *et al.*, 2009; Fierer & Jackson, 2006). The only clear treatment effect found in **Paper I** for the archaeal and crenarchaeal community was a negative effect of peat addition. This treatment gave rise to a high organic carbon content and C/N ratio in the soil, which might be a strong driver. Similarly, Nemergut *et al.*, (2010) reported a negative correlation between relative abundance of archaea and soil carbon and C/N ratio.

2.3 The nitrogen cycle in soil

When comparing the impact of anthropogenic activities on elemental biogeochemical cycles, it is the nitrogen cycle that is most strongly influenced (Arp & Stein, 2003). It is claimed that human activities have led to an approximate doubling of the rate by which nitrogen is entering the terrestrial nitrogen cycle (Schlesinger, 2009; Vitousek *et al.*, 1997). A major source of this increase is the worldwide use of nitrogen fertilizers (Galloway *et al.*, 2003). However, instead of being incorporated into belowground or aboveground biomass, much of the nitrogen entering the environment through human activities is lost, causing environmental problems (Schlesinger, 2009). Two ways by which nitrogen can be lost from soil and cause environmental problems is through formation of gaseous N_2O, which is emitted to the atmosphere, or through formation of soluble NO_3^-, which can be leached to surrounding water bodies. N_2O is a potent greenhouse gas, which is reported to have a global warming potential 310 times greater that of carbon dioxide (CO_2) (IPCC, 2007). Nitrate leaching can lead to eutrophication of lakes and streams, resulting in oxygen depletion of affected ecosystems (Kowalchuk & Stephen, 2001). In addition, an excess level of NO_3^- in drinking water is an important human health issue which can cause fatal conditions in infants (Schlesinger, 2009; Vitousek *et al.*, 1997).

A number of groups of microorganisms regulate the various steps of nitrogen transformations in the nitrogen cycle, but in soil it can be simplified to three main steps. The first process is biological nitrogen fixation, whereby atmospheric dinitrogen gas (N_2) is converted to ammonia (NH_3). The second process is nitrification, whereby NH_3 is converted into nitrite (NO_2^-) and NO_3^-. The third process, which completes the circle, is denitrification, whereby oxidized forms of nitrogen are converted to the gases nitric oxide (NO), N_2O and N_2. These processes can operate more or less optimally depending on surrounding factors, making it important to understand the links between management strategies and organisms driving the nitrogen cycle.

2.3.1 Oxidation of ammonia

Autotrophic nitrification is a two-step reaction in which NH_3 is converted via NO_2^- into NO_3^-. The first step is ammonia oxidation, considered to be the rate-limiting step, which is carried out by ammonia oxidizing bacteria and archaea belonging to the phylum Proteobacteria and Crenarchaeota/Thaumarchaeota, respectively. The second step is nitrite oxidation, which is carried out by the nitrite oxidizing bacteria and all known members of this group are today thought to belong to the phyla Proteobacteria and Nitrospira. Both steps in the nitrification process are aerobic and the organisms use inorganic nitrogen and CO_2 as their energy and carbon source, with oxygen being the terminal electron acceptor (Könneke *et al.*, 2005; Prosser, 1989). The autotrophic nitrification process has been studied for a long time, but it is today known that there are also other reactions by which ammonia or ammonium can be transformed.

Heterotrophic microorganisms can contribute to the nitrification process and members are found over a wide phylogenetic range of bacteria and fungi. However, the reactions of heterotrophic nitrification are reported to not be energy-yielding and thus not contribute to cellular growth. Both the phylogenetic diversity and physiological role of these microorganisms are unclear (Hayatsu *et al.*, 2008). Anaerobic ammonia oxidation (anammox), in which ammonium (NH_4^+) combined with NO_2^- is transformed into N_2 directly under anoxic conditions, is a process that contributes significantly to the cycling of nitrogen in marine environments. The role of anammox bacteria in soil is still unclear, although it is postulated that they might be important (Nannipieri & Eldor, 2009; Hayatsu *et al.*, 2008). However, organisms involved in heterotrophic nitrification and the anammox process were not included in the studies described in this thesis.

3 Ammonia oxidizing bacteria and archaea

The first step in the nitrification process is the oxidation of ammonia. The overall biochemical reaction of this step, which is conducted by the ammonia oxidizing microorganisms, starts with conversion of ammonia into hydroxylamine, which is further converted into nitrite (Figure 2). The process of oxidizing ammonia into hydroxylamine (1) is catalyzed by the enzyme ammonia monooxygenase (AMO), while the conversion of hydroxylamine (2) is catalyzed by the enzyme hydroxylamine oxidoreductase (HAO).

$$\mathbf{1)\ NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O}$$

$$\mathbf{2)\ NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-}$$

Figure 2. Overall transformation of ammonia into nitrite. The first step (1) comprises oxidation of ammonia into hydroxylamine, and the second step (2) oxidation of hydroxylamine into nitrite.

Ammonia oxidation was until recently considered to be solely performed by the ammonia oxidizing bacteria. The AOB were discovered in the late 1800s (Winogradsky, 1892), while it took over 100 years longer before the ammonia oxidizing archaea were found. This discovery was not the result of a specific search for ammonia oxidizing archaea, but rather a coincidental finding from two metagenomic studies on seawater (Venter *et al.*, 2004) and soil (Treusch *et al.*, 2005). These two studies revealed *amo*A genes, encoding the active site of ammonia monooxygenase, in uncultivated Crenarchaeota. At the same time, the first successful cultivation of an ammonia oxidizing archaea, *Nitrosopumilus maritimus*, was reported by Könneke *et al.*, (2005), who isolated and named the organism. To date, a number of published studies have addressed the relationship between these two groups of ammonia oxidizers in order to obtain more information on their ecology and activity (reviews by Francis *et al.,* (2007) and Prosser & Nicol, (2008) and references therein).

3.1 Ammonia oxidizing bacteria

Bacterial ammonia oxidizers are ubiquitous and yet they usually comprise <1% of the total bacterial community present (Francis *et al.*, 2007; He *et al.*, 2007). Much of our understanding of the AOB is based on results from studies of pure cultures. However, such studies are both difficult and time-consuming, since these organisms are slow-growing (Kowalchuk & Stephen, 2001). Consequently, much of the recent literature describing the AOB is based on information generated from molecular studies. Taxonomically, the AOB are found in any of the three genera *Nitrosomonas*, *Nitrosospira* and *Nitrosococcus*, with the first two genera belonging to the Betaproteobacteria (β-proteobacteria) and the third to the Gammaproteobacteria (γ-proteobacteria). Bacterial ammonia oxidizers belonging to *Nitrosomonas* or *Nitrosospira* have been found in a variety of ecosystems, while the AOB belonging to *Nitrosococcus* have so far only been found in marine habitats. Cell shape varies from the spherical *Nitrosococcus* to the straight rods of *Nitrosomonas* and the tightly coiled spirals of the *Nitrosospira*.

All known AOB use ammonia as their sole energy source and their optimal activity is, in general, at mesophilic temperature and neutral-alkaline pH. However, there are distinct differences between species with regard to ecophysiological characteristics, such as preferred substrate concentration, capacity to use urea as a source of ammonia and salt requirement, upon which members of the AOB can be identified. Maximum ammonium tolerance varies from 50 mM (NH_4Cl; pH 8.0) for *Nitrosomonas oligotropha* and *Nitrosospira multiformis* up to 1000 mM for *Nitrosococcus oceani*, while maximum salt tolerance varies from 100 mM for *Nitrosospira tenuis* to 1800 mM for *Nitrosococcus halophilus* (Koops *et al.*, 2006). The ecophysiological characteristics described for the different species generally follow the species phylogeny within the *Nitrosomonas*, but no such pattern has been found for the *Nitrosospira* or the *Nitrosococcus* (Campbell *et al.*, 2011; Koops & Pommerening-Röser, 2001). Preferred habitats for the defined AOB species are rather diverse and vary between sewage sludge, eutrophic freshwater, oligotrophic freshwater, soils, marine environments and salt lakes. These differences, combined with many other ecophysiological characteristics, are important aspects that influence the distribution of the AOB in nature (Koops *et al.*, 2006).

Table 1. *AOB isolated to date, with their current classification and geographical origins*

Organism	Class	Origin	Reference
Nitrosospira briensis	β-proteobacteria	Soil, Crete	Winogradsky & Winogradsky, 1933
Nitrosospira multiformis	β-proteobacteria	Soil, Surinam	Watson *et al.*, 1971
Nitrosospira tenuis	β-proteobacteria	Soil, Hawaii	Harms *et al.*, 1976
Nitrosomonas europaea	β-proteobacteria	Soil, France	Winogradsky, 1892
Nitrosomonas cryotolerans	β-proteobacteria	Kasitsna Bay, Alska	Jones *et al.*, 1988
Nitrosomonas communis	β-proteobacteria	Soil, Greece	Koops *et al.*, 1991
Nitrosomonas ureae	β-proteobacteria	Soil, Italy	Koops *et al.*, 1991
Nitrosomonas aestuarii	β-proteobacteria	Brackish water, Denmark	Koops *et al.*, 1991
Nitrosomonas marina	β-proteobacteria	Shell grit, Australia	Koops *et al.*, 1991
Nitrosomonas nitrosa	β-proteobacteria	Activated sludge, Germany	Koops *et al.*, 1991
Nitrosomonas eutropha	β-proteobacteria	Municipal sewage, Germany	Koops *et al.*, 1991
Nitrosomonas oligotropha	β-proteobacteria	Soil, Germany	Koops *et al.*, 1991
Nitrosomonas halophila	β-proteobacteria	North Sea	Koops *et al.*, 1991
Nitrosomonas mobilis	β-proteobacteria	South Pacific Ocean	Koops *et al.*, 1976
Nitrosococcus oceani	γ-proteobacteria	Atlantic ocean, USA	Watson, 1965
Nitrosococcus halophilus	γ-proteobacteria	Salt Lagoon, Italy	Koops *et al.*, 1990
Nitrosococcus watsonii	γ-proteobacteria	Mediterranean Sea, Egypt	Campbell *et al.*, 2011

3.2 Ammonia oxidizing archaea

Understandably, the number of isolated AOA is not as great as that of the AOB, due to their more recent discovery. To date, there is one isolated AOA, *Nitrosopumilus maritimus*, and two cultured representatives in enrichments that both originate from hot springs; *Nitrosocaldus yellowstonii* and *Nitrososphaera gargensis* (de la Torre *et al.*, 2008; Hatzenpichler *et al.*, 2008). In addition, *Crenarchaeum symbiosum*, the archaeal symbiont of the marine sponge *Axinella mexicana* (Preston *et al.*, 1996), has been reported to potentially function as an autotroph and harbour genes predicted to encode ammonia monooxygenase subunits. Thus, it is possibly able to use reduced nitrogen as an energy source, with autotrophic metabolism (Hallam *et al.*, 2006). However, this symbiont has not been cultivated to date.

Nevertheless, results from studies using a direct molecular approach show that the AOA are ubiquitous and they have even been found to outnumber the AOB in marine and most terrestrial ecosystems (Erguder *et al.*, 2009). Phylogenetically, there is an ongoing debate regarding where the AOA belong. The domain *Archaea* was long believed to be divided into the Euryarchaeota and the Crenarchaeota and the AOA were initially placed within the Creanarchaeota. Many retrieved *amoA* sequences from soil and marine environments form two separate clades of mesophilic crenarchaea (Prosser & Nicol, 2008). However, these mesophilic crenarchaea have recently been proposed to instead represent a novel archaeal phylum named Thaumarchaeota (Brochier-Armanet *et al.*, 2008). Based on the genomes of *Nitrosopumilus maritimus* and *Nitrososphaera gargensis*, the AOA have been suggested to belong to this new phylum (Spang *et al.*, 2010).

The cultured AOA, *Nitrosopumilus maritimus*, can grow under ammonia concentrations below the detection limit of 10nM, which is 100-fold lower than reported for cultivated AOB (Martens-Habbena *et al.*, 2009). However, it has a similar growth rate as found for the AOB (Könneke *et al.*, 2005). Archaeal *amoA* genes can be retrieved at wide temperature and pH ranges, suggesting broad ecological and phylogenetic diversity within this group of ammonia oxidizers too (Erguder *et al.*, 2009). However, the number of characterized AOA species needs to increase before explicit information regarding ecophysiological characteristics of the AOA can be obtained.

Table 2. *AOA isolated or cultured to date, with their geographical origins*

Organism	Origin	Reference
Nitrosopumilus maritimus[a]	Aquarium water, USA	Könneke *et al.*, 2005
Nitrosocaldus yellowstonii[b]	Terrestrial hot spring, USA	de la Torre *et al.*, 2008
Nitrososphaera gargensis[b]	Garga hot spring, Russia	Hatzenpichler *et al.*, 2008

[a] Isolated

[b] Enrichment culture

3.3 Targeting the AOB and AOA using molecular methods

Pure culture studies are important in order to obtain data on the ammonia oxidizing communities, since this will generate information regarding growth strategies and preferred niches. Nevertheless, the results from microcosm studies of pure cultures will most likely not reflect the response of the whole ammonia oxidizing community found in an agricultural field. Thus, studies of these organisms in their natural habitat are important in order to determine their ecological significance.

When using molecular methods to study the ammonia oxidizing community, the AOB can be targeted either by 16S rRNA genes or *amo*A genes. Targeting the 16S rRNA genes has been shown to result in superior resolution in tree topologies compared with targeting the *amo*A genes, although with high congruency between tree topologies (Purkhold *et al.*, 2003). However, there are some strong advantages with targeting the *amo*A genes, since this approach potentially decreases the risk of targeting taxonomically related organisms compared with the actual physiologically and ecologically different ammonia oxidizers present in a particular sample (Junier *et al.*, 2010). There is sufficient divergence between *amoA* genes in AOB and AOA so that this functional marker can be used as the target gene when distinguishing between the AOB and AOA communities in environmental samples (Leininger *et al.*, 2006; Treusch *et al.*, 2005; Rotthauwe *et al.*, 1997).

3.4 Relative contributions of AOB and AOA to ammonia oxidation in soils

Following the identification of the AOA, the question emerged as to whether this group is functionally important for ammonia oxidation in the environment. It is now clear that the AOA play an important role in ammonia oxidation in marine ecosystems (Erguder *et al.*, 2009; You *et al.*, 2009), but their role in soil is still being debated. There are many studies showing evidence of the AOA dominating in abundance over the AOB in soils (Di *et al.*, 2010; Adair & Schwartz, 2008; Chen *et al.*, 2008; Nicol *et al.*, 2008; Shen *et al.*, 2008; He *et al.*, 2007). This suggests that the AOA might be significant players in the nitrification process in some soil environments. Many have also reported shifts in the abundance and structure of both the AOB and the AOA communities as a result of different management strategies in soil. These changes have often been related to measured nitrification activity, but the results thus far have failed to present a unified picture regarding the group that contributes most to ammonia oxidation in soils. Evidence of functional dominance has been presented for both the AOB and the AOA (Zhang *et al.*, 2010; Jia & Conrad, 2009), and functional redundancy between the two groups has even been suggested (Schauss *et al.*, 2009). It is important to bear in mind that the presence of AOA may be unrelated to ammonia oxidation activity, since they may have alternative growth strategies to the AOB. However, several studies have suggested the contrary (Erguder *et al.*, 2009; You *et al.*, 2009; Hayatsu *et al.*, 2008; Prosser & Nicol, 2008; Leininger *et al.*, 2006).

A number of previous studies have described the differing contributions of the AOB and AOA to ammonia oxidation in the environment. It has been suggested that the AOB are favoured by nutrient-rich, high pH and moderate temperature conditions, while the AOA are favoured in soils with lower nutrient status, low pH and more extreme temperatures (Verhamme *et al.*, 2011; Di *et al.*, 2010; Schleper, 2010; Erguder *et al.*, 2009; Koops *et al.*, 2006). If there is a difference in habitat preference between the AOB and AOA, this could be the result of necessary specialization, since the two groups potentially compete across varying ecosystems. However, even though it seems that the AOA might be favoured in low energy conditions, such as unfertilized forest soils or oligotrophic waters, or that they might be adapted to more extreme conditions (Schleper, 2010; Valentine, 2007), this does not necessarily mean that the AOA are always the active nitrifiers in soil systems under more extreme growing conditions. This was shown in a study of a zinc-contaminated soil where the bacterial rather than the archaeal ammonia oxidizers restored nitrification in the soil, while the AOA were more sensitive to soil perturbation (Mertens *et al.*, 2009). Nevertheless, the role of the ammonia oxidizers in terrestrial ecosystems is today an active research area and conflicting findings are constantly being reported regarding the relative contributions of the AOB and AOA to terrestrial ammonia oxidation. This clearly shows that the topic needs further exploration.

4 Drivers and roles of the AOB and AOA in soils

It is essential to understand the links between important environmental drivers and the AOB and AOA in soils in order to further elucidate their roles with regard to distribution and activity. Thus it is important to study the effects on the two ammonia oxidizing communities of single environmental parameters, such as soil pH and temperature, as well as the effects of e.g. land management or the environmental impact of other human activities that can result in a change in multiple environmental parameters. Even though we have information from molecular studies on the dominance of the AOA over the AOB in many soil ecosystems (Erguder *et al.*, 2009), according to current literature no AOA have been isolated from soil. Our restricted knowledge of AOA physiology limits our understanding of the respective ecological roles of the AOB and AOA. However, the number of isolated representatives is likely to increase and this may greatly facilitate our understanding of this seemingly important group of ammonia oxidizers. Nevertheless, there is today a growing body of literature addressing questions regarding drivers and ecological roles of the AOB and AOA, both within and between the two groups, using non-cultivation based methods.

4.1 Environmental influences on AOB and AOA communities

4.1.1 Approaches to study changes in soil microbial communities

Various approaches can be used to study changes in microbial communities in soil and one way is to use model systems representing different soil types or management strategies. The subjection of agricultural soils to various treatments, such as different fertilization regimes, often results in distinct changes in soil properties, and these changes will also affect the soil microbial community. Managed agricultural soils undergoing long-term treatments can be valuable for identifying correlations between microbial composition and ecosystem function (Reed & Martiny, 2007). It is important to remember that identifying correlations is not the same as identifying causal relationships, but correlations can still indicate the factors that influence ecosystem processes and the microbial community.

Using temporal gradients is another approach to assess drivers of microorganisms in a particular soil system. This can be done with the aim of evaluating variation in a specific microbial community in the soil with regard to size or composition during a constantly occurring change, such as seasonal shifts, changes in temperature or precipitation. This approach can also be used to study variation in response over time, e.g. to monitor the recovery of an ecosystem following a single strong disturbance such as an oil spill.

A third possibility is to apply a spatially focused approach. Soil factors can vary over space in undisturbed systems due to natural fluctuations in soil parameters, but also in managed systems due to changing management strategies. Information on how these variations affect spatial patterns in the indigenous microbial community can be of interest in identifying important environmental drivers for microbial communities at different scales.

4.1.2 Treatment effects: Fertilization regime and soil amendment

The amount and source of nutrients and organic materials added to the soil depend on fertilization regime and have an influence on soil properties. When striving to achieve sustainable agricultural practices, important questions can be answered using model systems such as long-term treatment experiments, as was done in **Papers I** and **II**. The data presented in these studies originated from soil taken from the long-term soil organic matter field trials located in Ultuna, Sweden (Figure 1; Kirchmann *et al.*, 1994). These were established in 1956 to study the effects of different organic and inorganic fertilizers on soil properties and crop yields. The soil at the site is a clay loam and the field trial is a block design, with four independent replicate blocks. In **Paper I**, soil from this field site was used to study management effects on bacterial and archaeal groups defined at higher taxonomic ranks (discussed in section 2.2), while in **Paper II** the focus was on the ammonia oxidizing community. The effects of organic amendments with labile (straw) and more recalcitrant (peat) carbon, with or without addition of easily plant-available nitrogen, on the AOB and AOA communities in terms of abundance, structure and activity was evaluated in **Paper II**. Plots with added peat showed the strongest changes in soil characteristics, with a significant increase in soil organic carbon content and a significant decrease in soil pH. These changes were reflected in the ammonia oxidizing community. Substrate-induced ammonia oxidation rates were lowest in the peat-amended soil and the activity was correlated to the abundance of AOA, but not AOB. The same treatment also gave rise to the most differing AOB and AOA community structures. AOA abundance was positively related to addition of more labile carbon, which led us to suggest that type of organic matter could play a role in growth of the AOA community. Contradictory correlations between soil properties/activity and AOB and AOA abundance also indicated niche partitioning between the two groups.

4.1.3 Temporal and spatial heterogeneity

Exploring potential changes in microbial communities in different sampling years and in different seasons within years is interesting, since this provides information regarding natural fluctuations in microbial communities. The abundance of bacterial and archaeal phyla/classes in the long-term field trials at Ultuna (Figure 1) were compared for two sampling occasions five years apart in **Paper I**. Of the eight taxonomic groups targeted, two groups, the Actinobacteria and Bacteriodetes, changed significantly in abundance between the sampling occasions. There are many possible reasons for this, but one explanation could be changes in abiotic factors such as temperature and precipitation close to sampling times. The Actinobacteria have been shown to change with changing soil moisture (Alekhina *et al.*, 2001), while the Bacteriodetes are reported to be highly variable in soil (Fierer *et al.*, 2007). Interestingly, most of the groups were stable between sampling occasions, indicating robustness of the communities. However, the observed stability could be due to high physiological diversity within these phyla resulting in potential changes over this time span not being detected when targeting high taxonomic ranks.

It was recently shown by Drotz *et al.*, (2010) that, contrary to earlier beliefs, microbial processes can continue in frozen soil, making it interesting to study how seasonal changes influence soil microbial communities. In **Paper III** we examined the effect of seasonal changes on the ammonia oxidizing community in a peat soil, a habitat where the AOB and AOA are relatively unexplored. Peat soils play a significant role in global climate change due to their potential as a source or sink of greenhouse gases (Kasimir-Klemedtsson *et al.*, 1997). **Paper III** explored the relationships between temporal changes in substrate-induced ammonia oxidation rates or N_2O emissions and the AOB and AOA communities at the site. Soil was sampled in four different seasons, with two of these sampling occasions representing a cold period and the other two a warm period. No temporal differences with regard to substrate-induced ammonia oxidation rates were observed at the site, but the N_2O emissions increased with lower temperature, indicating temporal variation. Temporal differences were also observed for the AOB and AOA community structures, with the warmest sampling period producing the most differing community structures. In addition, size of the AOB community was found to vary over time, with lower abundance being observed during the cold period. In agreement, Rasche *et al.*, (2011) reported that AOB abundance in a temperate beech forest was significantly affected by seasonality, however, they showed a negative correlation between AOB abundance and temperature. In addition they also found that seasonality significantly effected AOA abundance which remained relatively unvaried throughout the sampling period in **Paper III**.

Studies of the distribution of bacteria and archaea in soil can provide information regarding biodiversity and also give an insight into the ecology and life-history strategies for non-cultured bacteria and archaea (Fierer *et al.*, 2009; Philippot *et al.*, 2009). In addition, understanding how microorganisms are spatially distributed at different scales might reveal important drivers for these communities, because factors shaping the community at the micro scale might not be the same at the macro scale. In other words, different factors could be important at different scales. Targeting groups based on a functional trait rather than taxon could further deepen our awareness of relationship between the ecology and activity of soil bacteria and archaea. Furthermore, targeting functional communities at larger scales can provide results of interest for management strategies. In **Paper IV**, spatial patterns of the AOB and AOA were studied at Logården, a 44-hectare experimental farm in Sweden with a silty clay loam soil. The experiments on the farm started in 1991 and comprise separate farming systems on two fields, one integrated (26 ha) and one organic (18 ha), with a different fertilization regime and individual 7-year crop rotation for each system. Spatial distributions of both the abundance and structure of the two communities were mapped, together with substrate-induced ammonia oxidation rates, measured *in vitro*, and nitrate leaching from the soil, measured *in situ*. The abundance and structure of the targeted AOB and AOA communities all exhibited spatial patterns at hectare scale, confirming findings by others of patterns of ammonia oxidizing communities at large scales (Bru *et al.*, 2010; Fierer *et al.*, 2009). The community structure patterns for both the AOB and AOA did not reflect the farming system, but were correlated to different soil parameters. Patterns of AOB and AOA abundances correlated differently to soil parameters and to measured community and ecosystem functions.

4.2 Drivers of AOB and AOA communities in soil ecosystems

Questions of what environmental factors that control the diversity and relative abundance of AOB and AOA in different systems have frequently been addressed. The determination of such factors could potentially identify important drivers for the two communities. Another frequently addressed issue is whether the AOB or AOA drive the oxidation of ammonia in a given soil. These are complex questions not possible to address fully in only one or a few studies, and there are still a lot of unknowns regarding the relationship between the two ammonia oxidizing groups. Many studies have reported contradictory findings regarding effects and responses of the AOB and AOA to various soil environmental conditions, which could be the result of soil heterogeneity.

4.2.1 Soil pH, temperature and moisture

Soil pH is known to affect microbial community composition in soil in general (Lauber *et al.*, 2009; Fierer & Jackson, 2006; **Paper I**) and has also been shown to be an important driver for the ammonia oxidizing community. A study on community structure and abundance of the AOB and AOA in a Scottish agricultural soil across a pH gradient found a clear difference between the community structures identified in acidic and neutral conditions (Nicol *et al.*, 2008). Two similar fertilization experiments, conducted on one acidic and one alkaline soil, revealed a changed community structure of only the AOB in the alkaline soil, but of only the AOA in the acidic soil (Shen *et al.*, 2008; He *et al.*, 2007). In **Papers II** and **IV**, soil pH was found to influence both the AOB and AOA community structures but no correlation was observed between soil pH and AOB abundance and contradictory correlations between soil pH and AOA abundance were found. Changes in abundance in the above-mentioned fertilizer experiment on acidic soil showed a positive correlation between soil pH and AOB and AOA abundance (He *et al.*, 2007). The fertilizer experiment on alkaline soil, on the other hand, exhibited a negative correlation between soil pH and AOB abundance (Shen *et al.*, 2008), while Nicol *et al.*, (2008) reported an increasing AOA abundance in acidic soil. Altogether, these findings support a strong link between soil pH and the ammonia oxidizing communities. However, the contradictory findings suggest that much is still uncovered in this area.

The temperature in soil systems may not vary greatly compared with that in aquatic systems such as hot springs and deep oceans, but there can still be relatively large variations between different terrestrial ecosystems. This makes temperature a potentially important factor affecting the ammonia oxidizing community. We found evidence of decreasing AOB abundance with decreasing temperature and that community structure of both AOB and AOA differed between cold and warm sampling periods in **Paper III**. On the contrary, another study has shown that AOB abundance increased with decreasing temperature (Avrahami & Bohannan, 2007). It has also previously been suggested that temperature is a selective factor for both AOB and AOA community structure (Fierer *et al.*, 2009; Tourna *et al.*, 2008; Avrahami & Conrad, 2003; Avrahami *et al.*, 2003). Further studies addressing the role of temperature for the ammonia oxidizing community are needed before conclusions can be drawn regarding its potential as a driver for the AOB and AOA. However, the results obtained to date indicate that differences in soil temperature could be an important factor for the AOB and AOA communities.

Soil moisture is another important factor to consider as a driver affecting AOB and AOA communities. It was found to influence the community structure of both the AOB and AOA in **Paper II** and of the AOB in **Paper IV**. In support, evidence has been presented of AOB and AOA community structures to separate clearly between 'wet' and 'dry' soils (Gleeson *et al.*, 2010). In **Paper II** soil moisture also showed a positive correlation to AOB abundance and a negative correlation to AOA abundance. This was partly supported by Avrahami & Bohannan (2007) who showed that the overall abundance of AOB increased with increasing soil moisture.

4.2.2 Inorganic nitrogen fertilization

A relatively well studied area with regard to effect and response of the ammonia oxidizing community is, understandably, the relationship between inorganic nitrogen fertilization and the ammonia oxidizing community. In contrast to the above-mentioned drivers which are all individual soil parameters, nitrogen fertilization could, in addition to changing nitrogen content, also potentially generate changes in several other soil parameters, such as e.g. pH, making it difficult to draw general conclusions. Nevertheless, nitrogen fertilization has been shown to induce changes in AOB community structure (Glaser *et al.*, 2010; Shen *et al.*, 2008) and in AOA community structure (Verhamme *et al.*, 2011; **Paper II**), indicating that both communities can alter in composition in response to inorganic nitrogen fertilization of the soil. In addition, many have reported changes in growth response by the communities to nitrogen fertilization. Much of the recent literature provides evidence of selection for growth of AOB in soil subjected to nitrogen fertilization resulting in high substrate conditions (Verhamme *et al.*, 2011; Di *et al.*, 2009). In contrast, AOA abundance has been shown to be unrelated to ammonia availability (Glaser *et al.*, 2010), or to be favoured by low ammonia substrate conditions (Di *et al.*, 2010; Erguder *et al.*, 2009). This suggests that inorganic nitrogen fertilization have an impact on both the AOB and AOA communities, but can potentially result in opposing effects.

4.2.3 Soil organic matter

It has been suggested that the AOA might be able to utilize organic material as a carbon source, and thus potentially be capable of mixotrophic or heterotrophic growth (Jia & Conrad, 2009; Hallam *et al.*, 2006). If true, this could furnish an explanation for the observed dominating abundance of AOA over the AOB in some soil systems. **Papers II** and **III** show signs of that the AOA could be negatively affected in terms of abundance by high organic carbon content in soil. The proportion of AOA decreased significantly in soil amended with the more recalcitrant carbon source peat, which generated a higher organic carbon content, compared to soil amended with straw in **Paper II**. This could indicate that the AOA are disadvantaged when competing for substrate in soil with high organic carbon content compared to in soil with more labile carbon sources. In support, it has been suggested that the AOA might be favoured by easily available exudates from plant roots (Chen *et al.*, 2008; Herrmann *et al.*, 2008). It could of course merely be exudation of oxygen and CO_2 that influences the growth of AOA, but it could also be the release of labile organic substrates if they can grow mixotrophically or heterotrophically.

Dominance of the AOB over the AOA was also observed in the peat soil studied in **Paper III**. Similar results have been found by others, but those results were attributed to high ammonia levels in the soil rather than organic carbon content (Höfferle *et al.*, 2010). This might also be a possible explanation for the results in **Paper III**, since the studied soil had relatively high ammonium levels. Nevertheless, there might still be a link between the AOA and amount or type of organic matter in soils, even though ammonium levels might be an overriding factor. For example, a microcosm experiment on acidic forest peat soil with high organic carbon content, but low ammonium concentration, showed growth of only the AOA, while AOB could not be detected. The authors hypothesized that a dominance of AOA in the studied soil could partly be due to a potential mixotrophic or heterotrophic growth of the AOA (Stopnisek *et al.*, 2010).

4.3 Ecological niches for AOB and AOA in soil

Much of the current literature reports contradictory findings regarding effects and responses of the two ammonia oxidizing communities to various environmental conditions in soil. This discrepancy could potentially be explained by niche differentiation between the AOB and AOA. Two papers in this thesis provide evidence supporting this theory. Differential correlation results between abundance data and soil parameter were identified at both the long-term Ultuna field site and the Logården site

(**Papers II** and **IV**). Spatial patterns of AOB and AOA abundance showed contrasting distributions (Figure 3) and the community structures of the two communities correlated differently to some soil properties at the Logården site (**Paper IV**).

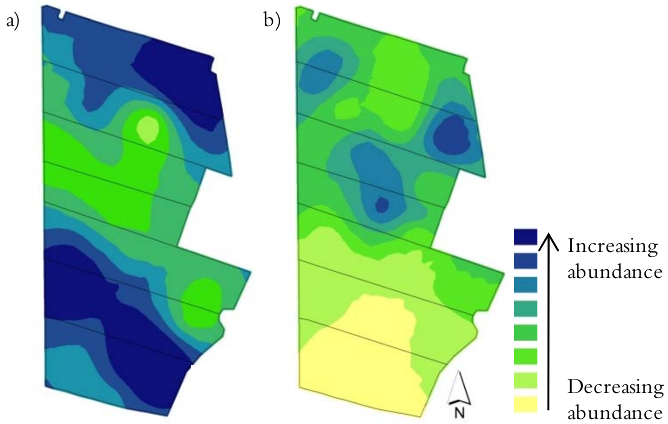

Figure 3. Kriged maps showing contrasting distribution patterns of the (a) AOB and (b) AOA abundances (*amoA* gene copy numbers per g dw soil) in seven fields at Logården experimental farm (Maps: M. Söderström, redrawn from **Paper IV**).

Even though findings in **Paper II** and **IV** are only based on correlation analysis, it is interesting that the soil parameters that were correlated with varying community size or structure did not overlap for the AOB and AOA. Similarly, a study targeting the size of nitrogen cycling communities at landscape scale reported no observed overlap between variables driving the variation in patterns of AOB and AOA community abundance (Bru *et al.*, 2010). Niche separation between the AOB and AOA has also been suggested by others (e.g. Schleper, 2010), and one proposed explanation for the division is based on nutrient status, with low nutrient habitats being the preferred environment for the AOA (Erguder *et al.*, 2009). This is supported by the finding that the isolated archaeal representative, *Nitrosopumilus maritimus*, can survive on ammonium levels well below the minimum concentrations required for growth of isolated AOB (Martens-Habbena *et al.*, 2009). The AOB have been found to grow substantially in soil with high substrate levels, while growth of the AOA community was only observed in soils without substrate addition (Di *et al.*, 2010). AOA growth has also been found to be unrelated to ammonium concentration (Verhamme *et al.*, 2011; Glaser *et al.*, 2010), further supporting the assumption that a high substrate level might not be an important factor in shaping a habitat for the AOA. Interestingly, both the AOB and AOA communities have been identified as being functionally dominant in soil (Zhang *et al.*, 2010; Jia & Conrad, 2009). However, there was an important difference between these two studies with regard to source of substrate, as Jia & Conrad (2009) fertilized the soil with ammonia, while the system used by Zhang *et al*,. (2010) derived ammonia from mineralization, thus generating lower substrate concentrations. This could explain the contradictory findings between the two studies, since the former experimental setup might favour the AOB, while the latter might favour the AOA. Together, the above findings suggest that the AOB and AOA co-exist in relatively high numbers in many soil ecosystems, but that they have differing habitat preferences.

4.4 Role of the ammonia oxidizing community in nitrogen losses from soils

In order to achieve a balance in the global nitrogen cycle, nitrogen use efficiency has to increase (Schlesinger, 2009). Losses of nitrogenous compounds through nitrification-related processes can be substantial. For example, it has been found that these processes can result in losses of nearly 70% of applied nitrogen fertilizers in managed ecosystems (Subbarao *et al.*, 2006). Thus we need to determine the role of the AOB and AOA in nitrogen losses from soils. Demonstrating a causal relationship between a group of organisms and their functions is not an easy task, and the ammonia oxidizing community is no exception. In **Paper II**, nitrification activity was measured as substrate-induced ammonia oxidation rate and a correlation was observed between the structure of the AOB and AOA community and between AOA community size and oxidation rates. These findings suggest that the AOA were functionally dominant at the site. Others have also reported the AOA to be functionally dominant in soil from the same site (Hallin *et al.*, 2009) as well as in other soils (Gubry-Rangin *et al.*, 2010; Offre *et al.*, 2009). Our measurements of substrate-induced ammonia oxidation rates in order to assess the activity of the AOB and AOA in another soil demonstrated a negative relationship between AOA abundance and oxidation rates (**Paper IV**). However, data on the amount of nitrate being leached from the field showed a positive correlation with AOA abundance. It was hypothesized that substrate-induced ammonia oxidation rates would be positively correlated with the leaching data, since nitrate is formed when nitrite is supplied through oxidation of ammonia. We concluded that the lack of correlation was a methodological problem, since substrate-induced ammonia oxidation rates were measured after addition of ammonia in excess, thus possibly favouring the AOB community over the AOA. Nevertheless, the findings in **Paper IV** indicate partitioning between the AOB and the AOA and suggest that the AOA were responsible for nitrate leaching in the studied soil.

In contrast, no correlation was found between nitrogen losses from the peat soil and the ammonia oxidizer community, either when measured as substrate-induced ammonia oxidation rates or as N_2O emissions in **Paper III**. This lack of correlation between N_2O emissions and ammonium oxidizers has also been reported previously (Regina *et al.*, 1996). An explanation could be that we did not capture the 'true' N_2O emission pattern due to high spatial and/or temporal variability in emission rates. Another explanation for the lack of correlation could be that the number of cells measured in the samples did not equal the number of active cells. This was discussed by Röling *et al.*, (2007), who argued that cellular activity depends on several factors, such as changes in e.g. temperature or pH. Other processes, such as the denitrification process, could also have been responsible for formation of N_2O in this particular soil.

The evidence to date demonstrates that functional dominance in soil ecosystems will vary depending on external environmental parameters, and that one group alone does not dominate the ammonia oxidation process in soil ecosystems. Differing results have been reported regarding functional dominance and activity has been coupled to the AOB in some soil systems (Glaser *et al.*, 2010; Di *et al.*, 2009; Jia & Conrad, 2009; Mertens *et al.*, 2009) but to the AOA in other soil systems (Gubry-Rangin *et al.*, 2010; Stopnisek *et al.*, 2010; Zhang *et al.*, 2010; Offre *et al.*, 2009). This provides evidence that the AOB and AOA occupy different ecological niches and that both can be responsible for the oxidation of ammonia which could result in nitrogen losses from soils. However, since the dominant group can vary between systems, it is important to include both groups when evaluating the role of ammonia oxidizers in soil nitrogen loss.

4.5 Using ammonia oxidizers as a bioindicator

The AOB community has previously been suggested as a model organism in microbial ecology and has been used as an indicator group to study different kinds of soil perturbations, such as the effects of effluent irrigation on soil (Oved *et al.*, 2001), application of herbicides (Chang *et al.*, 2001) and recycling of organic waste products to arable soil (Nyberg *et al.*, 2006). When humans alter terrestrial ecosystems and change land use, the status of the soil is likely to be affected too due to changes in patterns of e.g. land surface hydrology and biogeochemical cycles (Ellis & Ramankutty, 2008). Soils are vital for many ecosystem services upon which human societies worldwide depend, which increases the importance of finding ways to monitor soil status. One way to approach this is to use members of the soil biota, since these are likely to be affected in the case of soil disturbance. However, due to the complex nature of soil ecosystems, finding a suitable candidate is not an easy task. Nevertheless, Ritz *et al.*, (2009) surveyed 183 biological indicator candidates for national-scale soil monitoring and ranked the AOB as top of the list of suggested bioindicators. Now that there is increasing evidence that the AOA are equally widespread and that they are functionally important in most soil ecosystems, we suggest that the AOA be included with the AOB when using ammonia oxidizers as a bioindicator (**Paper V**).

The ammonia oxidizing community has been shown to exhibit patterns when monitored at larger scales, such as field and regional scale (Bru *et al.*, 2010; **Paper IV**). This further confirms their potential for use in monitoring soil status, since it is important that the chosen bioindicator can be targeted not only at the small scale. However, since we have not fully determined how these groups relate to each other, especially in soil ecosystems, a clear division between the two groups cannot be made and both the AOB and AOA need to be monitored together. In addition, there is the question of which method to use when targeting the community. It was previously suggested that genetic profiling in the form of T-RFLP should be used (Ritz *et al.*, 2009), but instead we propose that the size rather than the composition of the community be targeted using qPCR. This is because qPCR is a relatively cost-effective and high throughput method compared with T-RFLP, but also since it has been suggested to be well suited for large-scale screening (Smith & Osborn, 2009).

5 Concluding remarks

The effects of soil management on indigenous soil bacterial and archaeal communities were studied here, with emphasis on the AOB and AOA. Environmental drivers of certain bacterial and archaeal phyla/classes and of the AOB and AOA were explored and the ecological roles of these two groups in soil were analyzed.

∴ **Paper I** - Different long-term fertilization regimes altered soil characteristics, but total abundance of eight phyla/classes of bacteria and archaea commonly found in soil were affected in similar ways, irrespective of fertilization treatment. However, the relative abundance of certain taxa responded differently to varying fertilizer treatment, indicating ecological coherence between higher taxonomic ranks.

∴ **Paper II** - The AOB and AOA communities were affected differently by the different long-term fertilization treatments, and type of organic matter could play a role for AOA abundance. Contradictory correlation results between measured soil properties or substrate-induced ammonia oxidation rates and AOB and AOA abundance suggest niche partitioning between the two ammonia oxidizing groups.

∴ **Paper III** - The AOB outnumbered the AOA in a drained and forested peat soil, an effect which could be explained by relatively high ammonium levels at the site. Temporal variations were observed for AOB and AOA community structures and AOB abundance. Neither measured substrate-induced ammonia oxidation rates nor N_2O emissions could be coupled to the AOB or the AOA.

∴ **Paper IV** - The community abundance and structure of AOB and AOA exhibited spatial patterns at hectare scale and differed in correlations to soil parameters. Niche differentiation between the AOB and AOA was suggested in the agro-ecosystem studied, and the AOA were proposed to be the *in situ* contributors to nitrate leaching from the field by providing substrate for the nitrite oxidizers.

∴ **Paper V** - The environmental importance and ubiquity of the AOB and AOA and their potential as biological indicators in soil monitoring were demonstrated. It is proposed that the size of the ammonia oxidizing community be targeted to evaluate soil status, since this is a relatively cost-effective and high throughput method.

5.1 Challenges for the future

Many studies within this field are based on one sampling occasion and thus results represent only a snap-shot of the AOB and AOA community and their activity. It would be of great benefit for the field if sampling in different studies be expanded so that potential fluctuations over time can also be detected. This is an important issue, since it would be interesting to know more about the naturally occurring fluctuations in community size, structure and activity of the AOB and AOA communities.

More knowledge regarding ecophysiological characteristics of the AOA is needed. Even though sequence information supports a high abundance and ubiquity of the archaeal ammonia oxidizers in the environment, only a few AOA have been characterized to date and there is limited knowledge about AOA growth strategies. Increasing this knowledgebase might aid the identification of specific drivers and ecological niches for the AOB and AOA in terrestrial ecosystems.

In order to achieve a reduction of the excess amounts of anthropogenic nitrogen which is currently entering Earth's ecosystems, it is important that information of organisms responsible for different parts of the nitrogen cycle be continuously improved. This because as William H. Schlesinger wrote 'Humans are adding nitrogen to the Earth's surface; we do not know where it all goes, but we do know that increasing concentrations of nitrogen in unexpected places will cause significant environmental damage that we will all learn to regret'.

"I make no apologies for putting microorganisms on a pedestal above all other living things. For if the last blue whale choked to death on the last panda, it would be disastrous but not the end of the world. But if we accidentally poisoned the last two species of ammonia oxidizers, that would be another matter. It could be happening now and we wouldn't even know…"

Tom Curtis - 2006

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