Niche Differentiation of Ammonia-Oxidizing Microbial Communities in Arid Land Soils

by

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ABSTRACT

Human activity has increased loading of reactive nitrogen (N) in the environment, with important and often deleterious impacts on biodiversity, climate, and human health. Since the fate of N in the ecosystem is mainly controlled by microorganisms, understanding the factors that shape microbial communities becomes relevant and urgent. In arid land soils, these microbial communities and factors are not well understood.

I aimed to study the role of N cycling microbes, such as the ammonia-oxidizing bacteria (AOB), the recently discovered ammonia-oxidizing archaea (AOA), and various fungal groups, in soils of arid lands. I also tested if niche differentiation among microbial populations is a driver of differential biogeochemical outcomes.

I found that N cycling microbial communities in arid lands are structured by environmental factors to a stronger degree than what is generally observed in mesic systems. For example, in biological soil crusts, temperature selected for AOA in warmer deserts and for AOB in colder deserts. Land-use change also affects niche differentiation, with fungi being the major agents of N_2O production in natural arid lands, whereas emissions could be attributed to bacteria in mesic urban lawns. By contrast, NO_3^- production in the native desert and managed soils was mainly controlled by autotrophic microbes (i.e., AOB and AOA) rather than by heterotrophic fungi. I could also determine that AOA surprisingly responded positively to inorganic N availability in both short (one month) and long-term (seven years) experimental manipulations in an arid land soil,

while environmental N enrichment in other ecosystem types is known to favor AOB over AOA.

This work improves our predictions of ecosystem response to anthropogenic N increase and shows that paradigms derived from mesic systems are not always applicable to arid lands. My dissertation also highlights the unique ecology of ammonia oxidizers and draws attention to the importance of N cycling in desert soils.

ACKNOWLEDGEMENTS

Firstmost, I thank Drs. Sharon J. Hall and Ferran Garcia-Pichel. This thorough work would not have been possible without their expertise and support. The information and ideas in this document are only some of the elements that I have learned from my mentors. Dr. Hall has taught me to aim for excellence and not to settle on 'just good enough'. This perspective has allowed me to be successful and continuously seek out ways of improving myself as a graduate student and scientist. The training I received from Dr. Hall has built a strong foundation in recognizing and contributing to novel work. Subsequently, together with the guidance of Dr. Garcia-Pichel, I was able to propel further to reach my potential abilities. It was intriguing to see Dr. Garcia-Pichel become excited about figuring out the smallest details of a natural phenomenon. I began to appreciate that one has to know the basics to understand how the whole system works. His enthusiasm was obvious as soon as there was a slight sign of data from anyone in the lab group, giving an opportunity for discussion and learning moments. The science research that we carried out was enhanced by the fun atmosphere in the Hall and Garcia-Pichel labs.

I appreciate Drs. Jean McLain and Egbert Schwartz for excellent comments and guidance on my research. It was helpful having access to experts that are able to provide friendly feedback and gauging novelty of the research before my work goes out to the rest of the microbial ecologists in the world. I am grateful to Drs. Kevin McCluney (grad-student at the time) and John Sabo with whom my science career started at ASU. Likewise, Dr.

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One of my biggest acknowledgements is to Dr. Nancy Grimm, the Central Arizona Phoenix Long-Term Ecological Research (CAP LTER) program, and many scientists and staff involved in the CAP LTER, including Steven Earl, Marcia Nation, Quincy Stewart, as well as Dr. Dan Childers. The CAP LTER is the embodiment of my overall experience during graduate school. I have been exposed to the idea of collaborating with scientists and people outside of academia, with the goal of advancing ecological understanding and carrying out research that is sustainability-driven for the betterment of the world. I feel that this experience of interdisciplinary collaboration – building 'bridges' – will be something that I do for the rest of my career and life. I am also grateful for the CAP LTER being one of my biggest financial supporters. Likewise, the GPSA has funded numerous project efforts. My department SoLS has also provided support. Thank you Yvonne Delgado and Wendi Simonson for your administrative help.

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ABBREVIATIONS

(Frequently used terms only)

amoA	gene encoding subunit A of the ammonia monooxygenase enzyme
AO	ammonia oxidation
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
AOM	ammonia oxidizing microorganisms
AMO	ammonia monooxygenase enzyme
HAO	hydroxylamine oxidoreductase enzyme
BSC	biological soil crust
MAT	mean annual temperature
MAP	mean annual precipitation
N	nitrogen
C	carbon
SOM	soil organic matter
WHC	water holding capacity
NH_3	ammonia
NH_4^+	ammonium
N_2O	nitrous oxide
NO_2^-	nitrite
NO_3^-	nitrate
qPCR	quantitative polymerase chain reaction

OTU   operational taxonomic unit

DNA   deoxyribonucleic acid

1. Dissertation introduction

    1.1. Using ecological theory to advance understanding of ammonia oxidation

processes in arid land systems

       1.1.1. Niche differentiation

Niche differentiation can be used as an underlying theme to test hypotheses about the

relationships between environmental conditions, total and relative abundance of

populations, and ecosystem function. The premise of niche theory is that different

ecological traits among species allow for coexistence within a habitat (Hutchinson 1959).

For example, substrate availability may separate the niche of organisms into oligotrophs

(slow growth, high efficiency) and copiotrophs (fast growth, low efficiency) (Macarthur

and Pianka 1966; Grime 1977; Southwood 1977). Spatial and temporal changes within a

habitat may also select for unique populations. These interactions were initially studied

by plant ecophysiologists, meanwhile the discipline of microbial ecology was only

forming. Ultimately, the study of microorganisms and their environment expanded

(Brock 1966; Alexander 1971; Tiedje 1999), facilitating research about niche

specialization and building a connection between genes and ecosystems (Schimel and

Gulledge 1998; Fierer et al. 2007; Zengler 2009). In microbial communities, oligotrophs

in a nutrient-depleted soil may be rapidly outcompeted by copiotrophic populations upon

nutrient enrichment. This scenario may occur when some dormant microbial populations

instantaneously respond to the new, resourceful conditions (Lennon and Jones 2011). An oligotrophic community may also shift successionally to become a copiotrophic-type of community after recurrent or long-term exposure to nutrient-rich inputs (Jackson 2003; Fierer et al. 2010). Recognition of the microbial communities is central to predicting their differential responses to environmental factors that control their abundance and function in the ecosystem.

1.2. Aims of the dissertation

In this dissertation, the three major goals are to 1) expand fundamental understanding of core principles in microbial ecology, 2) fill in gaps of knowledge about an important group of microorganisms – the ammonia oxidizers, and 3) bring attention to research in arid land environments.

Each chapter in this work will highlight the role of niche differentiation, as it pertains to factors such as substrate availability, ecosystem type, and climate. To test ideas about the interactions of microbial populations and differential contributions to ecosystem function, we will study a specific step of the nitrogen (N) cycle – ammonia oxidation (AO). The influence of environmental conditions on the relationships between ammonia oxidizing microorganisms (AOM) and AO rates will be tested in the context of arid land soils. My research in arid areas becomes even more important considering that these ecosystems are widely distributed (Figure 1) but are some of the most understudied in the world

(Figure 2). The disparity in this research area has been documented (e.g. Maestre et al. 2012), and hopefully will gain more recognition as an outcome of my work.

1.3. Background on ammonia oxidation

1.3.1. The nitrogen cycle

All living cells require N as a component of protein and nucleic acid biomolecules. Assimilation of N by plants and microorganisms for biosynthesis comes from uptake of organic N, ammonium (NH_4^+), and nitrate (NO_3^-) (Geisseler et al. 2010). However, majority of the Earth's N exists as dinitrogen (N_2) in the atmosphere (79.5% of total N; Galloway 2003). Other reservoirs include sedimentary rocks (20.1%) and oceans (0.4%). The N in all biota and organic matter is only trace amounts of the total ($< 0.1\%$). Most organisms are not able to assimilate the elemental source of N directly. Thus, most life is essentially restricted to the activities of microorganisms that have the capacity to break the stable N-N triple bond, ultimately producing forms of N that can be metabolized (Howard and Rees 1996). Additionally, some other microorganisms need N sources for catabolic processes, yielding energy-rich compounds (e.g. adenosine triphosphate; ATP). In these reactions, the greater the reduction potential differences between oxidation-reduction couples, the greater the energy yield (Madigan et al. 1997). For example, aerobic respiration, using oxygen as an electron acceptor, will drive more ATP synthesis than in anaerobic metabolism such as respiration of NO_3^- (lower reduction potential than oxygen). Biological reactions are also able to generate more energy from oxidation of

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highly reduced substrates. Thus, aerobic respiration will have greater energy yield from oxidation of NH_4^+ compared to nitrite (NO_2^-), which is in an oxidized state of N. These redox reactions and other biological metabolism of N are the fundamental cellular processes that drive the N cycle.

The Earth's N cycle consists of numerous processes, including N_2 fixation, nitrification, and denitrification, which are among the first discovered and most studied (Klotz and Stein 2008; Galloway et al. 2013). To meet the global biological demand for N, some prokaryotes equipped with the nitrogenase enzyme are able to use N_2 fixation to reduce N_2 to ammonia (NH_3; Howard and Rees 1996; Zehr and Turner 2001). Fertility of all natural ecosystems, thus, to some degree depends on the occurrence, distribution, and activities of N_2-fixers (Cleveland et al. 1999; Vitousek et al. 2013). Nitrification is the aerobic oxidation of NH_3 to NO_2^- – discussed in detail later in this Chapter – followed by oxidation to NO_3^- (Prosser 1989). These two steps are carried out by AOM and nitrite oxidizing bacteria, respectively, creating the substrate for anaerobic denitrification. In classical denitrification, N returns to the atmosphere and closes the N cycle, as NO_3^- is reduced in a series of steps to N_2 (Tiedje et al. 1983). Conventional understanding has progressed to a more complete picture of the N cycle, uncovering a diverse array of N transformations that, as it turns out, are not only mediated by bacteria (Figure 3). For instance, novel molecular techniques have identified the role of other microbial domains with entirely different metabolic pathways, such as eukaryotes and archaea (e.g., fungal denitrification, archaeal ammonia oxidation; Shoun et al. 1992; Konneke et al. 2005).

While these studies show that several microbial groups other than bacteria have the potential to contribute to N cycling, less is known about their distribution in soils, relative importance to biogeochemical pathways, and responses to a changing environment.

1.3.2. Economic and environmental importance of the nitrogen cycle

Human activity has profoundly affected the N cycle during the last 100 years (Vitousek et al. 1997; Galloway and Cowling 2002). Growth in the human population has created a demand for more food production than could be sustained by natural biological N fixation in agricultural systems (Galloway 2003). The invention and commercialization of the Haber-Bosch process allowed for the production of N fertilizer, lessening some issues of food security. Over time, the development of anthropogenic N fixation has surpassed the biological source as the dominant input of reactive N globally. Consequently, prior to reaching the intended destination (i.e. food consumption), majority of the N from fertilizers is lost to the environment through several processes (Galloway and Cowling 2002). For example, while the NH_4^+ cation can be held by soils through bonding to the overall negatively charged soil colloids, the more mobile NO_3^- may leach through soils into groundwater and can be transferred downstream, where detriments for aquatic life can result such as eutrophication (Camargo and Alonso 2006). The N cycle also contains pathways of N loss to the atmosphere through the emission of nitric oxide (NO), nitrous oxide (N_2O), and nitrous acid (HONO) as gaseous intermediates or products that affect air quality and climate (Wrage et al. 2001; Seitzinger et al. 2006;

Oswald et al. 2013). Increased use of fossil-fuel combustion has also elevated the amount

of anthropogenic N that is entering the environment (e.g. deposition; Matson et al. 2002;

Fowler et al. 2013). Understanding the interactions between environmental factors and

the function of N cycling microorganisms is vital to help restore a balanced N cycle by

minimizing the unintended, cascading effects of N processing in the ecosystem

(Galloway et al. 2008).

1.3.3. Mechanisms of biological ammonia oxidation in microorganisms

Humans have thought about N for centuries (Galloway et al. 2013) and it was over 100

years ago that aerobic AO – one of the key steps of the N cycle – was shown to be carried

out by bacteria (Winogradsky 1890). Yet only in 2005, the archaea were first isolated and

shown to contribute to AO (Konneke et al. 2005), triggering scientists in many

disciplines to re-examine the understanding of the N cycle. Soon after, it was clear that

the AOA outnumbered AOB in most environments (Leininger et al. 2006). The AOA

were now implicated as one of the most relevant microbial groups that alter the fate of N

in the ecosystem. For this reason, the number of *amoA* database sequences (Figure 4;

Junier et al. 2010) and the number of studies on the N cycle, including the AOA, have

dramatically risen in recent years (Figure 5).

AO is catalyzed by the ammonia monooxygenase (AMO) enzyme for energy gain by

AOB and AOA (Klotz and Stein 2011; Offre et al. 2013). In AOB, NH_3 is initially

oxidized (by one atom of oxygen) to hydroxylamine (NH_2OH) which is then oxidized by the hydroxylamine oxidoreductase (HAO) enzyme, producing 4 electrons that are shuffled through cytochrome proteins and to the quinone pool (Figure 6; Figure 7). While 2 of the electrons are returned to AMO as reductant (for the remaining one atom of oxygen), the other 2 electrons are passed through the electron transport chain, leading to the proton motive force and ATP production (Arp and Stein 2003; Bock and Wagner 2006). This metabolism within the AOB is linked with fixation of carbon dioxide and is why the AOB are classified as chemolithoautotrophs (Arp et al. 2002). Assimilation of carbon (C) is via the Calvin Benson Bassham cycle, catalyzed by the type I ribulose biphosphate carboxylase/oxygenase (RubisCO; Wei et al. 2004). The AOA also oxidize NH_3 via AMO, ultimately producing ATP (Walker et al. 2010). However, the mode of C fixation and most other comparisons between the AOA and AOB reveal many differences (Hatzenpichler 2012).

Since the first hints of evidence that archaea are ammonia oxidizers (Venter et al. 2004; Treusch et al. 2005), research has focused advancing our understanding of the genomics, biochemistry, and ecology of AOA. There is no evidence of any type of bacterial HAO homolog in AOA, suggesting that the AOA and AOB have distinct machinery and mechanisms for energy generation (Stahl and de la Torre 2012). Additionally, the genes encoding the three subunits of AMO are arranged in different orders in the AOA and AOB, with one of them, *amoA*, known to exist as one copy in the genome of AOA but as multiple copies in AOB (2 for *Nitrosomonas* spp., 3 for *Nitrosospira* spp.; Norton et al.

2002; Walker et al. 2010). Metabolic regulation and processes contributing to energy generation in AOA are unclear. However, recent evidence shows that AOA may have mixotrophic capabilities, either switching to or supplementing growth with a heterotrophic metabolism (Tourna et al. 2011; Hatzenpichler 2012). The ecophysiology of AOA also differs from AOB, with clear findings of AOA growth at NH_3 concentrations and pH levels lower than what is known for the AOB (Martens-Habbena et al. 2009; Gubry-Rangin et al. 2011). Differential adaptations within the AOM may have significant consequences for the fate of N in the environment. Other characteristics of the AOA, such as their habitat range and contribution to AO in extreme environments (e.g. dryland soils), are currently unknown or not well understood.

1.4. Arid land environments

1.4.1. Specialized niches of microbial communities in ecosystems with low amounts of precipitation

Arid and semi-arid ecosystems cover one-third of Earth's land surface (Figure 1; Koohafkan and Stewart 2008) and are characterized by high spatial and temporal heterogeneity and extreme environmental conditions (Safriel et al. 2005; Schimel et al. 2007; Collins et al. 2008). These characteristics include high temperatures, frequent wetting/desiccating cycles, high soil pH, low soil C availability, and influence from urbanization. Soils that encounter extreme and fluctuating conditions may be ideal

systems to detect a shift in microbial population dominance and function (Wall and Virginia 1999; Austin 2011; Fierer et al. 2012).

Desert ecosystems are experiencing increased land-use change and intensive management, including both pulsed and chronic inputs of water and fertilizers (Warren et al. 1996; Grimm et al. 2008; Davies and Hall 2010). As a result, growth of cities and anthropogenic atmospheric deposition is altering resource availability and the N cycle in soils of water-limited environments (McCrackin et al. 2008; Hall et al. 2009; Hall et al. 2011). One could principally predict that native desert conditions, especially those with low N input in soils away from plants (Austin et al. 2004; Schade and Hobbie 2005), may select for the most oligotrophic ammonia oxidizers – the AOA (Martens-Habbena et al. 2009). On the other hand, rapid pulses of water (from precipitation or irrigation) and nutrients may favor a distinct group of microbes, such as *Nitrosomonas*-related AOB that respond more swiftly to activation after dormancy than other types of ammonia oxidizers (Bollmann et al. 2002; Placella and Firestone 2013). Given these trends, desert soils – particularly those near cities – may harbor novel or interesting microbial communities adapted to extreme and rapidly changing environmental conditions that are worth studying.

Microbial communities are especially important to productivity and nutrient cycling in arid lands since the distribution and growth of plants is more restricted than in mesic environments (Belnap et al. 2003; Collins et al. 2008; Bashan and de-Bashan 2010). Arid

lands can favor growth of some fungal populations (Cousins et al. 2003; Porras-Alfaro et al. 2011) due to unique qualities of fungi, including association with primary producers to increase resource acquisition (e.g. soil crust and rock surface communities; Green et al. 2008; Pointing and Belnap 2012) and survival under low water availability (Allen 2007; Collins et al. 2008). Recent experiments in several dryland and grassland sites have shown that fungi are unexpectedly important contributors to N cycling (McLain and Martens 2005; McLain and Martens 2006; Stursova et al. 2006; Crenshaw et al. 2008; Laughlin et al. 2009).

Biological soil crusts (BSCs) can cover large portions of the landscape in deserts (Belnap 1995; Pointing and Belnap 2012) and are important contributors to global C and N stock (Garcia-Pichel et al. 2003; Elbert et al. 2012). Pioneering cyanobacteria initiate the formation of BSCs by stabilizing loose soils (Garcia-Pichel and Wojciechowski 2009), allowing a succession of other microorganisms (Nagy et al. 2005), including fungi (Bates and Garcia-Pichel 2009) and archaea (Soule et al. 2009). The cyanobacterial communities are affected by climate and N enrichment, suggesting that future environmental change may lead to as-of-yet unknown consequences for these microbial groups and the ecosystem (Belnap et al. 2008; Garcia-Pichel et al. 2013). BSC topsoil assemblages play vital roles in biogeochemical and hydrological processes within arid ecosystems (Evans and Johansen 1999; Belnap and Lange 2003; Belnap 2006; Strauss et al. 2012). For instance, AO in BSCs directly impacts soil fertility (Johnson et al. 2005). AOB have been recovered from BSCs and are numerically abundant (Johnson et al. 2005; Gundlapally

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and Garcia-Pichel 2006). Although archaea are likely important soil ammonia oxidizers (Bates et al. 2011; Offre et al. 2013) and sizable archaeal populations have been reported from BSCs (Soule et al. 2009), little is known about the role of archaea in the N cycle of BSCs, and in arid lands in general.

1.5. Goals of each dissertation chapter

Chapter 1- Dissertation introduction

The current section reviews current knowledge on AO and highlights the gaps in knowledge about the ecology of ammonia oxidizers. This introductory chapter sets up the importance of using arid lands as a context for testing ecological theory related to AO.

Chapter 2- Ammonia-oxidizing archaea respond positively to inorganic N addition in desert soils

In mesic environments, changes in soil N availability affect AO rates by selecting for particular types of ammonia oxidizers. For example, AOB are typically favored over AOA when soils are fertilized with high concentrations of inorganic N. However, for unclear reasons, AOA abundance is often unresponsive to changes in the soil environment and is frequently poorly correlated with AO rates. Additionally, research has revealed inconsistent patterns in the responsiveness of AOA and AOB, and their subgroups, to N treatments. In some cases, AOA have been shown to respond positively to N inputs from organic sources, but no evidence thus far suggests that AOA respond solely to inorganic N inputs in nature. It is unknown if niche differentiation between

AOA and AOB will occur in arid land soils as well. In this section, we test if long-term N enrichment alters the abundance and composition of ammonia-oxidizing communities in Sonoran Desert soils. We also used a kinetics approach to measure the *in situ* function of ammonia oxidizers across a range of inorganic N concentrations.

Chapter 3- Niche differentiation of ammonia-oxidizing microorganisms during pulsed nitrogen inputs in a dryland soil

Arid land soils are frequently exposed to desiccation and wetting events that lead to rapid changes in soil conditions. Little is known how AOA and AOB respond to short-term shifts in N availability. After enriching the soil with one pulse of N and ample water, we explored community dynamics over the course of one month.

Chapter 4- Contributions of fungi, archaea, and bacteria to ammonia oxidation in southwestern US *[published in Soil Biology and Biochemistry; impact factor 3.65]*

Recent work has suggested that fungal contribution to N cycling may be especially relevant in dryland systems. Some fungi form hyphal networks that facilitate nutrient exchange between desert shrubs, biological soil crusts, and microbial communities across infertile soil spaces. Unique physiological traits of fungi may also allow them to adapt particularly well to the extreme conditions and pulse dynamics of arid lands. We ask, what is the distribution and potential contribution of different metabolic groups to the N cycle (e.g. autotrophic archaeal and bacterial nitrification, fungal denitrification) across the southwestern US. We sampled across diverse land-use types, including arid deserts

and semi-arid grasslands, as well as natural and urban soils, to also test if the ecosystem type plays a role in niche differentiation.

Chapter 5- Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands *[published in Ecological Processes; new journal started in 2012, no impact factor yet]*

Since BSCs are integral components of arid lands, characterizing the distribution and composition of these microbial communities is important to understand the N cycle of the entire desert landscape. We characterized the distribution of AOA and AOB in BSCs at a large geographical scale spanning the western US. Additionally, we quantified climatic variables in an effort to determine if biogeography is important to the structure of ammonia-oxidizing communities in BSCs.

Chapter 6- Ammonia oxidation: a meta-analysis including arid lands

Here, I synthesize the knowledge about ammonia oxidizing communities in arid land soils. The chapter is a combination of data reported from relevant literature in drylands, the patterns covered in Chapters 2 - 5, and a meta-analysis. The meta-analysis is carried out on literature data to evaluate the relationship between ammonia oxidizing community parameters (AOA abundance, AOB abundance, ratio of AOA:AOB) and environmental variables (soil properties: pH, concentration of NH_4^+; climate: temperature) on a global scale, including arid lands.

Chapter 7- Concluding remarks

The concluding section is a summary of the work presented in the preceding chapters, emphasizing the main points discovered in each project of my dissertation. I discuss the new knowledge revealed in my work in arid lands and the novel contributions of my work to the field of ammonia oxidation.
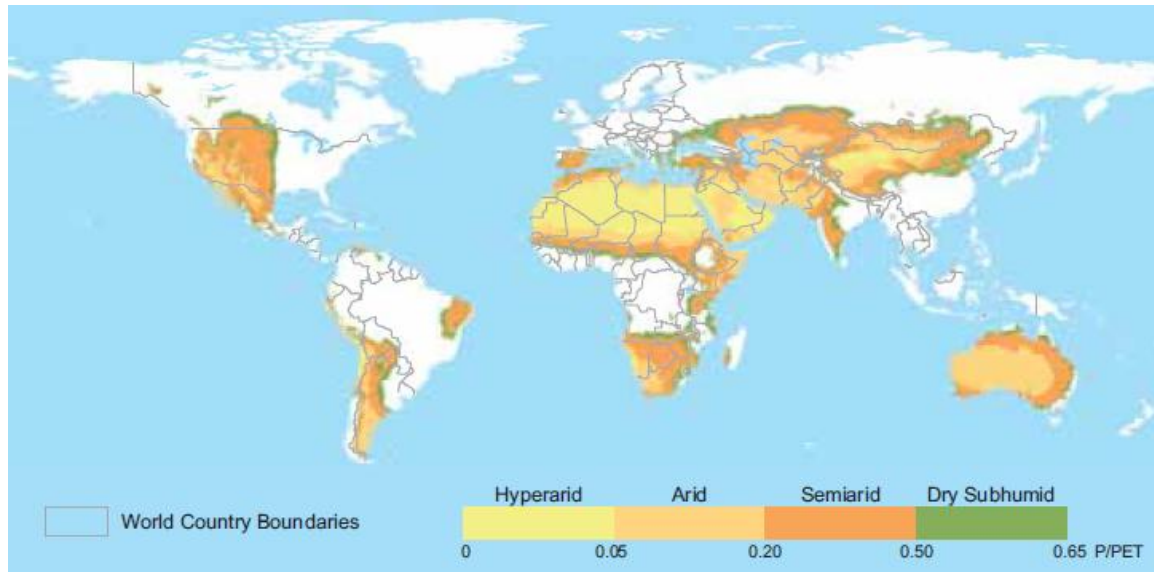
Figure 1. Distribution of drylands in the world. Legend indicates an aridity index, P/PET, which is calculated as annual precipitation divided by annual potential evapotranspiration. Figure from Koohafkan and Stewart 2008.
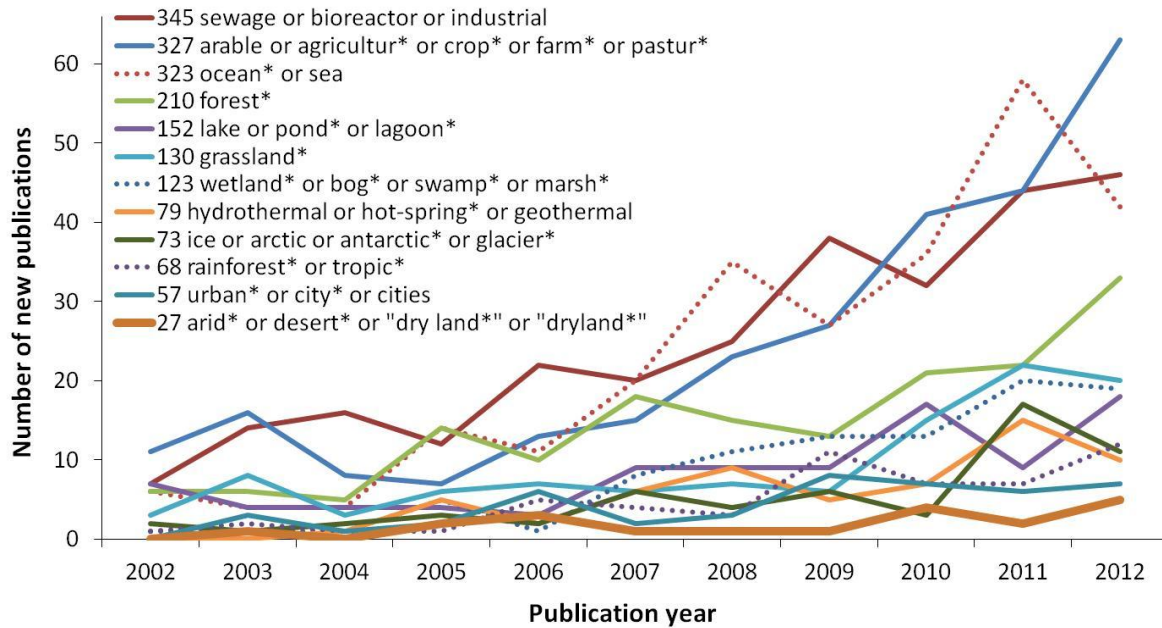
Figure 2. Number of publication search results per year in Web of Science using keywords related to ecosystem type. Each search was also paired with the following one set of terms: *amoa or aoa or aob or "ammonia oxidi*"*. Number in legend indicates total number of search results in decreasing order (top to bottom). The text after the number indicates the exact string of characters used for the search. Older data were excluded from the bar chart. The top set of terms (345 results) had an additional 575 results (that were excluded for simplicity) if the following terms would have been included: sludge or wastewater. A study may have overlap between multiple ecosystem types. * is used as a wildcard. Quotes are used to keep words together in a search.

Figure 3. Processes in the nitrogen cycle. Microbial: 1. dinitrogen fixation by bacteria and archaea; 2. oxidation of ammonia to nitrite by bacteria; 3. oxidation of nitrite to nitrate; 4. classic anaerobic denitrification; 5. anaerobic ammonium oxidation (anammox); 6. dissimilatory respiratory ammonification; 7. assimilatory nitrate reduction and ammonification; 8. oxidation of ammonia to nitrite by archaea; 9. oxidation of hydroxylamine to nitrous oxide. 10. aerobic denitrification by AOB and heterotrophs; 11. assimilation; 12. mineralization; 13. co-denitrification; 14. heterotrophic nitrification; 15. ammonia oxidation to nitrous acid by bacteria. 16. nitrite oxidation by purple sulfur bacteria. 17. denitrification by methanotrophs. 18. denitrification by foramanifera. Squiggly blue arrows represent transformations and inputs from abiotic and/or anthropogenic processes: 19. lightning; 20. leaching; 21. volatilization; 22. fertilizer production; 23. deposition of reactive nitrogen. 24. emission of reactive nitrogen. Dark arrows represent anaerobic processes. Light arrows represent aerobic processes. Dotted arrows represent aerobic, anaerobic, or micro-aerophilic processes. Oxidation states are indicated by dark red text. Enzymes carrying out reactions are indicated as name of functional gene or protein in green text. Figure adapted from Bock and Wagner 2006; Robertson and Vitousek 2009; Canfield et al. 2010; Klotz and Stein 2011; Baggs 2011; Butterbach-Bahl et al. 2013; Simon and Klotz 2013; Fowler et al. 2013.
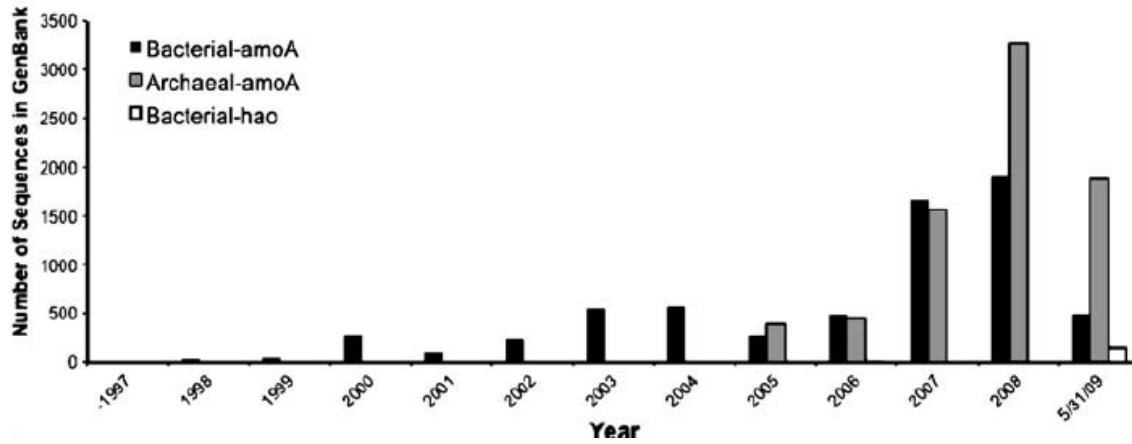
Figure 4. Number of functional gene sequences submitted to GenBank database over time. amoA = gene for ammonia monooxygenase enzyme; hao = gene for hydroxylamine oxidoreductase enzyme. Figure from Junier et al. 2010.
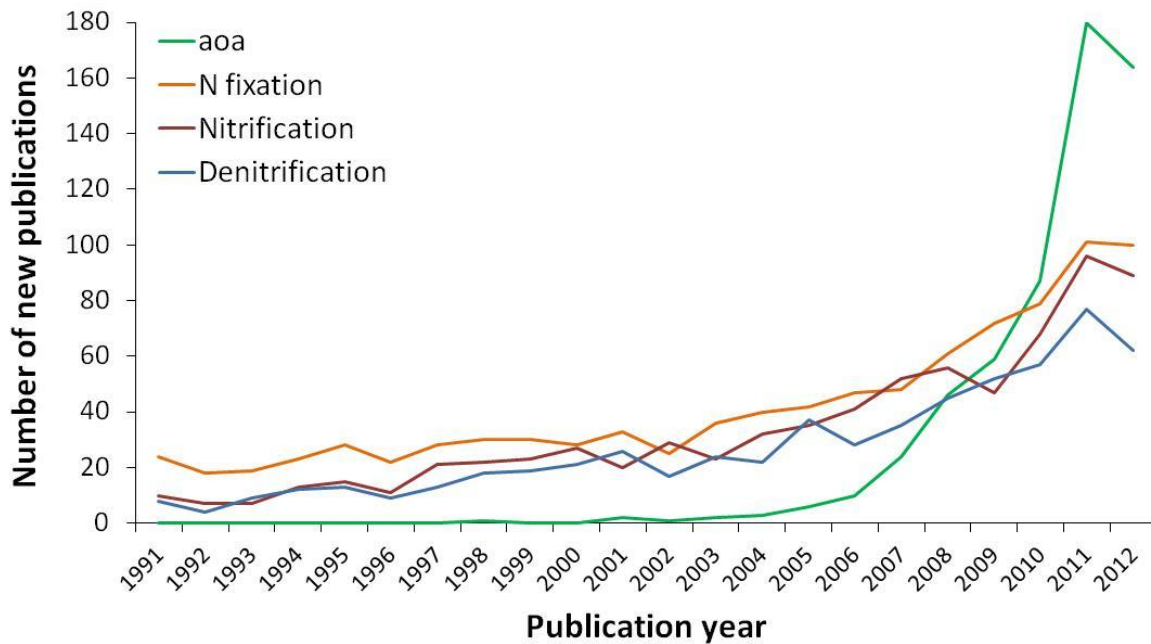
Figure 5. Number of publications per year in Web of Science using keywords for key aspects of the nitrogen cycle. The four terms were searched for separately through the Thomson Reuters database. The following keywords were used in the search: **aoa,** *archa* AND ("ammonia oxid*" or thaum* or aoa or aea); **N fixation,** ecolog* AND ("nitrogen fix*" or "n-fix*" or "n2-fix*"); **Nitrification,** ecolog* AND ("ammonia oxidi*" or "nitrif*"); **Denitrification,** ecolog* AND (denitrif*). Older studies were excluded from the figure. The search for aoa was intended to find all publications in any discipline about archaea that are potential ammonia oxidizers. The other three searches were each combined with "ecolog*" to be able to capture the most relevant publications in microbial ecology research, possibly excluding unidisciplinary studies such as from only genetics or biochemistry.

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$NH_3 + \frac{1}{2}O_2 \rightarrow NH_2OH$ (1)

$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$ (2)

$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$ (1 + 2) (3)

$NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^-$ (4)

$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$ (5)

$NH_2OH + 1\frac{1}{2}O_2 \rightarrow HNO_2 + 2e^- + 2H^+$ (4 + 5) (6)

$NH_3 + 1\frac{1}{2}O_2 \rightarrow HNO_2 + H_2O$ (7)
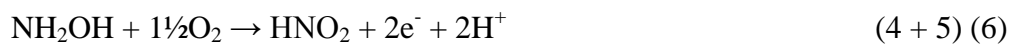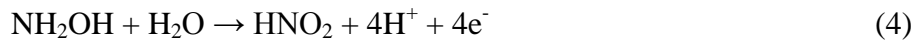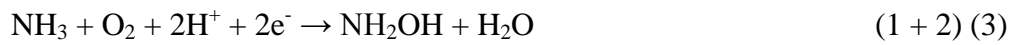
$\Delta G_0{'} = -235 kJ \cdot mol^{-1}$

Figure 6. Half reactions and sum reactions of aerobic ammonia oxidation to nitrite in ammonia oxidizing bacteria. Change in standard free energy is calculated for the final given reaction (Bock and Wagner 2006).
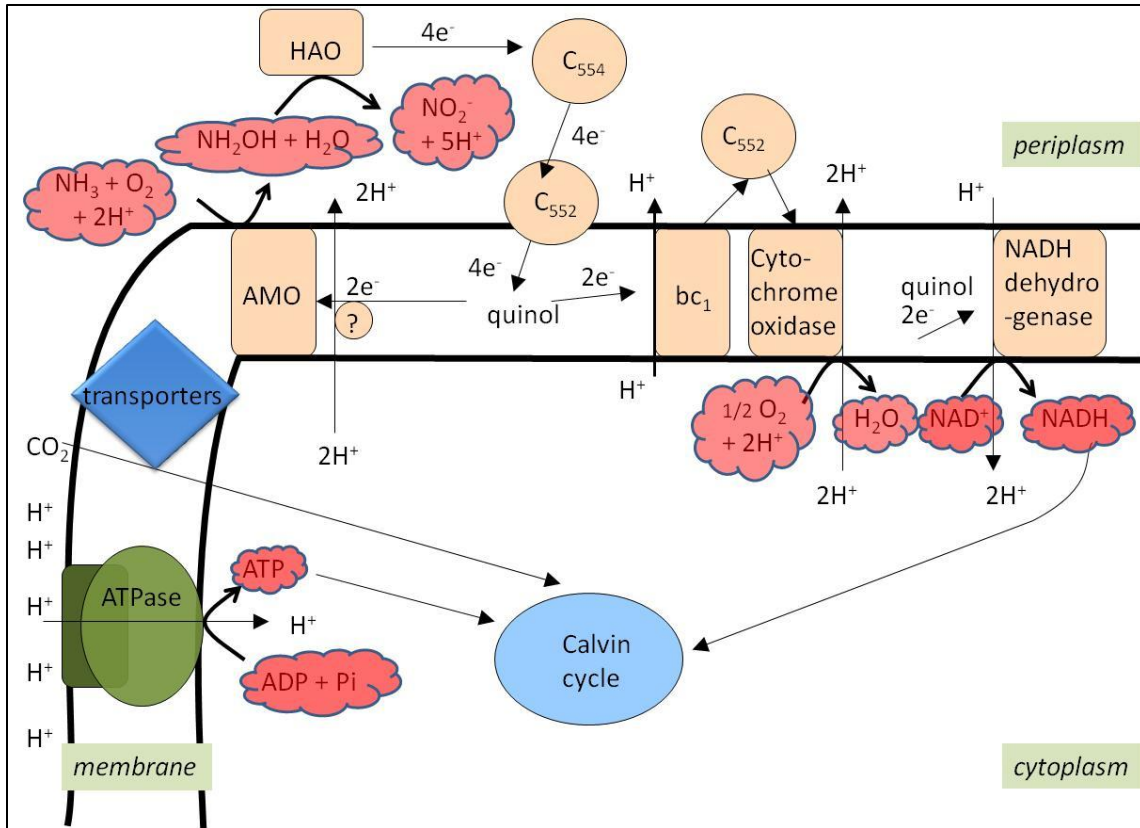
Figure 7. Electron transport chain and energy generation in an ammonia oxidizing bacterial cell. Figure redrawn based on Arp and Stein 2003; Klotz and Stein 2008.

1.7. References

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2. Ammonia-oxidizing archaea respond positively to inorganic N addition in desert soils

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2.1. Abstract

Nitrogen (N) addition typically enhances ammonia oxidation (AO) rates and affects ammonia-oxidizing community composition by increasing the population density of ammonia-oxidizing bacteria (AOB) but not that of archaea (AOA). We asked if inorganic N addition affects AO by enriching for particular physiological types of microorganisms (i.e. through niche differentiation). To answer this question, we used Sonoran Desert soils (Arizona, USA) that had been supplied with NH_4NO_3 for 7 years in comparison with unfertilized controls. In the lab, we determined community enzyme kinetics of AO. Based on the *amoA* gene, we used quantitative PCR to measure population size of AOA and AOB, and determined community composition and diversity with bioinformatics analyses of community DNA pyrosequencing. As expected, we found that N addition increased AO rates (based on maximal rates calculated from kinetics of AO) and abundance of bacterial *amoA* genes. Surprisingly, N addition also increased abundance of archaeal *amoA* genes. We also show that desert soil ammonia-oxidizing communities are dominated by AOA (*Nitrososphaera*-related phylotypes, 78% of entire *amoA*-encoded community), as expected, but are followed by a distribution of AOB that is unusual in terrestrial systems (*Nitrosomonas*, 18%; *Nitrosospira*, 2%). However, we did not detect any major effects of N addition on the composition of the ammonia-oxidizing community. This study highlights the unique responses and composition of ammonia-oxidizers in arid lands, which should be considered in predictions of AO responses to changes in N availability.

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2.2. Introduction

Since the early and influential work of Sergei Winogradsky (1890), bacteria were thought

to be the only biological agents of ammonia oxidation (AO). In the last three decades, the

deployment of molecular detection techniques has revealed that *Thaumarchaeota* in the

Archaeal domain contribute to AO as well (Konneke et al. 2005; Tourna et al. 2011).

High-throughput sequencing and molecular-fingerprinting studies show the presence of

genes attributable to diverse groups of ammonia-oxidizing archaea (AOA) and bacteria

(AOB) in a wide variety of environments (Purkhold et al. 2000; Leininger et al. 2006;

Prosser and Nicol 2008; Pester et al. 2012). Even though AOA outnumber AOB in many

ecosystems (Leininger et al. 2006; Adair and Schwartz 2008; Wessen et al. 2010), the

AOB are often the main contributors to AO (Jia and Conrad 2009; Di et al. 2009; Adair

and Schwartz 2011). It remains unclear why abundance of AOA is sometimes unrelated

to AO rates (Shen et al. 2008; Wessen et al. 2010). Differential substrate affinities and

ecophysiological sensitivities among and within the AOA and AOB may lead to AO

fluxes that depend not only on population size, but also on population composition

(Kowalchuk and Stephen 2001; Bollmann et al. 2002; Jia and Conrad 2009; Schleper and

Nicol 2010).

A review of literature reveals that mixed ammonia-oxidizer communities tend to show

dominance by one particular phylotype (Prosser 1989; Kowalchuk and Stephen 2001;

Zhalnina et al. 2012; He et al. 2012). However, it is uncertain how this outcome is

determined by potentially relevant environmental properties such as ammonium (NH_4^+)

content. For instance, while culture work shows that *Nitrosomonas* spp. (AOB) prefer

ammonia (NH_3)-rich conditions (Taylor and Bottomley 2006), *Nitrosospira*-related

clusters (AOB) commonly outnumber *Nitrosomonas* spp. in fertilized soils and also in

low-NH_4^+, pristine soils (Jordan et al. 2005; Chu et al. 2007). Additionally, AOB are

preferentially enriched after inorganic nitrogen (N) fertilization in the ecosystems studied

to date – most of which have low-pH soils that receive relatively high rates of

precipitation or water inputs – while AOA may respond positively only in some cases

when NH_3/NH_4^+ is supplied through organic matter mineralization (Offre et al. 2009; He

et al. 2012; Hatzenpichler 2012). These examples suggest that indeed changes in N

availability may control AO rates in soils through community compositional shifts

(Avrahami and Bohannan 2007; Tourna et al. 2010; Prosser and Nicol 2012).

Soils that encounter extreme and fluctuating conditions, such as those in some arid or

managed environments, may be ideal systems to detect a shift in microbial population

dominance and function (Wall and Virginia 1999; Fierer et al. 2012). Arid lands are

exposed to distinct conditions ranging from prolonged drought to rapid pulses of

precipitation and nutrients (Schimel et al. 2007; Collins et al. 2008). Desert soils are

typically dry with low organic matter and low N mineralization rates, especially in

interplant spaces away from plants (Austin et al. 2004; Schade and Hobbie 2005), which

may select for the most oligotrophic ammonia-oxidizers (i.e. the AOA). These

ecosystems also increasingly experience intensive management, including watering and

fertilizer inputs, both in agricultural and urban residential areas (Warren et al. 1996;

Davies and Hall 2010). As a result, anthropogenic activities and atmospheric deposition are altering resource availability and the N cycle in soils of water-limited environments (McCrackin et al. 2008; Hall et al. 2009; Hall et al. 2011; Marusenko et al. 2013a). Given these trends, desert soils – particularly those near cities – may harbor microbial communities adapted to rapidly changing environmental conditions.

We tested the effect of long-term inorganic N addition on AO processes and ammonia-oxidizing microorganisms (AOM) in arid land soils. In the lab, we used a short-term assessment of AO kinetics in bulk soil and also characterized AOA and AOB based on the *amoA* gene, which encodes a subunit of the ammonia-monooxygenase (AMO) enzyme. We hypothesized that N addition would cause the absolute and relative abundance of ammonia-oxidizers to shift from AOA-dominated in oligotrophic native (unfertilized) soils to AOB-dominated in NH_4^+-rich conditions. Consequently, this population replacement would enhance AO rates and *amoA* gene copy-specific AO rates, but decrease affinity between the enzyme and the substrate (increase K_m parameter; measured from kinetics of AO). Using common patch types in arid lands, we further expected that the decline of AOA relative to AOB under N addition would be less dramatic in relatively fertile soils under shrubs than in areas away from plants.

2.3. Materials and methods

2.3.1. Study area description

Our site is in the northern Sonoran Desert at ~620 m elevation in Lost Dutchman State Park, AZ, USA (coordinates: N 33.459372 S -111.484956), located downwind of the urban core and within boundaries of the Central Arizona–Phoenix Long-Term Ecological Research area (http://caplter.asu.edu). Soils are classified as Typic Haplargids subgroup of Aridisols. We measured soil AO rates and community parameters from a 20 m x 20 m plot that has been fertilized with N as NH_4NO_3 (added biannually at 60 kg N ha^{-1} yr^{-1} from 2005-2012; Hall et al. 2011), and compared data with an unfertilized plot (control). Plant cover (~60%) within our study plots is dominated by the native plants, creosote bush (*Larrea tridentata* [DC.] Coville), bursage (*Ambrosia* spp.), and N-fixers (*Prosopis* spp.). Mean annual temperature is 22.3°C, with the coldest and warmest months averaging 3.7°C and 41.9°C, respectively (2005-20012; NCDC, 2013). Mean annual precipitation is 272 mm but is highly variable year to year. Rainfall is bimodally distributed between summer monsoon events and low-intensity winter storms (WRCC, 1985).

2.3.2. Sample collection

Surface soil samples were collected in late January of 2012, one month after winter storms. In each of the control and N addition plots, soils were collected from two patch types: between plants (interplant) and under *L. tridentata* canopy (under plant), in order to explore N treatment effects in common patch types of Sonoran soils. Each soil sample consisted of two 0-5 cm (depth) x 7 cm (diameter) cores taken 5 cm apart. Twelve samples, processed independently for all analyses (soil properties, AO rates, quantitative PCR, pyrosequencing), consisted of three replicate soil samples from each plot (treatment, control) and patch type (interplant, under plant) (3 x 2 x 2 = 12 samples).

2.3.3. Laboratory methods and soil properties

Following collection, samples were transported on ice to the lab, sieved to < 2 mm, and homogenized. Soils were at 3-5% soil moisture upon collection and were analyzed within 24 h for all soil properties and processes. Two subsamples (2 g each) from each homogenized soil sample were frozen in liquid N and stored at -80°C until DNA extraction within one month. Duplicate DNA extracts were combined prior to molecular processing methods to obtain one determination per sample.

Soils were processed for pH (1:2 soil to DI H$_2$O), water holding capacity (% WHC; gravimetrically), organic matter content (% SOM; loss on ignition), and extractable

NH_4^+, nitrite (NO_2^-), and nitrate (NO_3^-) content (2M KCl extraction, colorimetric analysis), following standard, published methods (Marusenko et al. 2013a). Data reported for each of the three field replicates is an average of laboratory triplicates.

2.3.4. Potential rates of ammonia oxidation

In situ rates of *potential* AO were measured under various levels of N addition (see "Ammonia oxidation kinetics" below) using the shaken-slurry method, in which oxygen and substrate diffusion is not limited (Hart et al. 1994; Norton and Stark 2011). The direct product of AO was measured as NO_2^- accumulation after inclusion of chlorate ($NaClO_3$), a NO_2^--oxidation inhibitor (Belser and Mays 1980). Using NO_3^- as a proxy for AO was unsuitable since NO_2^- build-up is common in natural dryland conditions (Gelfand and Yakir 2008). The shaken-slurry assays contained 10 g soil in 100 mL solution of 0.015 mol·L^{-1} $NaClO_3$, and 0.2 mol·L^{-1} K_2HPO_4 and 0.2 mol·L^{-1} KH_2PO_4 to buffer pH at 7.2. Slurries and no-soil blanks were continuously aerated in solution by mixing at 180 rpm on a reciprocal shaker in the dark. Homogenized slurry aliquots were removed at four time points over 6 h and amended with several drops of $MgCl_2 + CaCl_2$ (0.6 M) to flocculate soil particles. Aliquots were then centrifuged at $3000 \times g$ and supernatant was filtered through pre-leached Whatman #42 ashless filters. The supernatants were stored at 4°C and analyzed within 24 h. Net rates of *potential* AO were calculated as the linear increase in NO_2^- content from 0 to 6 h, measured colorimetrically using a Lachat Quikchem 8000 autoanalyzer.

2.3.5. Actual rates of ammonia oxidation

We also measured AO following various levels of N addition in a modified method using NaClO$_3$ inhibition in static, aerobic incubations for 48 h of bulk soil (Nishio and Fujimoto 1990; Hart et al. 1994; Low et al. 1997). Although substrate diffusion here may be limited compared to the shaken-slurry assays, this method assesses AO in conditions more similar to those of native soils during microbial activity. Ten g of soil was brought to 60% WHC using water and NaClO$_3$ (15 mM) in plastic cups. Soil in one cup was extracted at the onset and a second cup extracted after incubation in the dark. Soils were extracted in 50 mL of 2 M KCl followed by shaking for 1 h and filtering through pre-leached Whatman #42 ashless filters. The extracts were stored at 4°C and analyzed colorimetrically within 24 h. Net rates of *actual* AO were calculated as the increase in NO$_2^-$ content between 0 and 48 h.

2.3.6. Ammonia oxidation kinetics

The relationship between substrate availability and reaction rate can be measured to discern functional parameters of microbial communities. We used calculations similar to those of enzyme kinetics to model relationships for AO in bulk soils based on the Michaelis-Menten equation (Martens-Habbena and Stahl 2011; Prosser and Nicol 2012):

$V = (V_{max} \times S) / (K_m + S)$

The NH_4^+ concentration (S) and AO rate (V) are used to estimate the maximum AO rate (V_{max}) and half-saturation constant (K_m; inverse of enzyme and substrate affinity). To estimate AO kinetics under oligotrophic conditions in the shaken-slurry assay, we removed pre-existing NH_4^+ from soils to obtain the least variable and lowest residual substrate availability (Widmer et al. 1989; Koper et al. 2010; Norton and Stark 2011). Prior to the shaken-slurry assay, 5 g soil was mixed in 45 mL of potassium phosphate solution and centrifuged at $3200 \times g$ for 1 min before the supernatant was removed. The resulting soil pellet from two preparations was combined to compose 10 g total soil from each plot (treatment, control), patch type (interplant, under plant), and soil sample replicate (x 3). Inorganic N was then supplemented as $(NH_4)_2SO_4$ mixed with DI water to eight final concentrations in the slurry ranging from 0-22.5 mM. In total we evaluated AO rates using 96 different soil preparations (12 soil samples x 8 NH_4^+ concentrations) per method (shaken-slurry assay, static incubation). In the static incubations, we excluded the N removal step as to minimize soil disturbance. Soils were supplemented with $(NH_4)_2SO_4$ in solution to produce final concentrations ranging from 0-50 µg NH_4^+-N g^{-1} (0-22.5 mM). The 0 µg NH_4^+-N g^{-1} addition (only includes pre-existing NH_4^+) was used to estimate background net rates of AO. As a rough indicator of N addition effect on the relative importance of NH_4^+ mineralization and nitrification, we also measured the net rate of NH_4^+ gain (production processes dominate) and loss (consumption processes dominate) during the static incubation experiment. In the assay, some of the NH_4^+ consumption processes are likely minimized due to sieving of soil (exclusion of large

NH$_4^+$-assimilating plant roots) and lower laboratory temperature compared to natural

conditions (reduced volatilization).

2.3.7. DNA extraction and purification

DNA was extracted using three freeze-thaw cycles followed by 30 m incubation at 50°C

with proteinase K and silica bead beating for chemical and mechanical cell lysis (Garcia-

Pichel et al. 2001). The lysate was purified by phenol:chloroform:isoamyl alcohol

(25:24:1) extraction, followed by DNA precipitation in 100% ethanol for 12 h at -80°C.

DNA concentration and quality was assessed on an agarose gel stained in ethidium

bromide and imaged using a Fluor-S Multi-Imager (BioRad Laboratories, CA, USA) with

an EZ Load Precision Molecular Mass Standard (BioRad). Bands of DNA were excised

from a low-melt agarose gel, homogenized with a tip in a microcentrifuge tube, allowed

to diffuse out into sterile H$_2$O for 12 h, and followed by 15 m centrifugation to collect

DNA in the supernatant.

2.3.8. Quantitative PCR

DNA was used for quantitative PCR (qPCR) with the following *amoA* primers:

CrenamoA616r (GCCATCCABCKRTANGTCCA; Tourna et al. 2008) and

CrenamoA23f for the AOA (ATGGTCTGGCTWAGACG); and amoA1f mod

(GGGGHTTYTACTGGTGGT; Stephen et al. 1999) and AmoA-2R' for the AOB

(CCTCKGSAAAGCCTTCTTC; Okano et al. 2004; Junier et al. 2008). qPCR reactions

contained 10 μL iTaq SYBRGreen Master Mix (BioRad), 250 nM final concentration of

each primer (AOA or AOB), 1 ng of environmental DNA, and molecular grade H_2O to

bring each reaction to a final volume of 20 μL. The reaction conditions were as follows:

initial denaturation for 150 s at 95°C followed by 45 cycles of 15 s at 95°C, 30 s at 55°C,

and 30 s at 72°C, and a final dissociation step to obtain the melting curve at 95°C, 60°C,

and 95°C for 15 s each. Standard curves were generated using templates from

Nitrosomonas europaea ATCC 19718 (bacterial *amoA*; $R^2 = 0.99$) and a putative AOA

clone (archaeal *amoA*; $R^2 = 0.99$) for a dilution series spanning 10^2-10^{10} gene copies per

reaction. Melting curves were checked to verify the quality of each reaction, and to

ensure the absence of primer-dimers. We report only determinations for which C_t values

could be interpolated within our standard curves. Each value reported is an average of

analytical triplicate qPCR reactions of the same DNA extract.

2.3.9. Pyrosequencing

Purified DNA extracts were shipped to a commercial laboratory for standard PCR and

bTEFAP pyrosequencing (Dowd et al. 2008). Commercial primers for PCR were amoA-

1F (GGGGTTTCTACTGGTGGT; Rotthauwe et al. 1997) and amoA-2R for AOB

(CCCCTCKGSAAAGCCTTCTTC), and Arch-amoAF

(STAATGGTCTGGCTTAGACG; Francis et al. 2005) and Arch-amoAR for AOA

(GCGGCCATCCATCTGTATGT) used with a HotStarTaq Plus Master Mix Kit (Qiagen,

CA, USA). PCR conditions were as follows: 180 s at 94°C followed by 28 cycles of 30 s

at 94°C, 40 s at 53°C, and 60 s at 72°C, and final elongation for 5 m at 72°C. PCR

amplicons were mixed in equal concentrations and purified using Agencourt Ampure

beads (Agencourt Bioscience Corporation, MA, USA). Sequencing utilized Roche 454

FLX titanium instruments and reagents.

2.3.10. Bioinformatics and phylogenetic analyses of *amoA*

Detailed steps of pyrosequencing data processing and analyses in Qiime (Caporaso et al.

2010b) are included as script in the Supplementary Files. Sequences (452 bp long) were

clustered into operational taxonomic units (OTUs; groups of sequences sharing > 97%

nucleotide similarity) using UClust (Edgar 2010). Representative sequences (one per

OTU) were aligned with Pynast (Caporaso et al. 2010a). Based on phylogenetic

classification for AOA in Pester et al. (2012), a taxonomic assignment was made for each

OTU using a reference database created from sequences of known pure isolates,

enrichments, and other characterized AOA from previous studies. The template

databases, including AOB based on Koops et al. (2006) and AOA, are described in the

Supplementary Files. For AOA, we excluded one replicate each in the interplant control

and N addition samples because of overall poor quality of the pyrosequencing data. The

minimum number of high-quality sequences after filtering was 525 for AOA and 950 for

AOB, with rarefied analysis producing 179 OTUs for AOA and 325 OTUs for AOB.

Phylogenetic analyses were carried out on a single alignment file (separately for AOA and AOB) that included sequences from our Qiime pipeline, as well as the reference sequences described above. All sequences were combined and realigned using default parameters for Muscle and analyzed by the tree-building module of the MEGA 5 software with the following parameters: Neighbor-joining statistical method, Jukes-Cantor nucleotide substitution model, 100 bootstrap replicates, uniform rates among sites, and pairwise gap-data deletion (Tamura et al. 2011). Representative sequences of phylogenetically distinct OTUs have been submitted to GenBank for dominant and novel archaeal (NCBI accession numbers: xxxx) and bacterial (xxxx) *amoA* genes (sequences will be submitted later).

2.3.11. Statistics

Statistical tests were carried out using Qiime for α and β diversity measures on processed pyrosequencing data, while all other analyses were in SPSS (v20.0 Windows). All soil properties, AO rates, and *amoA* abundance data were tested for linear model assumptions in SPSS using normal probability plots (for normality) and Levene's test (for equal variance), and transformed (natural log) when necessary. Individual two-way analysis of variance (ANOVA) tests were used to evaluate the effects of plants ('Patch') and N addition ('Treatment') on the following dependent variables: *amoA* copies per g soil, *amoA* copies per ng extractable DNA, AOA to AOB ratio, *potential* V_{max} AO rates, *actual* V_{max} AO rates, *amoA*-copy specific AO rates, net NH_4^+ change (averaged across

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supplemented NH_4^+ concentrations), and each of the soil properties. Significant

interactions were evaluated further for effects of Treatment using one-way ANOVA for

each patch type ($\alpha = 0.025$). The copy specific rates were calculated using V_{max} AO rate

(*potential* and *actual*) divided by the number of *amoA* copies per g soil. We used

bivariate Pearson correlations to assess relationships between soil properties vs.

community parameters (*amoA* data and AO rates) across all samples. We used linear

regression analyses to assess relationships between *amoA* abundance at the domain level

vs. V_{max} AO rates and also analyzed *amoA* abundance of individual OTUs vs. soil

properties and AO rates. In Qiime, we tested for Treatment effect on OTU-based

communities separately for AOA and AOB per patch type, using only strictly relevant

diversity metrics (Lozupone and Knight 2005; Caporaso et al. 2010b): α diversity

(Shannon's diversity, observed richness, and phylogenetic diversity [PD]); β diversity

(weighted and unweighted Unifrac, the multivariate group dispersion analogue of

Levene's test [PERMDISP], and analysis of similarity [ANOSIM]).

2.4. Results

2.4.1. Effects of N addition on the abundance of *amoA* genes and the

kinetics of ammonia oxidation

To aid in interpretation of long-term N addition effects on *amoA* abundance and AO

rates, we considered the influence of soil properties and the relative importance of

fertilizer N vs. ammonification as a possible NH_4^+ source. N addition slightly acidified the soils (Table 1) – an effect known to create less optimal conditions for AO (Arp and Stein 2003) – but resulted in an increase in AO rates here (Suppl. Table 1). Both maximum *actual* and *potential* AO rates were strongly predicted by pH and soil organic matter (negative and positive correlation, respectively), while background AO rates were most strongly related to NH_4^+ concentration (positively) across all patch types and N treatment. Additionally, N addition significantly increased net rates of NH_4^+ loss in both patch types (Patch, $P = 0.56$; Treatment, $P < 0.01$). These data suggest that N addition stimulated NH_4^+ change from consumption processes (e.g., NH_3/NH_4^+ oxidation, microbial immobilization) more dominantly than from organic N mineralizers.

As expected from other studies, long-term N addition increased the abundance of bacterial *amoA* gene copies per g soil and decreased the ratio of AOA to AOB in the relatively fertile soils under plant canopies (Fig. 1; Suppl. Table 2; N addition, 3.6 AOA/AOB; Control, 4.9 AOA/AOB). However, the effect of N addition on the ratio of AOA to AOB varied with plant patch type, with a general increasing trend in soils between plants (N addition, 6.2 AOA/AOB; Control, 4.3 AOA/AOB). Surprisingly, N addition also had a positive effect on the AOA: In soils between plants, both the abundance of archaeal *amoA* genes per g soil (Fig. 1) and archaeal *amoA* genes per ng DNA were higher in the N addition soils (904 archaeal *amoA* copies/ng DNA) than in the control (548 archaeal *amoA* copies/ng DNA).

Actual and *potential* maximal AO rates (i.e. at V_{max}) were significantly higher after long-term N addition compared to the control soils (Fig. 2; $P < 0.05$ in all cases). NH_4^+ supplementation in the short-term laboratory incubations enhanced AO, but only for the *actual* AO rates of the unfertilized soils (Fig. 2). This pattern shows that rates of AO in Sonoran Desert soils under undisturbed, background conditions are NH_4^+-limited and may be influenced by anthropogenic N addition. Taken together with the qPCR data, these results suggest that both AOA and AOB are likely contributors to AO and are both responsive to environmental change (Fig. 3), as N addition significantly increases AO rates as well as the abundance of archaeal and bacterial *amoA* genes.

To investigate the functional capacity of ammonia-oxidizing communities in bulk soils, we attempted to evaluate the affinity (K_m) parameter from the AO kinetics plots (Fig. 2), and normalized AO rates by the community size (Fig. 4). The K_m could only be estimated where NH_4^+ concentration was low enough to measure AO rate at $\frac{1}{2}V_{max}$. In the shaken-slurry assays (*potential* AO rates), residual NH_4^+ was detected as low as 17 µM (Fig. 2), signifying that the community K_m is at or below this low value (below K_m values for known AOB). In the static incubations (*actual* AO rates), the mean K_m was 2.6 and 1.2 mM NH_4^+ for the unfertilized (control) soils under plants and between plants, respectively, but effects of N addition could not be evaluated. However, *amoA* copy-specific AO rates were higher in N addition soils compared to control soils between plants (significant for the shaken-slurry assay only; Fig. 4; $P < 0.001$; $P > 0.2$ for Treatment effect in all other cases), suggesting a change in community function.

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2.4.2. Composition of the ammonia-oxidizing community

The community composition of AOM was minimally related to patch type or N addition when assessed at the level of OTUs, with no significant response by the dominant members (Fig. 5). All phylotypes detected were related to either *Nitrososphaera (Thaumarchaeota)* or *Nitrosomonas* and *Nitrosospira* (both *β-proteobacteria*), at a ratio of about 45:10:1, respectively (Fig. 5; Suppl. Fig. 1). One group, *Nitrososphaera* subcluster 1.1-related, accounted for 60% of all the AOM. In soil under plants, N addition decreased AOA phylogenetic diversity (PD) ($P < 0.001$) but increased the group variance for AOB (PERMDISP, $P = 0.037$). These results suggest that N addition increased AOA OTU community relatedness but decreased community relatedness in the AOB. However, all other α and β diversity metrics revealed that the composition of AOA and AOB was not related to N addition, nor patch type ($P > 0.1$ in all cases). Together – at least as far as one can detect based on the *amoA* gene sequences – these data suggest that long-term N addition had a minor effect, if at all, on AOM community structure.

Several patterns emerged in analyses between environmental parameters and *amoA* abundance at the individual OTU-level. AO rates were predicted by the number of *amoA* gene copies per g soil for particular individual OTUs (Suppl. Fig. 2), in support of domain-level trends where the net positive response to N addition is a sum of the entire community (Fig. 3). In some cases, individual OTUs have distinct relationships with AO

rates and soil properties, indicating that not all OTUs respond identically and that niche

segregation may occur within the subgroups of AOA and AOB.

2.5. Discussion

2.5.1. Source of N for ammonia-oxidizing microorganisms

AOA have been shown to be outcompeted by AOB and are unresponsive to inorganic N

addition in other studies from ecosystems distinct from the deserts studied here (e.g., Jia

and Conrad 2009; Di et al. 2009; Stopnisek et al. 2010; Xia et al. 2011; Verhamme et al.

2011; Levicnik-Hoefferle et al. 2012; and reviewed in Hatzenpichler 2012). A few

studies have shown that AOA may react favorably to organic N sources or N

mineralization (Chen et al. 2008; Schauss et al. 2009; Kelly et al. 2011; Lu et al. 2012;

Levicnik-Hoefferle et al. 2012). However, organic N inputs are relatively low in low-

productivity ecosystems such as deserts and other extreme environments (Schimel and

Bennett 2004; Booth et al. 2005). For example, although N inputs to arid lands

significantly increase productivity and N content of seasonal herbaceous annual plants

(particularly in wet years), net potential N mineralization in soil does not appear to be

consistently augmented by N addition – perhaps due to the frequency of water limitation,

the patchiness of plant growth, and litter and organic matter loss pathways such as

photodegradation and aeolian/hydrologic transport (Hall et al. 2009; Rao et al. 2009; Hall

et al. 2011). Even if N mineralization from organic matter is indeed a primary source of

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NH$_3$ for energy generation in AOA, our results highlight the unique, positive response of AOA to long-term inorganic N addition in deserts, only in the low organic matter patches away from shrubs.

In arid systems, AOA may require adaptive strategies to prefer inorganic sources of N. For example, pulses of resource availability from drying/wetting cycles in arid lands may force AOA to use inorganic N, since heterotrophs are likely the first to consume organic N upon metabolic activation after drought (Placella and Firestone 2013). Additionally, alkaline and hot environments may minimize NH$_3$ protonation to NH$_4^+$, and facilitate NH$_4^+$ from inorganic sources to deprotonate, leading to NH$_3$-gas diffusion throughout the soil matrix (McCalley and Sparks 2009; Geisseler et al. 2010) that could be freely used by both AOB and AOA. AOA may be capable of using either organic N or inorganic N by the same microbial cell, depending on environmental conditions, as shown *in vitro* for the only pure AOA isolate from soil, *Nitrososphaera viennensis* (Tourna et al. 2011), and *in silico* based on the genome of a recent enrichment culture, Candidatus *Nitrososphaera gargensis* (Spang et al. 2012).

2.5.2. Size, structure, and function of ammonia oxidizing communities in arid land soils

We hypothesized that long-term N addition selects for ammonia-oxidizers that are more copiotrophic (lower substrate affinity, higher per cell activity) than those in unfertilized

soils (Martens-Habbena et al. 2009; Prosser and Nicol 2012). Indeed, N addition elevated the AO rate per *amoA*–copy, but this effect was significant for only the least fertile parts of the landscape (in soil between plants; Fig. 4). However, the relative abundance of dominant *amoA* OTUs was constant across treatments, with small changes only in the minor members (Fig. 5). Of course we cannot fully discount the idea that perhaps the minor OTUs represent those that are ecologically relevant, while the numerically dominant groups are less efficient or inactive (Lennon and Jones 2011). This scenario has yet to be proven experimentally and is unlikely to be the case here since archaeal and bacterial *amoA* gene abundance – largely determined by the common OTUs – was positively correlated with AO rates (Fig. 3, Suppl. Fig. 2). Another interpretation of the community composition data is that AOM are regulating genes other than *amoA*, which have no major effect on relative abundance of *amoA* OTUs but contribute to instantaneous AO (Cho et al. 2006).

2.5.3. Evidence of unique ammonia oxidation patterns in deserts

Arid land soils face extreme environmental conditions that may select for unique phylogeny and niche separation. Terrestrial studies worldwide have revealed that the "marine" clade AOA (Group I.1a) are often the main responders to environmental changes in soil and contributors to AO (Hatzenpichler 2012), despite being outnumbered by the "soil" clade (Group I.1b; Verhamme et al. 2011; Isobe et al. 2012; Long et al. 2012; Zhang et al. 2012; Lu and Jia 2013). Here, we show that AOA within the "soil"

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clade responded significantly to N addition, and the abundance of this group was positively related to AO rates in desert soil. We also found that *Nitrosomonas* sequences outnumbered *Nitrosospira*, a rarity for soil systems. *Nitrosomonas* spp. dominance appears to be limited to alkaline, high-salt, and sometimes high-NH_4^+ conditions (Webster et al. 2005; Koops et al. 2006; Cantera et al. 2006; Ke and Lu 2012). Pulsed resource availability – a characteristic of arid lands – may also drive this distribution, since *Nitrosomonas* strains have advantages over *Nitrosospira* such as faster growth responses after starvation (Bollmann et al. 2002). Additionally, in most soils studied previously, AOA outnumber AOB to a greater extent than found in our study (Leininger et al. 2006). In cases where AOB outnumber AOA, typically up to 10-fold in terrestrial systems (e.g. Di et al. 2009), other arid lands also have a novel distribution as AOB outnumber AOA by 100-fold in cold desert biocrusts (Marusenko et al. 2013b). Overall, atypical ammonia-oxidizing communities appear to occupy desert soils.

This type of study refines predictions of how environmental change, such as N addition, affects community dominance and AO rates. Growth and activity characteristics derived from culture experiments can be combined with our environmental data, as described below, to explore relationships between AOA and AOB at the physiological and ecosystem scale (Stark and Firestone 1996; Schauss et al. 2009; Prosser and Nicol 2012). For example, since maximum AO activity per cell is higher for *Nitrosospira* and *Nitrosomonas* strains than for AOA by 10 and 35-fold, respectively, AOA must occur more abundantly than AOB to contribute equally to AO. Considering that *Nitrosomonas*

outnumber *Nitrosospira* by 10:1 in our soils, we estimate that the maximum cell activity

for *Nitrosomonas* plus *Nitrosospira* is 33-fold higher than for AOA (as a weighted

average). Based on 1 *amoA* copy per AOA cell and a weighted average of 2.1 *amoA*

copies per AOB cell (2 and 3 *amoA* copies per cell for *Nitrosomonas* and *Nitrosospira*,

respectively; Norton et al. 2002), we calculate that the maximum AO activity per *amoA*

copy is 16-fold higher for AOB than AOA. Assuming equal numbers of genomes and

amoA transcripts per cell, the AOA outnumbering AOB (as *amoA* gene copies) by 4.3-

fold in our soils equates to the AOB contributing to AO rates 3.7-fold more than AOA.

These calculations are consistent with our data, which show that AOB contribute ~3

times more to AO rates than AOA under plants and ~6 times more than AOA in the

spaces between plants (compare slopes in Fig. 3). We show that niche differentiation

plays a role in desert N cycling and that both the AOA and AOB contribute to AO in arid

lands.

2.5.4. Conclusion

N addition affects arid land N cycling primarily through changes in community size, but

less so through changes in community composition. This study is the first to show

significant and positive effects of inorganic N addition on abundance of *Nitrososphaera-*

related AOA in soils with low inputs of N from organic sources, which may be a unique

pattern specific to desert soil conditions. Increased anthropogenic activity resulting in

environmental N enrichment may continue to alter ecosystem function through responses

by both the AOA and AOB. These results stress the importance of research in arid lands and related extreme environments that may elucidate dynamics of AO and AOM in soils. For example, agricultural and pastoral systems in drylands occupy ~32% of the terrestrial land surface worldwide and often contain alkaline soil that is routinely exposed to high temperatures (Koohafkan and Stewart 2008). These systems may contain AOM communities more similar to hot deserts than to arable lands from more mesic environments. Despite covering one-third of terrestrial land globally and providing ecosystem services with economic value (Kroeger and Manalo 2007; Rola-Rubzen and McGregor 2009), arid and semi-arid ecosystems are still understudied (Martin et al. 2012).

Although our results highlight patterns at the long-term scale, it remains to be seen whether population dominance also shifts during short-term N changes associated with pulsed moisture fluctuations that are characteristic of arid lands. Further research is also necessary to predict the AOM contribution to ecologically and atmospherically important gases such as N_2O or NO from nitrifier denitrification and nitrification in these desert soils.

Acknowledgements

2.6. Tables/Figures

Table 1. Soil characteristics from plots used in this study.

Soil patch type[c]	Treatment[d]	pH Mean	pH SD	WHC (%)[a] Mean	WHC (%)[a] SD	SOM (%)[b] Mean	SOM (%)[b] SD	NO_2^- ($\mu g\ N{\cdot}g^{-1}$) Mean	NO_2^- SD	NO_3^- ($\mu g\ N{\cdot}g^{-1}$) Mean	NO_3^- SD	NH_4^+ ($\mu g\ N{\cdot}g^{-1}$) Mean	NH_4^+ SD
Interplant	Control	8.39	0.07	32.0	4.5	2.02	0.57	0.13	0.08	1.40	0.41	0.42	0.17
Interplant	N addition	8.21	0.08	35.1	2.8	2.45	0.44	1.58	0.71	36.10	21.37	22.47	21.31
Under plant	Control	8.25	0.11	42.1	3.3	3.26	0.37	0.03	0.01	4.50	4.63	1.06	0.04
Under plant	N addition	8.11	0.06	45.2	6.9	3.59	0.55	0.16	0.19	29.12	5.11	12.77	9.39
		Two-way ANOVA results, P value											
Patch x Treatment		0.651		0.992		0.868		0.171		0.459		0.464	
Treatment		0.007		0.284		0.214		0.002		0.002		0.036	
Patch		0.034		0.006		0.003		0.002		0.772		0.519	

[a] WHC = water holding capacity. [b] SOM = soil organic matter. [c] Soils were collected from spaces between plants or under the canopy of *Larrea tridentata* shrubs. [d] N addition plots were treated with 60 kg of N (as NH_4NO_3) ha$^{-1}{\cdot}$yr^{-1} during 2005-2012. SD = standard deviation. n = 3.
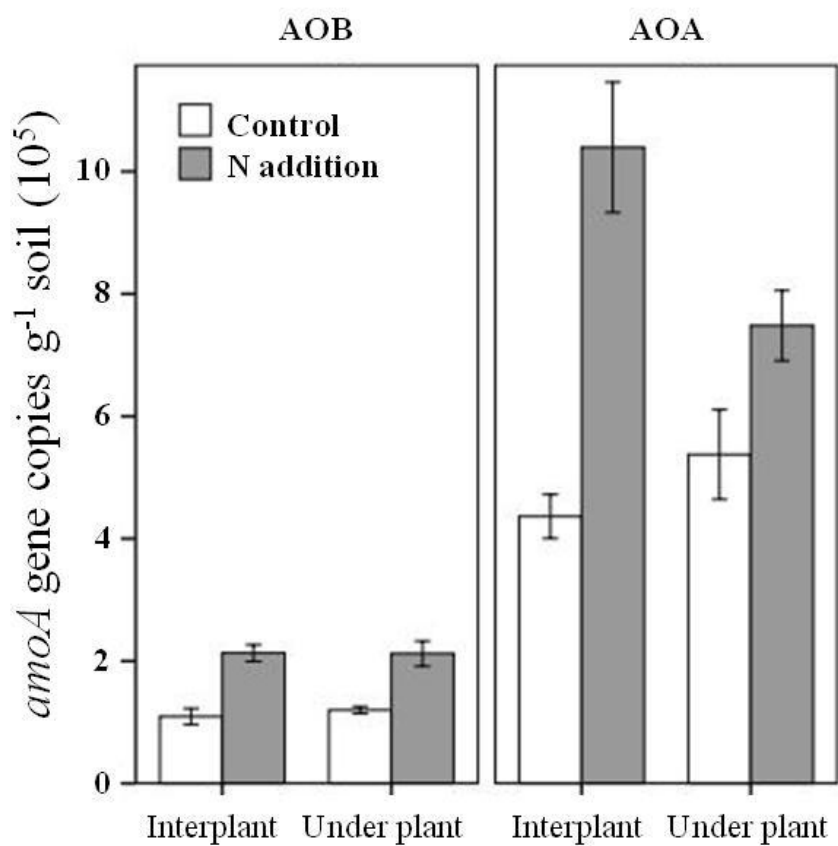
Figure 1. Quantification of *amoA* gene copy numbers for AOB and AOA from Sonoran Desert soil in N addition and control plots. Error bars are standard errors of independent field triplicates.
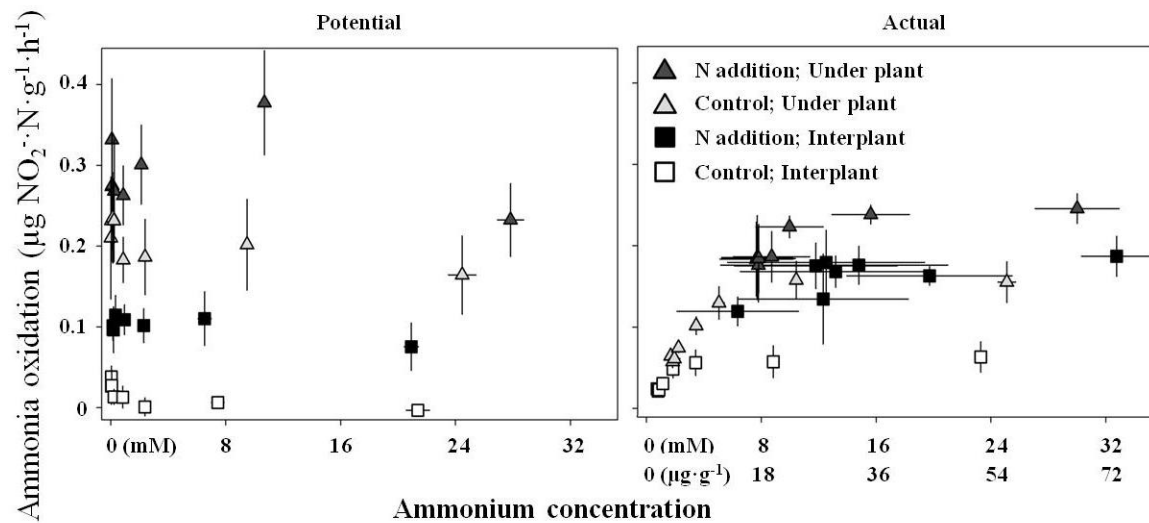
Figure 2. Kinetics of ammonia oxidation using the shaken-slurry assay for potential rates (left) and the static incubation method for actual rates (right). To test the effect of long-term N addition on ammonia oxidation rates, a range of NH_4^+ concentrations was supplemented in the short-term laboratory methods to measure kinetics of ammonia oxidation. NO_2^- accumulation is measured after sodium chlorate inhibition as a proxy for ammonia oxidation. For actual rate values, NH_4^+ is calculated as molar concentration based on soil moisture content at 60% water holding capacity and as µg NH_4^+-N·g^{-1}. Bi-directional error bars are standard deviations of independent field triplicates to show variation in supplemented NH_4^+ and measured ammonia oxidation rates.
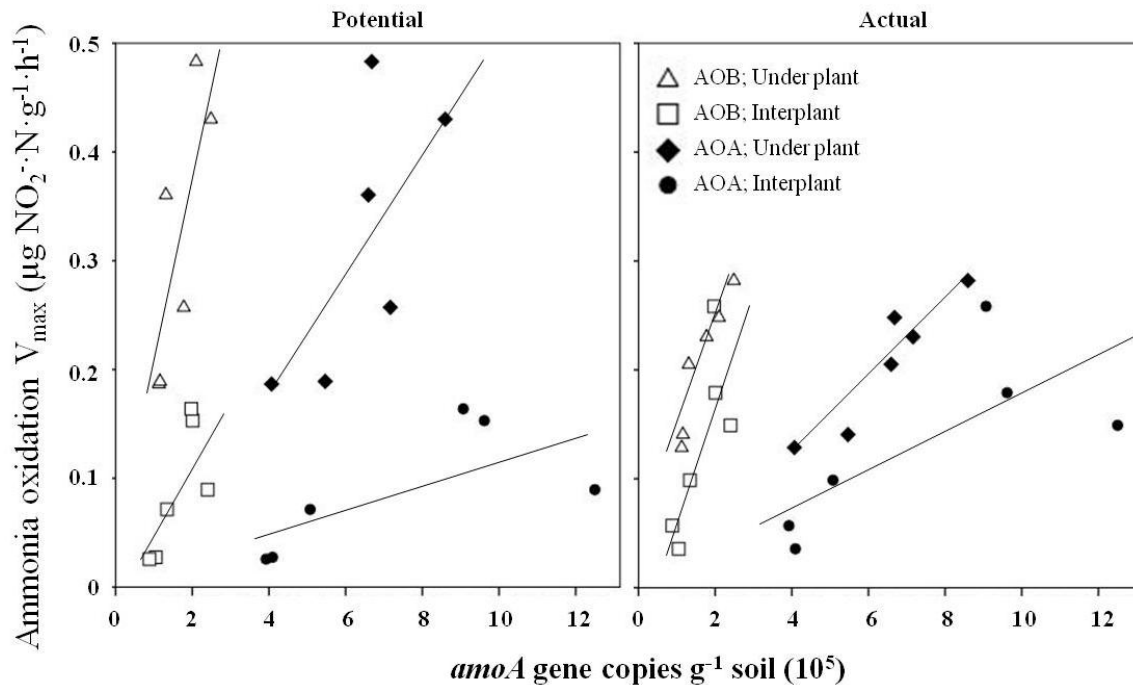
Figure 3. Relationship between *amoA* gene copy numbers and maximum (V$_{max}$) ammonia oxidation rates in soil across control and N addition plots. V$_{max}$ is calculated separately for potential (left) and actual (right) rate assays. Regression statistics for potential and actual rate assays, respectively: AOB Under plant, $R^2 = 0.63$ P $= 0.059$, $R^2 = 0.88$ P $= 0.005$; AOB Interplant, $R^2 = 0.59$ P $= 0.075$, $R^2 = 0.61$ P $= 0.068$; AOA Under plant, $R^2 = 0.49$ P $= 0.119$, $R^2 = 0.88$ P $= 0.005$; AOA Interplant, $R^2 = 0.49$ P $= 0.123$, $R^2 = 0.52$, P $= 0.104$.
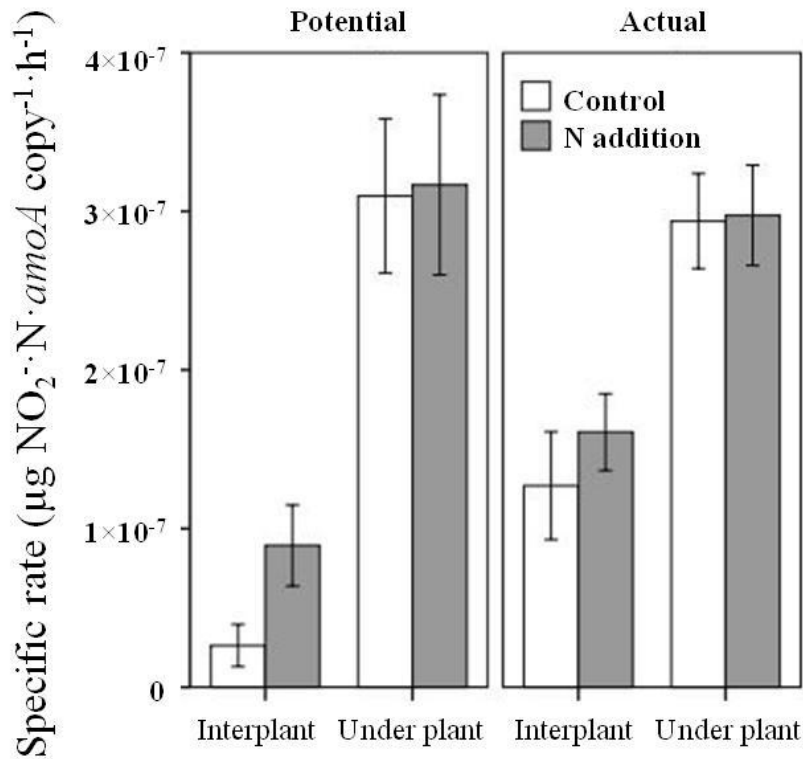
Figure 4. Effects of N addition on the function of the *amoA* gene-containing community using estimates of copy-specific ammonia oxidation rates. Specific rates were calculated as maximum ammonia oxidation rate (V_{max}), separately for the shaken-slurry assay (potential) and static incubation (actual), divided by *amoA* gene copy number per g soil. Error bars are standard errors of V_{max} and *amoA* calculations for independent field triplicates.
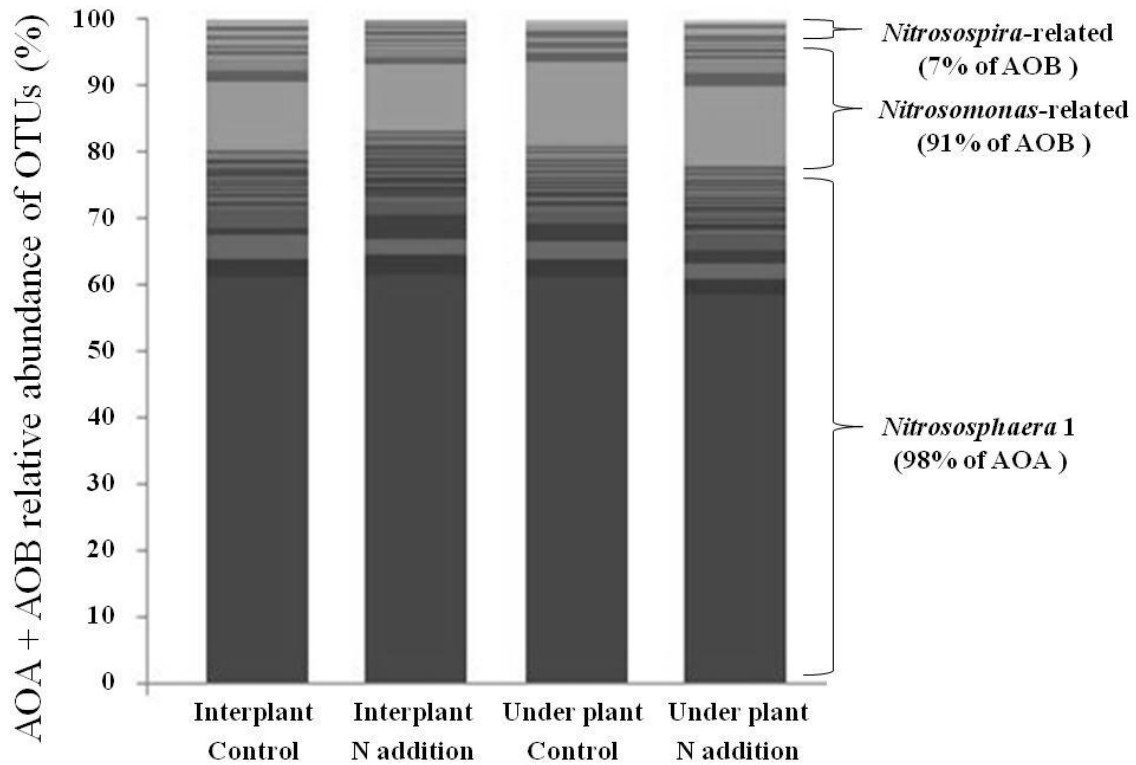
Figure 5. Community composition of OTUs (clustered at 97% nucleotide similarity) based on bioinformatics of *amoA* gene pyrosequencing. Diversity measures were analyzed separately for AOA and AOB prior to combining the two groups based on quantitative PCR data (using relative abundance of AOA to AOB). Brackets include taxonomic classifications and percent of phylotype out of AOA or AOB as an average across all treatment and patch replicates. The remaining 2% of AOB were unclassified to the species-level. The remaining 2% of AOA are identified under *Nitrososphaera* subclusters 2, 8, and 9. Each bar is the average of independent field and pyrosequencing triplicates (excluding one replicate each in the interplant control and N addition samples for the AOA).

Supplementary Table 1. Bivariate Pearson correlations for variables across N addition and control plots.

	Soil properties							amoA gene copy numbers			Ammonia oxidation rates			amoA copy-specific rates	
	pH	SOM	NO_3^-	NO_2^-	NH_4^+	Total iN	NH_4^+ change	AOB	AOA	AOA/AOB	Actual V_{max}	Potential V_{max}	Background	Actual	Potential
pH		-.526	-.713**	-.070	-.500	-.639*	.493	-.673*	-.535	.082	-.883**	-.683*	-.683*	-.551	-.490
SOM	.079		.065	-.195	-.072	.002	-.155	.304	.072	-.201	.623*	.838**	.446	.744**	.831**
NO_3^-	.009	.842		.382	.870**	.977**	-.669*	.783**	.726**	.005	.734**	.288	.723**	.128	.067
NO_2^-	.828	.544	.220		.243	.354	-.323	.567	.798**	.765**	.118	-.237	.176	-.388	-.405
NH_4^+	.098	.823	.000	.243		.955**	-.638*	.490	.518	-.012	.536	.095	.774**	-.047	-.001
Total iN	.025	.996	.000	.446	.000		-.680*	.688*	.672*	.016	.668*	.205	.768**	.045	.030
NH_4^+ change	.104	.630	.017	.259	.025	.015		-.654*	-.501	.061	-.499	-.224	-.831**	.102	.009
AOB	.017	.337	.003	.306	.000	.013	.021		.873**	.038	.749**	.485	.596*	.216	.186
AOA	.073	.824	.008	.054	.106	.017	.097	.000		.391	.551	.172	.464	-.010	-.063
AOA/AOB	.801	.530	.987	.002	.084	.960	.849	.907	.208		-.120	-.361	-.038	-.348	-.356
Actual V_{max}	.000	.030	.007	.715	.073	.017	.098	.005	.063	.711		.812**	.757**	.700*	.647*
Potential V_{max}	.014	.001	.364	.458	.768	.523	.483	.110	.592	.249	.001		.515	.837**	.909**
Background	.014	.146	.008	.584	.003	.004	.001	.041	.129	.906	.004	.087		.244	.385
amoA -Actual	.063	.006	.693	.213	.886	.889	.753	.500	.975	.268	.011	.001	.445		.892**
amoA -Potential	.106	.001	.835	.191	.999	.927	.979	.562	.847	.256	.023	.000	.216	.000	

Significance P-values are on the bottom-left section. Pearson R coefficients (corresponding to the P-values) are on the top-right section. ** Significant at α = 0.01. * Significant at α = 0.05. NH_4^+ change = Maximal net NH_4^+ production. n = 12 for each comparison.

Supplementary Table 2. ANOVA P-values for effects of N addition and patch type on *amoA* gene abundance.

	amoA gene copy number per g soil		*amoA* gene copy number per ng DNA		Ratio of AOA
	AOB	AOA	AOB	AOA	to AOB
Patch x Treatment	0.700	0.027	0.412	0.031	0.009
Treatment	0.001		0.113		
Patch	0.742		0.006		
Soil between plants[a]		0.006		0.023	0.109
Soil under plants[a]		0.086		0.790	0.022

[a] Significant interactions ($\alpha = 0.050$) in the two-way ANOVAs were evaluated further with a one-way ANOVA, using Treatment as the factor, to test effects of N addition separately for each patch type (soils between plants and under plant canopies). $\alpha = 0.025$ for one-way ANOVA significance.
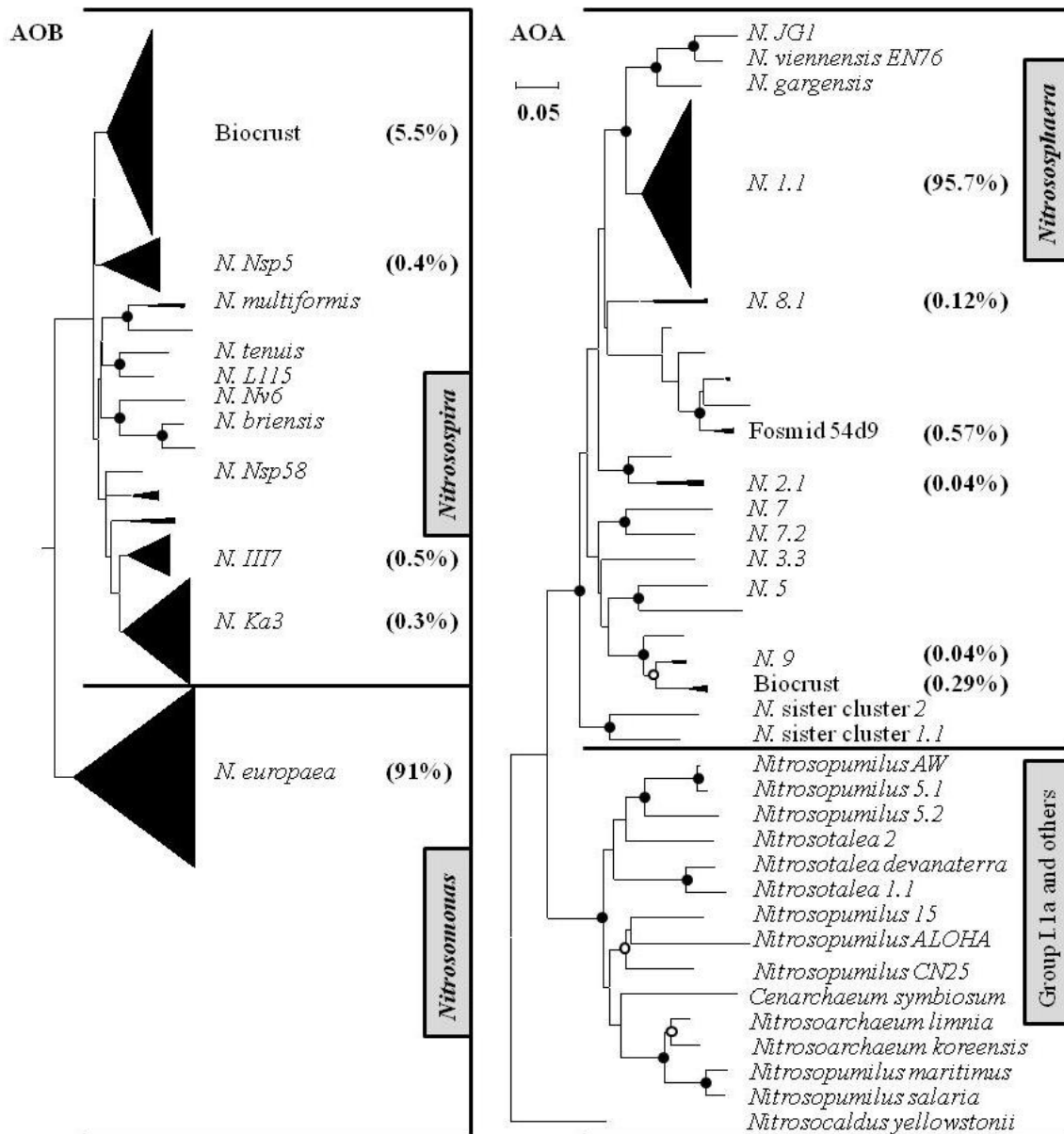
Supplementary Figure 1. Neighbor-joining phylogenetic tree of archaeal and bacterial *amoA* gene sequences. Sequences for this study were obtained from pyrosequencing of the *amoA* gene. Sequences of known strains and subclusters are used as reference groups. OTUs were clustered at 97% nucleotide similarity and taxonomy classified at multiple phylogenetic levels within the *Thaumarchaeota* (formerly named *Crenarchaeota*) as proposed by Pester et al. (2012) and within the AOB (Purkhold et al. 2000). The relative abundance of phylogenetic groups within the AOA or AOB detected in this study is shown in parentheses next to the clade name. Height of clades is proportional to the OTU richness. Bootstrap support is represented by full (75-99%) and empty (50-75%) markers at the nodes.
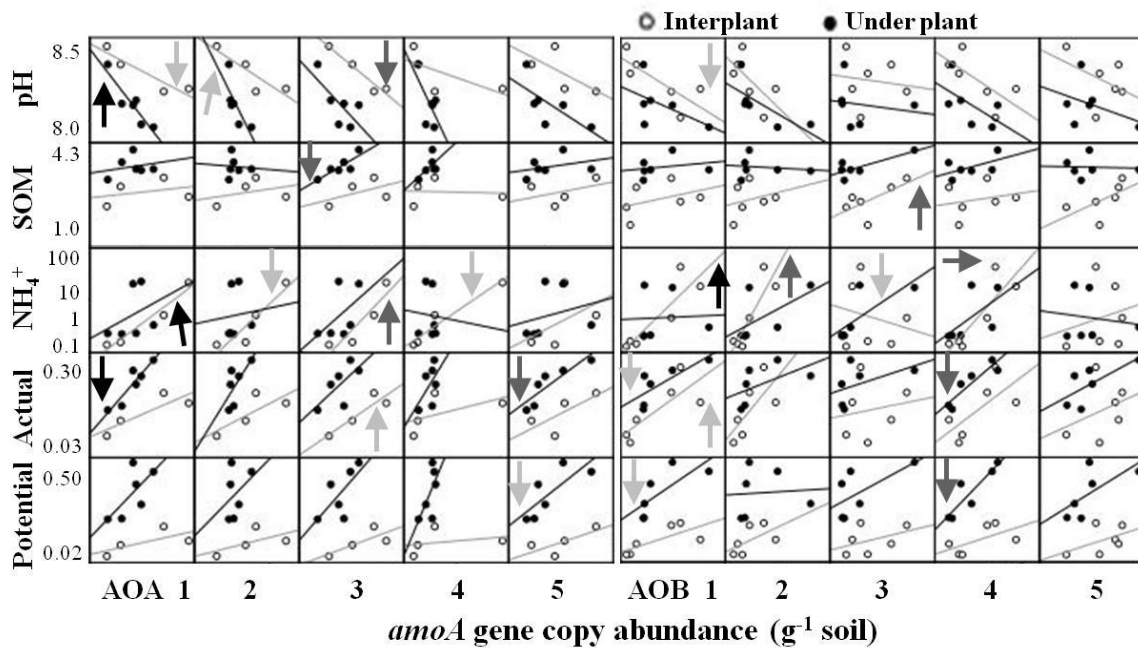
Supplementary Figure 2. Correlation of *amoA* gene copies for individual OTUs of dominant AOA and AOB versus maximum (V$_{max}$) ammonia oxidation rates and soil properties. Trend lines are plotted across control and N addition plots, separately for plant patch types (Interplant and Under plant). Arrows point at regressions with P-values of 0.01 (black), 0.05 (dark gray), and 0.10 (light gray). All AOA are *Nitrososphaera* subcluster 1.1-related. Top four AOB are *Nitrosomonas*-related. AOB #5 is related to *Nitrosospira* from US Southwest biocrust. These ten OTUs (five most abundant OTUs from each domain) comprise 85% of the *amoA*-containing community.

Supplementary File:

The following text includes Qiime format script and notes for pyrosequencing data

processing and bioinformatics.

[END OF MANUSCRIPT, SECTION BELOW IS BIOINFORMATICS

PROCESSING]

Supplementary: Qiime code

START OF QIIME CODE AND QIIME NOTES

#NOTES: This document contains script used in Qiime version 1.5 in VirtualBox through
Ubuntu on a Windows computer for paper entitled " Ammonia-oxidizing archaea respond
positively to inorganic nitrogen fertilization in a desert soil" by Yevgeniy Marusenko,
Ferran Garcia-Pichel, and Sharon J. Hall. Each step of the pyrosequencing data
processing pipeline first describes any script notes following the "#" string, then followed
by "$" and the script line. The $ is written instead of the "working directory" for brevity
since all script calls are within the Qiime directory and use documents/folders within this
working directory. For example, "Archaea" is the working directory in the following
directory: qiime@qiime-VirtualBox:~/qiime_software/qiime-1.5.0-
release/amoaproject/Archaea$

$ ls
072712NMarc-mapping.txt 072712NMarc-full.fasta 072712NMarc-full.qual
amoa_db_template.fas IDtaxonomy2.txt

$ split_libraries.py -m 072712NMarc-mapping.txt -f 072712NMarc-full.fasta -q
072712NMarc-full.qual -M 2 -a 6 -H 6 -s 25 -l 250 -L 452 -o split_library_output

#amoa_db_template.fas was manually created as a reference database of archaeal amoA
sequences from BLAST in GenBank. This template database replaces the use of other

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databases used by Qiime (greengenes, RDP, etc), allowing to use identical Qiime scripts but for a functional gene (amoA) instead of 16S. IDtaxonomy2.txt was manually created with 6 levels of taxonomic classification and short ID matching to ID in amoa_db_template for each sequence. See Supplementary Files to download these two files. The following steps were used to create reference database: 1) Based on published pure isolates, enrichment cultures, and other notable AOA such as those fitting into subcluster classifications identified by Pester et al. 20012, the amoA sequences were obtained from GenBank using searches for AOA name, author name, or accession numbers. These sequences were exported from GenBank to a text file in fasta format with a manually created ID for each sequence. 2) Sequences were aligned in MEGA using default ClustalW parameters. There were no gaps in the alignment for all sequences with one exception: N.yellowstonii inserted 3 gaps into all other sequences in one set of positions. All sequences were 451-453bp long (Qiime errors occurred if template sequence lengths were longer than lengths of the provided unknown sequences). 3) The MEGA alignment was exported as .fas into the Qiime working directory. Common problem: check mapping file and if necessary in the names of the sample change underscore "_" to period "." in SampleID column. All AOB data was processed independently of AOA data, following identical steps described here for AOA.

#Number of AOA sequences: 7,896 Min: 525 Max: 1087

The file seqs.fna was manually moved through the folder interface from split_library_output/ to the working directory.

#We used a high similarity threshold (97%) for de novo clustering to ensure that differences in ammonia-oxidizers will be picked up and grouped with as many OTUs as necessary. In a downstream step, all of the OTUs will be classified to phylogenetic assignments and will reveal the relatedness of distinct OTUs that otherwise may have been grouped into a single OTU at a lower similarity threshold. Less than 2% of all rare sequences, adding up to 104 OTUs for AOA and 178 OTUs for AOB, were retained for downstream analyses to ensure community patterns will not be missed. Rare sequences were identified as singletons occurring in ≤ 3 of the 12 samples, and doubletons occurring at least once.

$ pick_otus.py -i seqs.fna -o uclust_picked_otus_97/ -s .97
$ pick_rep_set.py -i uclust_picked_otus_97/seqs_otus.txt -f seqs.fna -o rep_set1.fna

#Since the template database is manually created and is not an exhaustive of all ammonia-oxidizers, we used Pynast alignment with a conservative threshold at 70% similarity to make sure that most sequences are retained in the alignment and any non-matching sequence is placed into a "failures" file. The only sequences (362 sequences) that failed to match were manually checked in a MEGA alignment file to ensure they are not novel amoA sequences. These "failures" were not filtered in the earlier processing

steps, but they were composed of many discontinuous 10-12nt fragments matching to parts of amoA with overall low coverage.

```
$ align_seqs.py -i rep_set1.fna -t amoa_db_template.fas -o pynast_aligned_70/ -p 70.0
$ assign_taxonomy.py -i rep_set1.fna -r amoa_db_template.fas -t IDtaxonomy2.txt -c 0.7
-o rdp_assign_70/
```

#using conservative -g and -e filter parameters to remove excessive gaps and high entropy positions.

```
$ filter_alignment.py -i pynast_aligned_70/rep_set1_aligned.fasta -s -o
filtered_alignment/ -g 0.95 -e 0.005
$ make_phylogeny.py -i filtered_alignment/rep_set1_aligned_pfiltered.fasta -o
rep_phylo.tre
$ make_otu_table.py -i uclust_picked_otus_97/seqs_otus.txt -t
rdp_assign_70/rep_set1_tax_assignments.txt -o otu_table.biom –e
pynast_aligned_70/rep_set1_failures.fasta
```

#Number of sequences: 7534 Number of OTUs: 205

```
$ make_otu_heatmap_html.py -i otu_table.biom -o OTU_Heatmap/
```

#edited mapping file 072712NMarc-mapping.txt manually to include separate columns for "Treatment", "Patch", combination of both as "TrPa". Category names were written as IN, IC, PN, and PC listed across the replicates for appropriate samples.

```
$ summarize_taxa_through_plots.py -i otu_table.biom -o wf_taxa_summary -m
072712NMarc-mapping.txt
```

#beta diversity analyses

```
$ single_rarefaction.py -i otu_table.biom -o otu_table_525.biom -d 525
```

#Number of sequences: 5250 (from 10 samples, 2 samples did not produce pyrosequencing data). Number of OTUs: 179.

```
$ make_prefs_file.py -m 072712NMarc-mapping.txt -b
"Treatment,Patch,Treatment&&Patch" -o prefs_TrPa.txt
$ beta_diversity.py -i otu_table_525.biom -m euclidean -o beta_diveuclidean/
$ beta_diversity.py -i otu_table_525.biom -m weighted_unifrac -o beta_divweighted/ -t
rep_phylo.tre
$ principal_coordinates.py -i beta_diveuclidean/euclidean_otu_table_525.txt -o
beta_div_euclideancoords.txt
```

$ principal_coordinates.py -i beta_divweighted/weighted_unifrac_otu_table_525.txt -o beta_div_weightedcoords.txt
$ make_2d_plots.py -i beta_div_euclideancoords.txt -m 072712NMarc-mapping.txt -b 'Treatment&&Patch' -o 2dplots/
$ make_2d_plots.py -i beta_div_weightedcoords.txt -m 072712NMarc-mapping.txt -b 'Treatment&&Patch' -o 2dplots/Weighted/
$ make_distance_histograms.py -d beta_divweighted/weighted_unifrac_otu_table_525.txt -m 072712NMarc-mapping.txt -f "TrPa" -o distancehistogram
$ upgma_cluster.py -i beta_diveuclidean/euclidean_otu_table_525.txt -o beta_div_euc_upgma.tre
#beta_significance.py repeated for p-significance, and unweighted, weighted, and normalized weighted unifrac
$ beta_significance.py -i otu_table_525.biom -t rep_phylo.tre -s unweighted_unifrac -o unw_sig.txt

#manually edited mapping file 072712NMarc-mapping.txt to parse P and I patch type for separate analyses, renamed mapping files containing only PC_PN or IC_IN
#For anosim: may need to manually modify distance matrix beta_divweighted/weighted_unifrac_otu_table_525.txt to remove any rows/columns with variables that are not part of the analysis. Retain only PC PN, or IC IN, to test for N fertilization effect within each patch type. Make sure no spaces within the distance matrix in the end of the document.

$ compare_categories.py --method anosim -i beta_divweighted/weighted_unifrac_otu_table_525_PC_PN.txt -m 072712NMarc-mapping_PC_PN.txt -c TrPa -o anosim_out_PC_PN -n 999
$ compare_categories.py --method anosim –I beta_divweighted/weighted_unifrac_otu_table_525_IC_IN.txt -m 072712NMarc-mapping_IC_IN.txt -c TrPa -o anosim_out_IC_IN -n 999
$ otu_category_significance.py -i otu_table_525.biom -m 072712NMarc-mapping_IC_IN.txt -s ANOVA -c TrPa -o single_anova_IC_IN.txt -f 0
$ otu_category_significance.py -i otu_table_525.biom -m 072712NMarc-mapping_PC_PN.txt -s ANOVA -c TrPa -o single_anova_PC_PN.txt -f 0

#alpha diversity analysis. May need to edit mapping file to remove any samples (entered as rows) that may be present in mapping file but absent from OTU table (due to low number of sequences from pyrosequencing that was reduced down to zero after filtering steps).

$ multiple_rarefactions.py -i otu_table.biom -m 10 -x 1087 -s 10 -n 10 -o rarefied_otu_tables/
$ alpha_diversity.py -i rarefied_otu_tables/ -m observed_species,shannon,PD_whole_tree -o alphadiversity/ -t rep_phylo.tre

```
$ collate_alpha.py -i alphadiversity/ -o collated_alpha/all
$ make_rarefaction_plots.py -i collated_alpha/all/ -m 072712NMarc-mapping.txt -b
Treatment,Patch,TrPa -o Rarefactionplots/all/
$ compare_alpha_diversity.py -i collated_alpha/shannon/PD_whole_tree.txt -m
072712NMarc-mapping.txt -c TrPa -d 520 -o alphadivstats.txt
```

#convert OTU table (or rarefied OTU table) to text file to use in excel. Will need to convert total abundance into relative abundances.

```
$ convert_biom.py -i otu_table_525.biom -o otu_table_525.txt -b --header_key taxonomy
-- output_metadata_id "Consensus Lineage"
```

END OF QIIME CODE

2.8. References

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3. Niche differentiation of ammonia-oxidizing microorganisms during pulsed nitrogen inputs in a dryland soil

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3.1. Abstract

Long-term nitrogen (N) addition typically favors ammonia-oxidizing bacteria (AOB) over ammonia-oxidizing archaea (AOA) in soils. However, it is unclear what types of ammonia-oxidizing community shifts occur at short-term scales, and if there are any consequences for ammonia oxidation (AO) rates. Moreover, few studies have explored these relationships in arid lands, which face relatively extreme conditions compared to other terrestrial systems. Arid land soils may have distinct niches for ammonia-oxidizers due to exposure to high temperatures, low moisture availability, high pH, frequent desiccation, and low productivity, which is then punctuated by fluctuations in the soil environment after precipitation. We added inorganic N (as ammonium sulfate) to a Sonoran Desert soil in a microcosm and measured AO dynamics throughout one month. As expected, occurrence of AO led to soil acidification and a decline in both ammonium availability and net AO rates over time. Surprisingly, the temporal soil changes were associated with a decrease in the relative importance of AOA to AOB, with stronger community shifts by the AOA than the AOB. These results highlight the presence of unique ammonia-oxidizers in desert soils and suggest that niche separation should be considered both within and among the AOA and AOB for understanding the effects of N inputs on AO rates in arid land ecosystems.

3.2. Background

Ammonia-oxidizing archaea (AOA) have a lower ammonia (NH_3) demand and lower

ammonium (NH_4^+) inhibition tolerance than ammonia-oxidizing bacteria (AOB)

(Martens-Habbena et al. 2009; Hatzenpichler 2012; Prosser and Nicol 2012). Since lower

pH decreases NH_3 availability by ionization to NH_4^+ (Frijlink et al. 1992), ammonia

oxidation (AO) is less favorable for AOB than for AOA in acidic soils. In relatively

mesic and acidic environments (e.g., soils receiving high rates of irrigation or

precipitation), environmental data confirm that fertilization with inorganic nitrogen (N)

sources, such as NH_4^+ salts, favors AOB over AOA (reviewed in He et al. 2012).

However, some studies report that AOA may respond positively to N additions, but only

when N is supplied as organic fertilizers or when NH_3 is a product of ammonification

(Stopnisek et al. 2010; Lu et al. 2012; Levicnik-Hoefferle et al. 2012). Recent studies

from arid ecosystems have also revealed a unique pattern where seven years of inorganic

N addition (NH_4NO_3) increased AO rates and community size of AOA, particularly in

low-organic matter soils away from vegetation (Marusenko et al. 2013a). Additionally,

these soils had an unusual dominance of AOA and *Nitrosomonas*-related AOB, but not

Nitrosospira. These results suggest that the metabolism and ecology of ammonia-

oxidizing microorganisms (AOM) typical of arid lands may be unique.

Dryland systems may offer novel perspectives in understanding microbial dominance and

functional shifts that occur temporally and spatially with fluctuating conditions (Fierer et

al. 2012; Rajeev et al. 2013). For instance, the Sonoran Desert experiences temperatures exceeding 45°C and severe summer drought followed by precipitation pulses where the soil remains moist for periods as brief as minutes to hours (Young and Nobel 1986; Sponseller 2007). Upon soil wetting, a swift activation response will enable *Nitrosomonas*-related AOB to use NH_3 before other AOB and AOA (Wilhelm et al. 1998; Bollmann et al. 2005; Pellitteri-Hahn et al. 2011; Placella and Firestone 2013). However, once NH_4^+ is diluted in water-saturated soils and/or depleted after metabolic activation of heterotrophs (Parker and Schimel 2011; Sullivan et al. 2012), the AOA will have a competitive advantage due to their high affinity for NH_4^+ (Martens-Habbena et al. 2009). When soils dry, NH_4^+ concentration will rapidly increase in solution within certain soil micro-sites (Parker and Schimel 2011), inhibiting some AOM (Koper et al. 2010). AOB tolerant of high-NH_3 conditions, including *Nitrosomonas*-related groups (Taylor and Bottomley 2006b), will have another opportunity for short-term dominance until moisture and diffusion limitation restrains the entire soil community (Stark and Firestone 1996; Dechesne et al. 2008). Since soils are dry for months at a time, the survival of archaea in general, including AOA, is likely favored over their bacterial counterparts due to physiological advantages in "extreme" environments (van de Vossenberg et al. 1998; Sher et al. 2013). To determine if these dynamics are responsible for niche separation of ammonia-oxidizers in arid lands, we ask how AOM communities and their function respond to a pulse of inorganic N accompanied with ample water availability.

3.3. Methods

We studied temporal dynamics over the short-term to determine the dominance of AOA and AOB and their activities in a native Sonoran soil, AZ, US. Methods are mostly followed from Marusenko et al. (2013a), with full descriptions and references in Supplementary Methods. Fourteen randomly distributed surface soils (0-5 cm) within a 5 x 5 m area, away from vegetation canopy (by at least 1 m), were collected in September 2012 one week after a summer monsoon rain event. Moisture and organic matter content in the soil was 3.0% and 1.9%, respectively.

In the lab, soils were aggregated and homogenized, and 10 g placed into a plastic cup (one of many cups) for incubation for *potential* and *actual* AO assays, as well as standard soil properties and molecular analyses (10 g soil destructively used for each measurement). A subset of cups was sampled at day 0 for background data, while all remaining cups were fertilized once with $(NH_4)_2SO_4$ in nanopure H_2O, maintained at 55-60% water holding capacity (WHC) throughout the experiment, and collected for measurements on each sampling day (0, 1, 5, 10, 24 d). We used the nitrite (NO_2^-) accumulation method with < 24 h shaken-slurry assays (*potential* AO rates) and 48 h aerobic, static incubations at 60% WHC (*actual* AO rates) to measure kinetics of AO at 11 concentrations of supplemented NH_4^+ for each sampling day. For the shaken-slurry assay, we used 20 g soil in 2 liters of buffer to dilute NH_4^+ to the lowest and least variable starting concentrations prior to NH_4^+ supplementation, then aliquoted 100 mL slurry per

determination. Additional aerobic, static incubations were used without N

supplementation as a proxy for *background* AO rates and net NH_4^+ change.

We used quantitative PCR (qPCR) and pyrosequencing for community total and relative

abundance based on bacterial and archaeal *amoA* genes. We also extracted RNA for

reverse transcription cDNA synthesis, followed by standard PCR (using same primers as

qPCR) for *amoA* to be used in denaturing gradient gel electrophoresis (DGGE). Bacterial

cDNA *amoA* amplification was unsuccessful. Nineteen dominant bands were excised and

sequenced from the reverse and forward direction for AOA classification.

Linear regression analyses were conducted to test the effects of the N pulse on soil

variables over the entire course of the experiment. Significant differences between days

were assessed with Bonferroni-corrected post-hoc tests after a one-way analysis of

variance (ANOVA) using time (days) as the independent variable and soil variable (soil

properties, AO rates, and AOM community parameters) as the dependent variable.

Metrics for α and β diversity were analyzed on pyrosequencing-processed data.

3.4. Results and discussion

The inorganic N addition with adequate soil moisture was followed by changes in soil

properties and processes, including an expected decrease in NH_4^+ concentration over time

and a pH drop by 0.73 units during days 1-5 (Table 1). Although patterns based on

absolute abundance of *amoA* were complex, the changing soil conditions resulted in a community shift in favor of, surprisingly, the AOB over AOA in general (Table 2), and also shifts within the AOA. These results are in support of a previous report from desert soils that showed a positive relationship between inorganic N availability and AOA (total abundance, abundance relative to AOB, and abundance relative to extractable DNA; Marusenko et al. 2013a), which highlights that the novel findings may be specific to arid lands. These soil and community changes also co-occurred with a shift in function, as *background* (i.e. unamended) AO rates decreased over the course of the experiment (P = 0.001).

3.4.1. Functional dynamics after a N pulse in arid land soil

The relative magnitude of NH_4^+ consumption and production processes seem to differ between the early and later stages of the pulse. During days 1-5 (Table 1; Table 2), *background* AO rates accounted for 96% of the net decrease in NH_4^+ content, indicating that the magnitude of N mineralization may be relatively low compared to nitrification during N-saturated conditions (Schimel and Bennett 2004; Hall et al. 2011). However, *background* AO rates could account for only 20% of the net NH_4^+ decrease during days 5-24 (the remaining NH_4^+ possibly due to microbial assimilation). Furthermore, in the 48 h aerobic incubations for each sampling day, net NH_4^+ change was positive (indicating dominance of mineralization) only for days 24-26. These results together suggest that AOM in arid lands may have the capacity to use NH_3 for energy generation both from

organic N sources and from the supplemented inorganic N (Marusenko et al. 2013a),

depending on if conditions are conducive for ammonification.

To investigate the functional capacity of ammonia-oxidizing communities under different

substrate availability (during the laboratory measurements), we measured kinetics of AO

in bulk soils. The native soils used here appeared to be NH_4^+-limited since *background*

AO rates were 31% ($\pm$ 15% SD) lower than the maximal (V_{max}) *actual* AO rates (Table

2). Additionally, maximal *actual* AO rates were 57% ($\pm$ 2.7%) of the maximal *potential*

rates during days 0-5 but only 17% ($\pm$ 2.4%) during days 10-24. This result suggests that

the conditions in the shaken-slurry assay (i.e. buffered pH, higher availability and

diffusion of oxygen, phosphorous, and NH_4^+) were more optimal than in the static

incubations, especially in the later stages of the pulse. We also estimated the K_m of bulk

soil where AO rates were augmented by the supplemented NH_4^+ in the laboratory assay,

which was the case only for static incubations at days 0 and 24: The substrate affinity of

the community was similar for both days, with a K_m of about 0.38 µg NH_4^+-N$\cdot$g^{-1}. Other

parameters of community function and abundance of AOM must then explain why AO

processes differ between days.

After an initial growth period lasting through day 1, the archaeal and bacterial *amoA* gene

abundance dropped dramatically from days 1 to 5 (Table 2). Abundance of both AOA

and AOB then increased from days 5 to 24, returning near background levels. Since

background AO rates decreased over time, the incongruent changes in *amoA* abundance

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suggest that the N pulse may be creating some community functional shifts. Specifically, distinct AOM populations may be active throughout time and/or the same populations are active but function differently (i.e. metabolic regulation). Indeed, maximal *actual* AO rates correlated strongly with the ratio of AOA to AOB (P = 0.008; R^2 = 0.91; Fig. 1). The shift in community function is also evident by the higher amount of NH_3 oxidized to NO_2^- per copy of *amoA* gene (i.e. copy-specific AO rates) during day 5 compared to any of the other days (Suppl. Fig. 1; P < 0.001). These results suggest that the contribution of AOA and AOB to AO activities may depend on conditions associated with a N pulse, the effects of which are likely concomitant (e.g. soil pH and NH_4^+ availability). For instance, the AOA to AOB ratio was significantly related to pH over time in this desert soil (P = 0.001; R^2 = 0.98). The opposite pattern occurred – where the AOA to AOB ratio increased with decreasing pH – in other types of ecosystems (Verhamme et al. 2011; Yao et al. 2011).

3.4.2. Characterizing the ammonia-oxidizing community

Phylogenetic analyses of *amoA* genes revealed that niche differentiation may have also occurred within the AOA, as several of the most abundant OTUs responded differently over time (Fig. 2). Particular AOA phylotypes are known to specialize in certain environmental conditions, such as in different pH (Gubry-Rangin et al. 2011) and NO_2^- concentration (Auguet and Casamayor 2013). Conditions during the first day favored NO_2^- build-up in the soil microcosm (P < 0.001), which rarely occurs in natural terrestrial

environments but may select for NO_2^--tolerant AOM (Gelfand and Yakir 2008a; Cua and Stein 2011). The overall decline in AOM from days 1 to 5 may be a consequence of NO_2^- toxicity. Alternatively, AOM community size may have been lowered by predatory grazers that were activated and no longer limited by mobility in soil micro-pockets (Parker and Schimel 2011). Environmental selection of AOM may also occur due to changes in NH_4^+ availability (Prosser and Nicol 2012; Marusenko et al. 2013a). The AOA community structure changed throughout the experiment after experiencing a pulse of N, with the most notable difference between days 0 and 24 (Fig. 3; $P < 0.05$ for both the weighted normalized and unweighted UNIFRACs; Lozupone and Knight 2005). By contrast, the relative abundance of each of the top 20 AOB OTUs (total 80% of all AOB) remained unaltered throughout the experiment. These results suggest that the AOB are unaffected while the AOA are more specialized to conditions associated with a N pulse. Consistent with this pattern, the diversity of AOA also appears to be more responsive than that of AOB, with a decline in Shannon's index over time for AOA ($P = 0.026$, AOA; $P = 0.5$, AOB; Table 1).

Interestingly, other findings raise questions that warrant further investigation in arid land soils. For example, previous experiments from the same site reported a dominance of *Nitrososphaera* subcluster 1.1 (AOA) and *Nitrosomonas* spp. (AOB) (Marusenko et al. 2013a). Instead, *Nitrososphaera*-related AOA and *Nitrosospira*-related AOB dominated here, with both groups relating to phylotypes that are detected in biological soil crusts (Soule et al. 2009; Marusenko et al. 2013b). The sampling here and in Marusenko et al.

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(2013a) occurred during different times of the year (September and January, respectively), in which seasonal effects (e.g. temperature, precipitation) may explain differences in the composition of ammonia-oxidizing communities (Sher et al. 2013). Additionally, two of the thirteen AOA phylotypes identified from *amoA* transcripts were related to the sister cluster of *Nitrososphaera* but were unrelated to any group detected from *amoA* genes (Suppl. Fig. 2). Although issues with primer bias and constraining DGGE analyses to dominant members limits our interpretations, the detection of *amoA* genes does not always indicate presence of viable ammonia-oxidizers and same patterns of activity as based on *amoA* transcripts (Nicol et al. 2008). Since most environments and current clades do not have an AOA culture representative yet, it remains to be seen whether particular AOA have adaptations that are specific to extreme and rapidly changing conditions as in arid lands. Other pulse dynamics, including moisture fluctuation (Gleeson et al. 2010) and repeated cycles of wetting and drying in natural conditions (Miller et al. 2005), may also affect AO processes and ammonia-oxidizing communities in drylands.

3.4.3. Conclusion

Following the addition of N, depletion of NH_4^+ over time corresponded to a decrease in relative importance of AOA to AOB and a decrease in AO rates. This pattern is generally consistent with previous descriptive findings at the same site showing that AO rates and AOA are positively associated with elevated long-term inorganic N availability

(Marusenko et al. 2013a). Niche differentiation of AOA and AOB to pulsed events may lead to a shift in population dominance that affects the fate of N in arid soils. Consequently, one can postulate that AOM may influence biological activity in arid lands. Further research of these processes may improve our understanding of ecosystem responses to changes in anthropogenic activities (e.g., increase in nitrogenous atmospheric deposition and fertilizer-use), climate fluctuations, or shifts in plant and biocrust communities, and aid land managers in improving dryland agricultural management in systems that are more similar to the desert soils here than to mesic environments.

Acknowledgements

3.5. Tables/Figures

Table 1. Soil properties throughout N pulse experiment.

Days after pulse	NH_4^+-N $(ng \cdot g^{-1})$	NO_2^--N $(ng \cdot g^{-1})$	Soil pH
0[a]	396 ± 25^C	12.7 ± 0.1^{BC}	7.83 ± 0.05^A
1	15197 ± 1501^A	474 ± 50^A	7.53 ± 0.05^A
5	13141 ± 147^A	44.4 ± 1.6^B	6.80 ± 0.09^B
10	6457 ± 234^B	16.3 ± 2.0^B	6.72 ± 0.03^B
24	75 ± 84^C	5.36 ± 4.4^C	6.76 ± 0.08^B
	Linear-regression analysis		
P[b]	$< 0.001^c$	$< 0.001^c$	0.024
Adj. R^2	0.920	0.769	0.425

[a] Pre-pulse. [b] P-values and adjusted R^2 from linear-regression analysis separately for each soil variable. [c] Tests carried out on data from days 1-24. All values reported as mean $\pm$ SD. Capital letters indicate significant differences between days within columns (Bonferroni-corrected post-hoc test after one-factor ANOVA; soil variable x days; $p \leq 0.05$).

Table 2. Soil ammonia-oxidizing community and function parameters throughout N pulse experiment.

Days after pulse	AOA (*amoA* gene copy·g⁻¹ soil)	AOB (*amoA* gene copy·g⁻¹ soil)	AOA: AOB[b]	Background AO (ng·g⁻¹·d⁻¹)	Actual[c] AO V_{max} (ng·g⁻¹·d⁻¹)	Potential[c] AO V_{max} (ng·g⁻¹·d⁻¹)	AOA[d] Shannon diversity	AOB[d] Shannon diversity
0[a]	$4.1 \pm 0.9 \times 10^{7\,B}$	$3.7 \pm 0.5 \times 10^{6\,C}$	11.2	348 ± 43^{A}	594	1100	5.4^{A}	4.9^{A}
1	$9.0 \pm 2.2 \times 10^{7\,A}$	$9.4 \pm 2.0 \times 10^{6\,A}$	9.6	495 ± 155^{A}	613	1035	5.4^{A}	4.1^{D}
5	$3.9 \pm 1.0 \times 10^{6\,E}$	$8.5 \pm 2.3 \times 10^{5\,D}$	4.6	285 ± 79^{AB}	352	608	4.5^{B}	4.7^{B}
10	$1.1 \pm 0.3 \times 10^{7\,D}$	$4.0 \pm 0.9 \times 10^{6\,BC}$	2.7	98 ± 24^{B}	144	779	4.6^{B}	4.2^{C}
24	$2.1 \pm 1.0 \times 10^{7\,C}$	$6.2 \pm 2.0 \times 10^{6\,AB}$	3.4	73 ± 9^{B}	171	1129	3.9^{C}	5.0^{A}

AO ammonia oxidation; AOA ammonia-oxidizing archaea; AOB ammonia-oxidizing bacteria. [a] Pre-pulse. [b] The AOA:AOB ratio is calculated using abundance means of six replicates each for AOA and AOB. [c] Actual and potential AO is calculated as the maximal rates (i.e. at V_{max} according to Michaelis-Menten kinetics) using eleven NH_4^+ concentrations for each AO kinetics assay. [d] Calculated in Qiime as mean of ten iterations. All other values reported as mean ± SD. Capital letters indicate significant differences between days within columns (Bonferroni-corrected post-hoc test after one-factor ANOVA; soil variable x days; p ≤ 0.05).
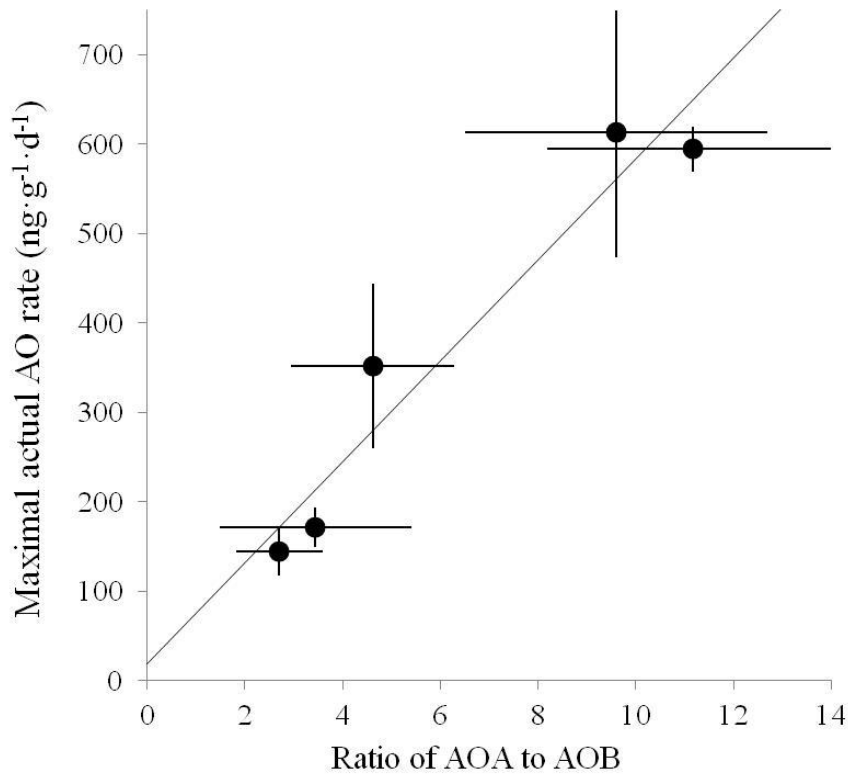
Figure 1. Correlation between AOA:AOB and maximal ammonia oxidation (AO) rate. Correlation significance was calculated based on means for each variable using five total replicates over time. Error bars are included to show variability in the data. Vertical error bars are calculated as the standard deviation of V_{max}, which is estimated from AO rates measured at a range of eleven NH_4^+ concentrations. Horizontal error bars are based on the relative error calculated from the means of *amoA* gene abundance for AOA and AOB (six qPCR replicates for each group per time point).
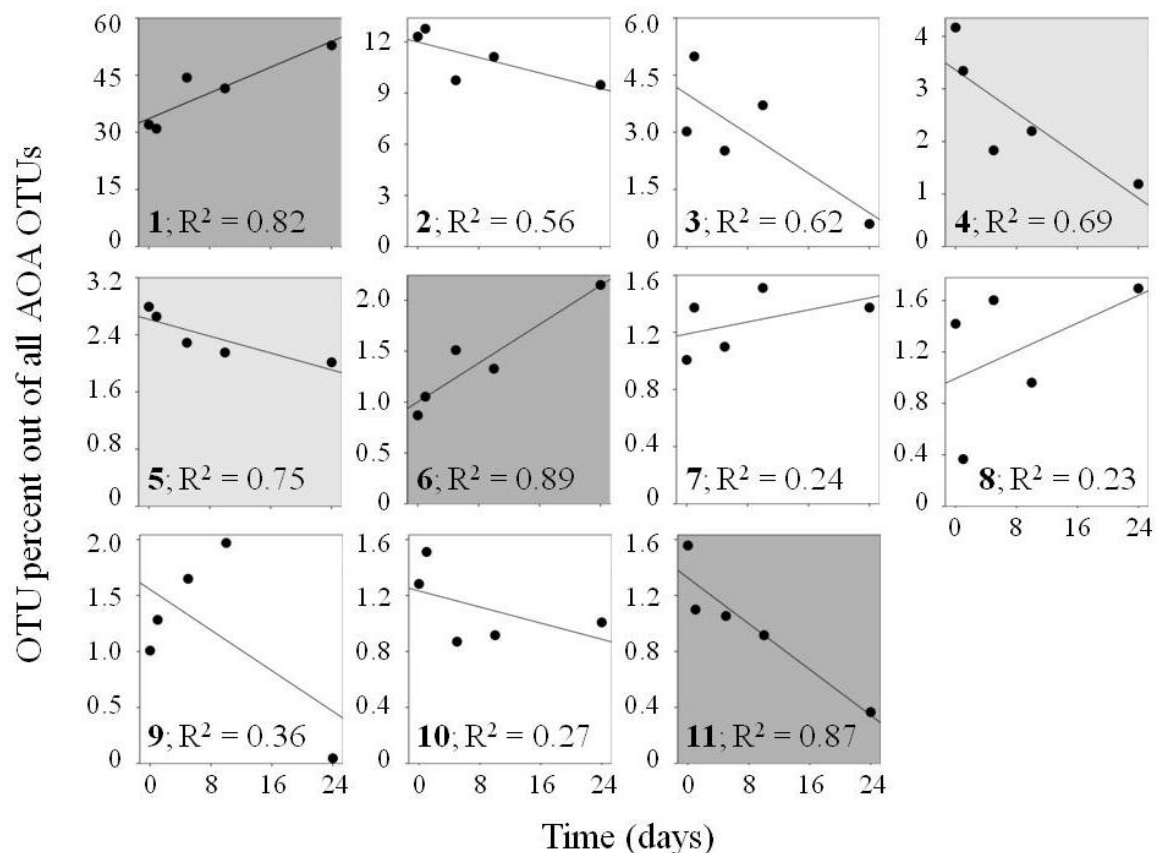
Figure 2. Changes in relative abundance for the eleven most abundant AOA groups throughout the experiment. Operational taxonomic units (OTUs; 97% nucleotide similarity) were considered most abundant if accounting for > 1% of all AOA when averaged across time. Eleven dominant OTUs (11/797 OTUs; separate panels) comprise 67% of all AOA detected. Panel shading indicates significant relationship at $\alpha = 0.05$ (dark gray) and other potentially relevant relationships that were revealed despite the low sample size are indicated at $\alpha = 0.10$ (light gray). Taxonomic classifications are assigned to *Nitrososphaera* subcluster 3.3 for OTU #3, *N. gargensis* for OTU #4, *N.* subcluster 2.1 for OTU #9, and *N.*-related biocrust phylotypes for all other OTUs. Note y-axis scale change to easily see significant patterns from most abundant (OTU #1) to less abundant (OTU #11) AOA groups.
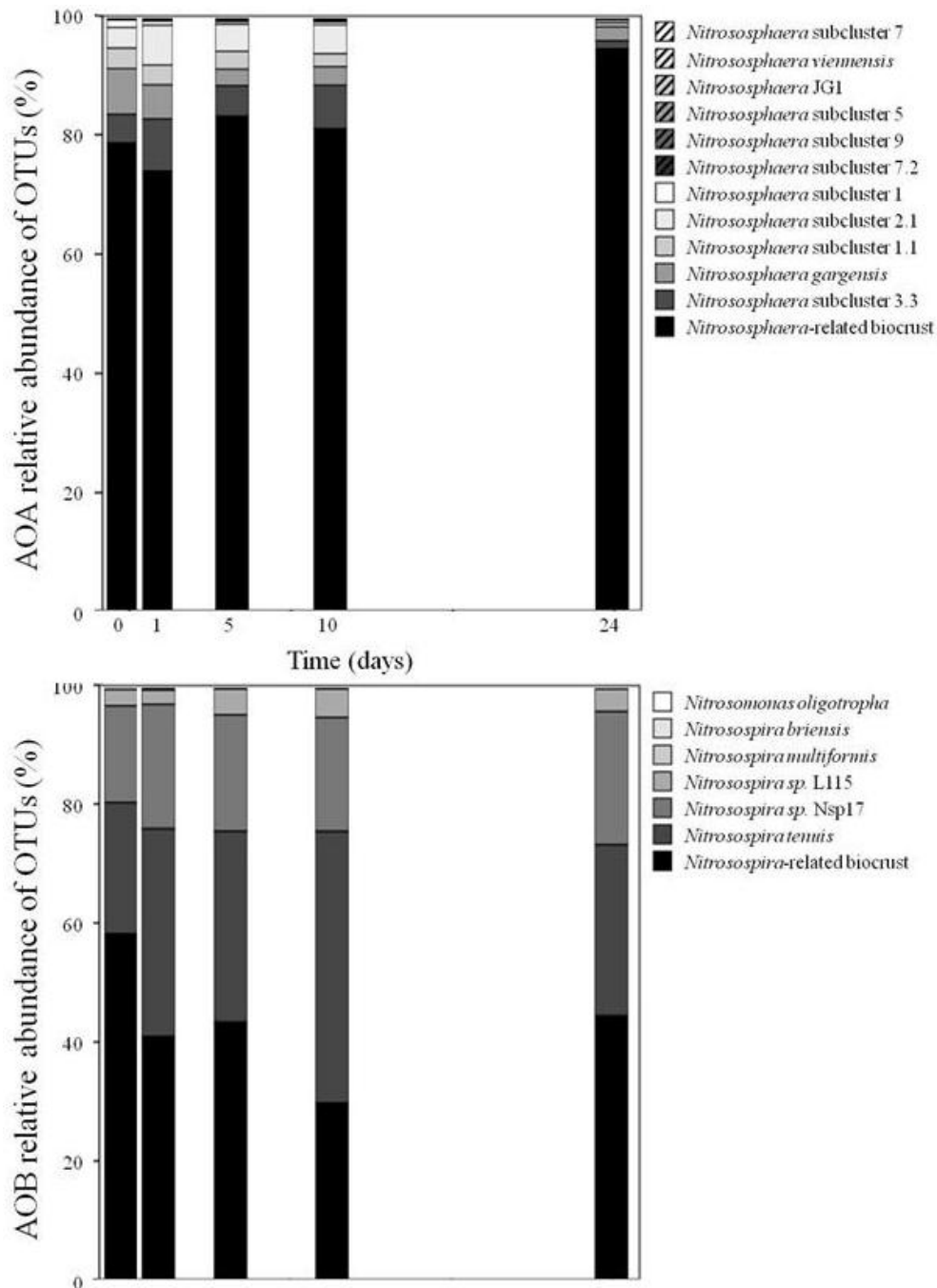
Figure 3. Community composition of OTUs (clustered at 97% nucleotide similarity) from bioinformatics of *amoA* gene pyrosequencing for AOA (top) and AOB (bottom). Taxonomy is based on sequences from previous studies including a Qiime template database (Marusenko et al. 2013a), AOA subclusters (Pester et al. 2012), and phylotypes from biological soil crusts (Marusenko et al. 2013b). Legend shows all detected phylogenetic groups, including minor community members that may not be easily seen in the figure relative to the dominant OTUs.

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3.6. Supplementary materials

3.6.1. Supplementary figures
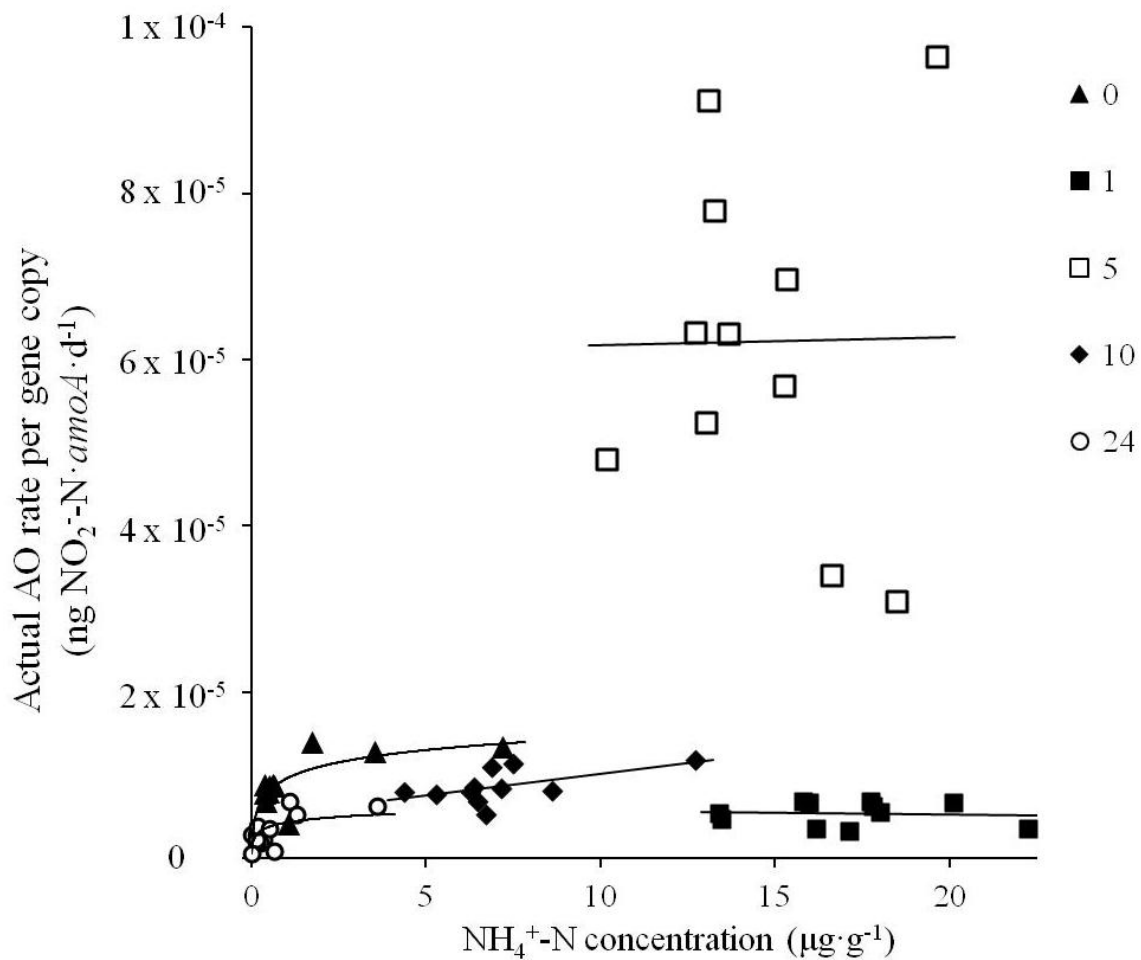
Supplementary Figure 1. Kinetics of *amoA*-copy specific ammonia oxidation rates.
Rates were obtained from dividing each *actual* AO rate (from aerobic, static incubations)
by community size of AOA and AOB combined (from qPCR data for *amoA* genes) for
each sampling day (0, 1, 5, 10, 24). K_m values could only be calculated for day 0 and day
24, the only data sets where NH_4^+ concentration was low enough to measure decreasing
rates and to apply the Michaelis-Menten model.
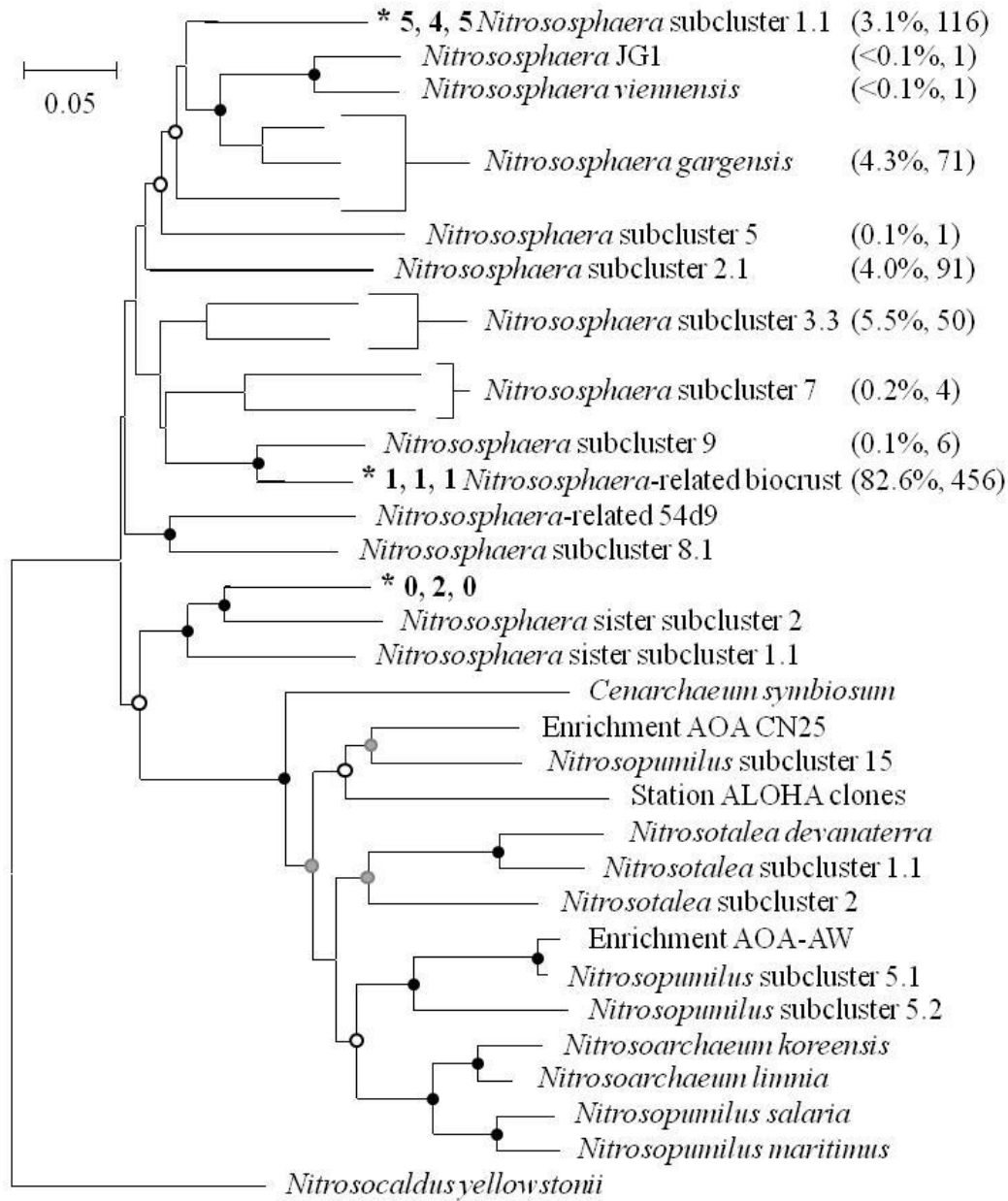
Supplementary Figure 2. Neighbor-joining phylogenetic tree of archaeal *amoA* gene and transcript sequences. Sequences for this study were obtained from pyrosequencing and denaturing gradient gel electrophoresis (DGGE). DGGE bands were excised after running PCR amplicons from archaeal *amoA* transcripts (RNA reverse transcribed to cDNA) and *amoA* genes. Alignment was carried out in MEGA 5 software using default Muscle parameters, including the following sequences: the consensus sequences from reverse and forward directions (from DGGE), the representative sequences of operational

taxonomic units (OTU) clustered at 85% nucleotide similarity from processed pyrosequencing data (filtering described in Marusenko et al. 2013a), and sequences from characterized AOA and those identified by Pester et al (2012) for *Thaumarchaeota* subclusters. Bootstrap support from 500 permutations is represented by black ($\geq$ 90%), gray ($\geq$ 75 %), and white ($\geq$ 50 %) circles at the nodes. Numbers in parentheses shows the percentage of that phylotype out of all AOA and the OTU richness, respectively, in this study (based on 97% nucleotide similarity). * Indicates the number of dominant bands detected for the cDNA at 5 d, cDNA at 24 d, and DNA (present in all time points), respectively.

3.6.2. Supplementary methods

3.6.2.1 Study area description

Our site is in the northern Sonoran Desert at ~620 m elevation in Lost Dutchman State Park, Apache Junction, AZ, USA (coordinates: N 33.459372 S -111.484956). Soils are classified as Typic Haplargids subgroup of Aridisols. Mean annual temperature is 22.3°C, with monthly means as low as 3.7°C and up to 41.9°C (2005-20012; NCDC, 2013). Mean annual precipitation is 272 mm but is highly variable year to year. Rainfall is bimodally distributed between summer monsoon events and low-intensity winter storms (WRCC, 1985). Surface soil samples were collected in September 2012 at least 1 m away from any shrub or tree canopy within a 5 m x 5 m area. Each soil sample consisted of fourteen 0-5 cm deep cores to be used in the laboratory microcosm experiment.

3.6.2.2. Laboratory methods and soil properties

Following collection, samples were transported on ice to the lab, sieved to < 2 mm, and homogenized. Soil was distributed among many cups for a subset of these soils to be destructively used at each sampling day. Soils were measured at day 0 for background data, while all remaining cups were fertilized once with $(NH_4)_2SO_4$ in nanopure H_2O, and maintained at 55-60% water holding capacity (WHC) throughout the experiment for additional sampling on days 1, 5, 10, and 24.

Soils were processed for pH (1:2 soil to DI H_2O), water holding capacity (% WHC; gravimetric), organic matter content (% SOM; loss on ignition), and extractable NH_4^+ and nitrite (NO_2^-) content (2M KCl extraction, colorimetric analysis), following standard, published methods (Marusenko et al. 2013a). Duplicates were used for pH and triplicates for other properties.

3.6.2.3. Potential rates of ammonia oxidation

In situ rates of *potential* AO were measured under various levels of N addition (see "Ammonia oxidation kinetics" below) using the shaken-slurry method (Hart et al. 1994; Norton and Stark 2011a). NO_2^- accumulation was measured as a proxy for AO after inclusion of chlorate ($NaClO_3$), a NO_2^--oxidation inhibitor (Belser and Mays 1980). The shaken-slurry assays contained 1 g soil in 100 mL solution (described below) of 0.015

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mol·L^{-1} NaClO$_3$, and 0.2 mol·L^{-1} K$_2$HPO$_4$ and 0.2 mol·L^{-1} KH$_2$PO$_4$ to buffer pH at 7.2.

Slurries and no-soil blanks were continuously aerated in solution by mixing at 180 rpm

on a reciprocal shaker in the dark. Homogenized slurry aliquots were removed at four

time points over 20 h and amended with several drops of MgCl$_2$ + CaCl$_2$ (0.6 M) to

flocculate soil particles. Aliquots were then centrifuged at 3000 $\times$ g and supernatant was

filtered through pre-leached Whatman #42 ashless filters. The supernatants were stored at

4°C and analyzed within 24 h. Net rates of *potential* AO were calculated as the linear

increase in NO$_2^-$ content from 0 to 20 h, measured colorimetrically using a Lachat

Quikchem 8000 autoanalyzer.

3.6.2.4. Actual rates of ammonia oxidation

We also measured AO following various levels of N addition in a modified method using

NaClO$_3$ inhibition in static, aerobic incubations for 48 h of bulk soil (Nishio and Fujimoto

1990; Hart et al. 1994; Low et al. 1997). Ten g of soil was brought up to 60% WHC using

water and NaClO$_3$ (15 mM) in plastic cups. Soil in one cup was extracted at the onset and

a second cup extracted after incubation in the dark. Soils were extracted in 50 mL of 2 M

KCl followed by shaking for 1 h and filtering through pre-leached Whatman #42 ashless

filters. The extracts were stored at 4°C and analyzed colorimetrically within 24 h. Net

rates of *actual* AO were calculated as the increase in NO$_2^-$ content between 0 and 48 h.

3.6.2.5. Ammonia oxidation kinetics

K_m and V_{max} were estimated for kinetics of AO (Marusenko et al. 2013a). To estimate AO kinetics under oligotrophic conditions in the shaken-slurry assay, we lowered the pre-existing NH_4^+ concentration in soil to obtain the least variable and lowest residual substrate availability (Norton and Stark 2011a). We used 20 g soil in 2 liters of potassium phosphate buffer, then aliquoted 100 mL slurry each into a separate flask. Inorganic N was then supplemented as $(NH_4)_2SO_4$ to each flask as eleven final concentrations in the slurry ranging from 0-4 mM. In total we evaluated AO rates using 55 different soil preparations (5 time points x 11 NH_4^+ concentrations) per method (shaken-slurry assay, static incubation). In the static incubations, we excluded the N dilution step as to minimize soil disturbance. Soils were supplemented with $(NH_4)_2SO_4$ with final concentrations ranging from 0-8 μg NH_4^+-N g^{-1} (0-2.5 mM). The 0 μg NH_4^+-N g^{-1} addition (only includes pre-existing NH_4^+) was used to estimate *background* net rates of AO. As a rough indicator of the relative importance of NH_4^+ mineralization and nitrification, we also measured the net rate of NH_4^+ gain (production processes dominate) and loss (consumption processes dominate) during the static incubation experiment. In the assay, some of the NH_4^+ consumption processes are likely minimized due to sieving of soil (exclusion of large NH_4^+-assimilating plant roots) and lower laboratory temperature compared to natural conditions (reduced volatilization).

3.6.2.6. Preparation of DNA and cDNA

During each sampling day, 2.5 g of homogenized soil sample was used for DNA and RNA extraction following standard manufacturer guidelines from MoBio PowerSoil Kit. Nucleic acid concentration and quality was assessed on an agarose gel stained in ethidium bromide and imaged using a Fluor-S Multi-Imager (BioRad Laboratories, CA, USA) with an EZ Load Precision Molecular Mass Standard (BioRad). Bands of DNA and RNA were excised from a low-melt agarose gel, homogenized with a tip in a microcentrifuge tube, allowed to diffuse out into sterile H_2O for 12 h, and followed by 15 m centrifugation to collect DNA and RNA in the supernatant. RNA was reverse transcribed to cDNA following standard protocols with random hexamer primers (iScript; BioRad), which was only successful for AOA but not for AOB after multiple attempts.

3.6.2.7. Quantitative PCR

DNA was used for quantitative PCR (qPCR) with the following *amoA* primers: CrenamoA616r (GCCATCCABCKRTANGTCCA; Tourna et al. 2008) and CrenamoA23f for the AOA (ATGGTCTGGCTWAGACG); and amoA1f mod (GGGGHTTYTACTGGTGGT; Stephen et al. 1999) and AmoA-2R' for the AOB (CCTCKGSAAAGCCTTCTTC; Okano et al. 2004; Junier et al. 2008). qPCR reactions contained 10 µL iTaq SYBRGreen Master Mix (BioRad), 250 nM final concentration of each primer (AOA or AOB), 1 ng of environmental DNA, and molecular grade H_2O to

bring each reaction to a final volume of 20 µL. The reaction conditions were as follows: initial denaturation for 150 s at 95°C followed by 45 cycles of 15 s at 95°C, 30 s at 55°C, and 30 s at 72°C, and a final dissociation step to obtain the melting curve at 95°C, 60°C, and 95°C for 15 s each. Standard curves were generated using templates from *Nitrosomonas europaea* ATCC 19718 (bacterial *amoA*; $R^2 = 0.97$) and a putative AOA clone (archaeal *amoA*; $R^2 = 0.98$) for a dilution series spanning 10^2-10^{10} gene copies per reaction. Melting curves were checked to verify the quality of each reaction, and to ensure the absence of primer-dimers. We report only determinations for which C_t values could be interpolated within our standard curves.

3.6.2.8. Denaturing gradient gel electrophoresis

We used purified DNA extracts for PCR with primers described above (qPCR) to obtain amplicons for denaturing gradient gel electrophoresis (DGGE). PCR conditions were as follows: 180 s at 94°C followed by 28 cycles of 30 s at 94°C, 40 s at 53°C, and 60 s at 72°C, and final elongation for 5 m at 72°C. Nucleic acid concentration and quality was assessed on an agarose gel stained in ethidium bromide and imaged using a Fluor-S Multi-Imager with an EZ Load Precision Molecular Mass Standard. Bands of DNA were excised from a low-melt agarose gel, homogenized with a tip in a microcentrifuge tube, allowed to diffuse out into sterile H_2O for 12 h, and followed by 15 m centrifugation to collect DNA in the supernatant. Purified PCR products were applied to a DGGE following standard procedures with a 6% acrylamide gel for 6 h at 200 V (Nagy et al.

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2005). Nineteen dominant bands were excised and sequenced from the reverse and forward direction for AOA taxonomic classification.

3.6.2.9. Pyrosequencing

Purified DNA extracts (following DNA extraction step) were shipped to a commercial laboratory for standard PCR and bTEFAP pyrosequencing (Dowd et al. 2008). Commercial primers for PCR were amoA-1F (GGGGTTTCTACTGGTGGT; Rotthauwe et al. 1997) and amoA-2R for AOB (CCCCTCKGSAAAGCCTTCTTC); and Arch-amoAF (STAATGGTCTGGCTTAGACG; Francis et al. 2005) and Arch-amoAR for AOA (GCGGCCATCCATCTGTATGT) used with a HotStarTaq Plus Master Mix Kit (Qiagen, CA, USA). PCR conditions were as follows: 180 s at 94°C followed by 28 cycles of 30 s at 94°C, 40 s at 53°C, and 60 s at 72°C, and final elongation for 5 m at 72°C. PCR amplicons were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Sequencing utilized Roche 454 FLX titanium instruments and reagents.

3.6.2.10. Bioinformatics and phylogenetic analyses of *amoA*

Detailed steps of pyrosequencing data processing and analyses in Qiime (Caporaso et al. 2010b) are described with script by Marusenko et al. (2013a). Sequences (452 bp long) were clustered into operational taxonomic units (OTUs; groups of sequences sharing >

97% nucleotide similarity) using UClust (Edgar 2010). Representative sequences (one per OTU) were aligned with Pynast (Caporaso et al. 2010a). Based on phylogenetic classification for AOA in Pester et al. (2012) and AOB in Koops et al. (2006), a taxonomic assignment was made for each OTU using a reference database created from sequences of known pure isolates, enrichments, and other characterized AOM from previous studies. These analyses were repeated at > 85% nucleotide similarity for OTU clustering.

Phylogenetic analyses were carried out on a single alignment file (separately for AOA and AOB) that included sequences from our Qiime pipeline, AOA sequences from DGGE, as well as the reference sequences described above. All sequences were combined and realigned using default parameters for Muscle and analyzed by the tree-building module of the MEGA 5 software with the following parameters: Neighbor-joining statistical method, Jukes-Cantor nucleotide substitution model, 500 bootstrap replicates, uniform rates among sites, and pairwise gap-data deletion (Tamura et al. 2011). Representative sequences of phylogenetically distinct OTUs have been submitted to GenBank for dominant and novel archaeal (NCBI accession numbers: xx) and bacterial (xx) *amoA*.

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3.6.2.11. Statistics

Statistical tests were carried out using Qiime for α and β diversity measures on processed pyrosequencing data, while all other analyses were in SPSS (v20.0 Windows). All soil properties, AO rates, and *amoA* abundance data were tested for linear model assumptions using normal probability plots (for normality) and Levene's test (for equal variance), and transformed (natural log) when necessary. We used bivariate Pearson correlations to assess relationships between soil properties, AOM community parameters, and measurements of AO across time. We used linear regression analyses to assess relationships between each dependent variable and time. Linear regression analysis was used to compare the intercepts of *amoA* gene copy-specific AO rate at different sampling days, in which the replicates for rates were across the range of NH_4^+ concentrations, excluding values at or below ½V_{max}. To assess significance between days, we used Bonferroni-corrected post-hoc tests after a one-way analysis of variance (ANOVA) using time (days) as the independent variable and soil variable (soil properties, AO rates, and AOM community parameters) as the dependent variable. In Qiime, we tested for effect of time on OTU-based communities separately for AOA and AOB, using α diversity (Shannon's diversity) and β diversity (weighted and unweighted Unifrac).

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4. Contributions of fungi, archaea, and bacteria to ammonia oxidation in southwestern US

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Highlights:

-Fungi are responsible for N_2O production in drylands but not managed grass soils

-Autotrophic bacteria or archaea control nitrification in dryland and managed soils

-These patterns are consistent both under shrub canopies and in spaces between plants

-Fungi contribute to N_2O flux across variable moisture conditions in arid ecosystems

-Land-cover changes associated with urbanization in drylands alter N cycling pathways

4.1. Abstract

Nitrification and denitrification are key processes that control nitrogen (N) availability and loss in terrestrial systems. Nitrification in soils is thought to be performed primarily by chemoautotrophic prokaryotes such as bacteria and archaea, and denitrification by bacterial heterotrophs. However, recent work in drylands suggests that fungi may play a larger role in N transformations than previously recognized. Arid and semi-arid ecosystems experience extreme temperatures and low moisture conditions, both of which favor growth and survival of some fungi, but the extent of fungal nitrification and denitrification in these soils has not been explored. We investigated the role of fungi in nitrate (NO_3^-) and nitrous oxide (N_2O) production in soils from regions across the southwestern US. Soils were collected from urban and Sonoran Desert sites within the Phoenix metropolitan area in Arizona, and from grasslands in Arizona and New Mexico. Rates of potential nitrification were measured in soils subjected to biocide treatments (fungal and bacterial inhibitors) and acetylene, an inhibitor of autotrophic ammonia oxidation. Nitrous oxide production was measured from soils incubated at two moisture levels in the presence of the biocide treatments. Rates of potential nitrification decreased significantly in all soils with additions of acetylene, indicating that autotrophic microorganisms may dominate ammonia oxidation and NO_3^- production rather than heterotrophs (e.g., fungi). Similarly, fungi played a minor role in N_2O production in urban turfgrass soils that were highly managed with irrigation and fertilizers. In contrast, fungi were major sources of N_2O production in desert and semi-arid grassland soils

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across a range of soil water content. We conclude that fungi are likely responsible for denitrification in aridland soils, and that land-use changes associated with urbanization alter the biotic pathways responsible for N cycling.

4.2. Introduction

Nitrification and denitrification are important processes in the terrestrial nitrogen (N) cycle that are mediated by microorganisms. Both nitrification and denitrification represent important pathways of N loss to the atmosphere, affecting air quality and climate through the emission of nitric oxide (NO) and nitrous oxide (N_2O) as gaseous intermediates or products (Wrage et al., 2001; Seitzinger et al., 2006). Nitrate (NO_3^-) produced through nitrification can also be transferred to aquatic systems with consequences for primary production (Camargo and Alonso, 2006). The conventional N cycle is thought to be controlled primarily by bacteria, in part due to numerous studies in managed and relatively mesic ecosystems such as agriculture and grasslands where bacterial abundance and activity is high (Hayatsu et al., 2008; Klotz and Stein, 2008). Recently, however, novel molecular techniques are helping to identify new N transformations and highlighting the role of other microbial domains with entirely different metabolic pathways, such as fungi and archaea (e.g., fungal denitrification, archaeal ammonia oxidation; Konneke et al., 2005; Leininger et al., 2006; Mulder et al., 1995; Shoun et al., 1992, 2012). While these studies show that different microbial groups have the potential to contribute to N cycling, less is known about their distribution in soils and relative importance to biogeochemical pathways. Recognition of the

microorganisms responsible for N cycling, and the differential responses to factors that control their abundance and activity, is central to predicting ecosystem function during environmental change.

Arid and semi-arid ecosystems cover one-third of Earth's land surface and are characterized by high spatial and temporal heterogeneity and extreme environmental conditions including high temperatures, infrequent and pulsed precipitation, low soil moisture, high soil pH, and low soil carbon (C) availability (Safriel et al., 2005; Collins et al., 2008). These conditions can favor growth of some fungal populations relative to bacteria due to various adaptations, including sporulation to increase survival, association with primary producers to increase resource acquisition (e.g., soil biological crusts), and production of extracellular enzymes that can degrade recalcitrant organic molecules to obtain nutrients that are unavailable to bacteria (Allen, 2007; Cousins et al., 2003; Green et al., 2008; Porras-Alfaro et al., 2008, 2011). Recent experiments in several dryland and other sites have shown that fungi are important players in the N cycle, including N_2O production and nitrification (Crenshaw et al., 2008; Laughlin et al., 2009; McLain and Martens, 2006; Stursova et al., 2006; Ma et al., 2008; Herold et al., 2012; Seo and DeLaune 2010). Fungi have been shown to produce N_2O through dissimilatory NO_3^- or nitrite reduction under low O_2 conditions in the laboratory (Shoun et al., 1992; Sutka et al., 2008; Zhou et al., 2010; Prendergast-Miller et al., 2011; Shoun et al., 2012). Additionally, while autotrophic nitrification uses C mainly from the atmosphere (Prosser, 1989), the heterotrophic metabolism of fungi links NO_3^- production to soil C availability

(Schimel et al., 1984; Castaldi and Smith, 1998; McLain and Martens, 2006). Thus, the tight coupling of organic C and N cycling associated with fungal metabolism alters predictions of ecosystem functioning in soils dominated by fungal-mediated processes. Arid and semi-arid terrestrial ecosystems globally are experiencing changes due to extended drought, grazing, biological invasions, shrub or grass encroachment, land-use and land-cover change, and atmospheric pollution from urbanization, all of which are likely to affect soil microbial C and N transformations (Brown et al., 1997; Ojima et al., 1999; Kaye et al., 2005; Pennington and Collins, 2007).

Recognizing the potential importance of fungi to aridland ecosystem function, Collins and colleagues expanded on the existing pulse-reserve paradigm (Reynolds et al., 2004), developing the Threshold-Delay Nutrient Dynamics (TDND) model in which soil conditions after precipitation events drive asynchrony in plant and microbial activity (Collins et al., 2008). Specifically, fungi may control biogeochemical cycling when soils dry. Even though some fungi prefer high moisture conditions (e.g., Frey et al., 1999; Egerton-Warburton et al., 2007), metabolism of other fungal groups may be less restricted than bacterial and plant processes under low water potential (Austin et al., 2004; Allen, 2006, 2007; Jin et al., 2011; Lennon et al., 2012). However, despite recent evidence in support of the TDND model, the extent of fungal nitrification and denitrification in arid and semi-arid ecosystems is unclear. Some drylands harbor fungal communities within a heterogeneous mosaic of lichen-dominated biocrusts and mycorrhizal perennial shrubs and grasses (e.g., Tabernas Desert in southeast Spain,

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Chihuahuan Desert, Great Basin Desert, and Colorado Plateau). Conversely, fungi may be less important in deserts that support cyanobacteria-dominated biocrusts and low grass cover (e.g., the Sonoran and Mojave Deserts; MacMahon, 2000; Green et al., 2008; Maestre et al., 2011; McLain and Martens, 2006; Porras-Alfaro et al., 2007; Belnap and Lange, 2001; Strauss et al., 2012). The composition of primary producers, partially driven by the environmental and climatic differences between diverse deserts, may differentially affect heterotrophic activity and the fungal contribution to N transformations (Schlesinger et al., 1996; MacMahon, 2000; Belnap and Lange, 2001; Austin et al., 2004; Collins et al., 2008; Veresoglou et al., 2012).

Drylands are also home to a third of the world's people and support among the fastest rates of population growth globally (Warren et al., 1996; Safriel et al., 2005). Land-use and land-cover change associated with urban development can both directly and indirectly affect soil microbial communities and element cycling (Kaye et al., 2005; Groffman et al., 2006). For example, the intensive and regular use of water and fertilizers in agriculture, mesic lawns, and residential landscapes directly modifies soil microbial community structure, distribution, and function (Hall et al., 2009; Lorenz and Lal, 2009). Indirectly, urbanization can alter microclimates (Bang et al., 2010), faunal populations (Bang et al., 2012; Faeth et al., 2005), and the composition and rate of deposition of atmospheric compounds (Kaye et al., 2011; Lohse et al., 2008), all of which can affect soil microbial community composition and biogeochemical cycling in remnant native

landscapes that are exposed to the urban environment compared to more rural sites (McCrackin et al., 2008; Hall et al., 2009).

In this research, we explored whether fungi play a significant role in soil N cycling across a range of current or former dryland ecosystems in the southwestern US, including high elevation grasslands in Arizona and New Mexico, native soils in the Sonoran Desert, and desert soils that have been influenced directly by human activity through land-use change and indirectly through exposure to the urban environment. In two laboratory experiments, we measured rates of potential nitrification and N_2O production from soils incubated under a range of moisture conditions with either autotrophic, bacterial, or fungal inhibitors. We hypothesized that production of NO_3^- and N_2O in native dryland soils would be dominated by fungal communities preferentially over prokaryotes. In contrast, we predicted that rates of N cycling would increase but fungal dominance would decline along a resource gradient associated with urbanization, from relatively resource-poor native desert outside the urban airshed, to remnant native desert within city boundaries ('indirect' effect of urbanization), to managed desert-style residential yards and fertile, irrigated landscapes such as lawns ('direct' effect of urbanization). Finally, in native desert soils, we expected that patterns of N_2O production would be organized spatially around shrub resource islands and temporally around pulse rain events (tested here with soils incubated under high and low water content), with the highest rates but lowest fungal contributions under high soil moisture conditions and in relatively fertile patches under shrub canopies.

4.3. Material and methods

4.3.1. Study area description

We collected soils from six common ecosystems within Arizona and New Mexico (USA) to determine the relative contributions of different microbial groups to soil N transformations (Figure 1, Table 1). These ecosystems included two semi-arid grasslands, one within the Agua Fria National Monument (AFNM) in central AZ (hereafter as 'grassland AZ') and one within the Sevilleta National Wildlife Refuge (SNWR) in central New Mexico ('grassland NM'). The grassland AZ site is located 80 km north of the Phoenix metro area and receives on average 412 mm of precipitation annually. This region was occupied by native human populations between 1200-1450 AD (Spielmann et al., 2011), although our samples were collected from areas that do not show archaeological evidence of human settlement (Trujillo, 2011). Soils at the grassland AZ site are classified as silty clay vertisols (> 30 % clay) with soil pH of 7.2 (Trujillo, 2011). Plant cover is dominated by annual and perennial grasses, including tobosa grass (*Pleuraphis mutica* Buckley) and grama species (*Bouteloua* Lag. spp.; Briggs et al., 2007), and shrubs (*Acacia greggii* [A.Gray] and *Juniperus* spp.). The grassland NM site in the Chihuahuan Desert is slightly drier, receiving approximately 255 mm of precipitation annually, with soil pH of 8.5 (Crenshaw et al., 2008). Common ground cover includes both blue grama (*Bouteloua gracilis*) and black grama (*Bouteloua eriopoda*) grasses (Pennington and Collins, 2007). This site was used as a 'positive

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control' since previous work in this region of the SNWR has shown that N_2O production is controlled primarily by fungi (Crenshaw et al., 2008).

The other four ecosystem types in this study are arrayed along a rural-urban gradient in the northern Sonoran Desert in AZ, at elevations ranging from 300-600 m within the boundaries of the 6,400-km^2 Central Arizona–Phoenix Long-Term Ecological Research site located in and surrounding metropolitan Phoenix (CAP LTER; http://caplter.asu.edu; Hall et al., 2009; Figure 1; Table 1). These sites include native Sonoran Desert outside of the urban area ('outlying desert'), protected native desert areas within the city ('remnant desert'), desert-style residential yards with an agricultural and lawn history but re-landscaped with native plants and drip irrigation ('xeriscape'; Davies and Hall, 2010), and managed (i.e. irrigated and fertilized) grass lawns within municipal parks ('lawn'). Soils in the outlying desert, remnant desert, xeriscape, and lawn have a soil pH of 7.6, 7.6, 8.1, and 7.5, respectively (Hall et al., 2009). The desert sites sampled in this study had no history of irrigation or fertilization, and were > 20 m distance from visible paths and roads (Hall et al., 2011), while the xeriscape sites spent between 21-64 y minimum as agriculture, 11-44 y as lawn, and 5-25 y as desert-landscaped yards (Davies and Hall, 2010). Plant cover in the outlying and remnant desert sites is dominated by the common native shrubs creosote bush (*Larrea tridentata* [DC.] Coville) and bursage (*Ambrosia* spp.). The most common turfgrass species planted in lawns include Bermuda grass (*Cynodon dactylon* [L.] Pers.) in the summer and ryegrass (*Lolium* spp.) overseeded in the winter. Vegetation in the xeriscape sites included native trees such as palo verde

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(*Parkinsonia* spp.) and brittlebush (*Encelia farinosa* A. Gray ex Torr.). Plant growth

patterns in the CAP LTER are regulated in part by the precipitation regime in the

Sonoran Desert (Sponseller et al., 2012), with highly variable inter-annual and seasonal

rainfall that averages 193 mm per year (NOAA, 2009). Rainfall in the Sonoran Desert is

bimodally distributed, split between summer monsoon events from July to September and

low-intensity storms from the Pacific Ocean between November and March (WRCC,

1985).

4.3.2. Potential nitrification assays

4.3.2.1. Sample collection

Surface soil samples were collected from 0-5 cm depth during the pre-monsoon season in

late June/early July of 2007. Soils were collected from two patch types in the desert and

xeriscape sites (Table 1), between plants (interplant; 'IP') and under plant canopies

(under plant; 'P'), in order to explore the effects of resource islands on the relative

importance of fungal and bacterial contributions to N cycling (Schlesinger et al., 1996).

For the samples under plants, soils were collected under *L. tridentata* in desert sites and

under *E. farinosa* in the xeriscape sites. For further details about patch selection criteria

in these ecosystem types, see Hall et al. (2009) and Davies and Hall (2010). Lawn and

grassland AZ soils were collected from under grass and thus grouped with the P patch

type for the purposes of comparative analyses (no IP patch type present). Each soil

sample consisted of two soil cores located within 5 cm of each other and homogenized in a plastic bag. In total, five replicate soil samples were collected per patch type in each of the outlying and remnant desert sites. Additionally, five replicate soil samples were collected from lawns, two samples were collected from grasslands in AZ, and four samples were collected from each of the two patch types in xeriscapes. Soils from the grassland NM site were not included in the potential nitrification assays.

4.3.2.2. Laboratory methods

Following sample collection, soils were placed in coolers with ice, transported to the lab at Arizona State University (ASU), and sieved to < 2 mm within 48 h. Lawn soils were moist upon collection (11-22% soil moisture) and were stored on ice for up to 48 h before assaying rates of potential nitrification. Soils from all other sites, which were collected prior to lawn soils, were dry upon collection (0.4-1.3% soil moisture) and were further air dried for 3 weeks before N cycling assays. Soil moisture in dryland soils was not significantly different after air drying. Lawn soils were processed differently than other soils to minimize environmental effects on the microbial community due to storage compared to native conditions. After the potential nitrification experiments, the remaining soil from all sites was air dried and stored for further analysis of soil properties.

In-situ rates of potential nitrification were measured using the shaken-slurry method. Soils in this assay are supplemented with NH_4^+ and constantly aerated to minimize N immobilization and denitrification (Schimel et al., 1984; Hart et al., 1994; Kuenen and Robertson, 1994; Stark, 1996). The two biocide treatments and a control included: cycloheximide ($C_{15}H_{23}NO_4$; a fungicide) at 1.5 mg·g^{-1} (Castaldi and Smith, 1998; Laughlin and Stevens, 2002), acetylene at 1% of headspace (approximately 1 kPa C_2H_2; inhibits autotrophic nitrification; Mosier, 1980), and no inhibitor as a control. The concentration of cycloheximide used is within the range that inhibits eukaryotes but not prokaryotes. Additionally, cycloheximide does not affect the metabolism of archaea (e.g., Rasche and Ferry, 1996; Konneke et al., 2005; Tourna et al., 2011), with the exception of one study suggesting that cycloheximide inhibited one cultured archaeon (Taylor et al., 2010). The shaken-slurry assays were performed by adding 10 g soil to a 100 mL solution of 50 mmol·L^{-1} $(NH_4)_2SO_4$, 0.2 mol·L^{-1} K_2HPO_4, and 0.2 mol·L^{-1} KH_2PO_4, with a pH of 7.2. Soil slurries were shaken at 180 rpm on a reciprocal shaker. Homogenized aliquots of slurry were removed at five time points over 24 h and amended with several drops of 0.6 M $MgCl_2$ + $CaCl_2$ to flocculate soil particles. Aliquots were then centrifuged at 3000 RCF (Centra CL3 Thermo IEC, Massachusetts, USA) and supernatant was filtered through Whatman #42 ashless filters (Fisher Scientific, Pennsylvania, USA) that were pre-leached with 2 M KCl. The extracts were frozen until colorimetric analysis for NO_2^- + NO_3^- on a Lachat Quikchem 8000 autoanalyzer at ASU (Hach Company, Colorado, USA). Rates of potential nitrification were calculated as the linear increase in NO_2^- + NO_3^- concentration over time (μg N g^{-1} dry soil h^{-1}).

4.3.3. N_2O production assays

4.3.3.1. Sample collection

Surface soil samples were collected to capture the zone of active microbial populations in each ecosystem type. The sampling included soils from 0-2 cm depth in outlying and remnant desert sites (Belnap et al., 2003; Sponseller, 2007), 0-7 cm in lawns and the grassland AZ, and 0-10 cm in the grassland NM. To explore temporal variability in microbial contribution to N_2O production, soils were collected prior to the first monsoon rains in August 2008, July 2009, and August 2009. As described for nitrification soil sampling (section 2.2.1.), soils were collected from IP and P patch types. Soils from the grassland NM site were collected from under blue grama grass and thus were grouped with the P patch type for the purposes of comparative analyses (no IP patch type present). In all sites, each sample consisted of five soil cores located within 5 cm of each other and homogenized together in a plastic bag. Bulked soil samples from each site were later divided into subsamples as laboratory replicates for the N_2O production experiment.

4.3.3.2. Laboratory methods

Following sample collection, grassland NM soils were placed on ice in a cooler and shipped to ASU overnight. All AZ soils were placed in coolers with ice, transported to the lab, and sieved to < 2 mm within 48 h. Lawn soils were moist upon collection (12-

14% soil moisture) and were stored on ice for up to 48 h before assaying N_2O production. Soils from all other sites were dry upon collection (0.6-1.7% soil moisture) and were stored for up to 48 h before N cycling assays. Following N_2O production incubations, remaining soil was air dried and stored for further analysis of soil properties.

For the N_2O production experiments, subsamples of the composited soil were used as laboratory replicates (n = 4 homogenized soil subsamples from each ecosystem and patch type per microbial inhibitor). Soils were incubated in the laboratory in 500 mL airtight Nalgene containers (Nalgene, New York, USA) fitted with black butyl rubber septa across a range of soil moisture conditions with two biocide treatments and a water control. These treatments included: cycloheximide at 1.5 $mg \cdot g^{-1}$ in water, streptomycin sulfate ($C_{42}H_{84}N_{14}O_{36}S_3$; a bactericide) at 3.0 $mg \cdot g^{-1}$ in water (Castaldi and Smith, 1998; Laughlin and Stevens, 2002), and a DI water control (water added at the same rate as biocide treatments but with no inhibitor). The concentration of streptomycin used is within the range that inhibits bacterial but not archaeal protein synthesis (Tourna et al., 2011), preventing N metabolism in most bacteria (Weisblum and Davies, 1968). The volume of water used with each inhibitor was determined by the different water treatments: to test the microbial contribution to N_2O production at different water contents that are typical of dryland and managed soils following precipitation events, soils were incubated under relatively dry conditions (10-35% of the soil water-holding capacity; WHC; hereafter as 'low water') and wet conditions (47-77% of the soil WHC; 'high water'). Lawn samples were already moist and thus were incubated at 30-60%

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WHC to represent the low water range and 60-100% WHC to represent the high water range that is experienced by lawns immediately after irrigation. Results from our incubations confirmed that N_2O production rates in all ecosystems, patch types, and seasons were significantly higher in the high water range compared to the low water treatments (Figure 2; Kolmogorov-Smirnov test, p value < 0.001). These findings are consistent with earlier studies showing a positive relationship between water content and N_2O production (Firestone et al., 1980; Linn and Doran, 1984; Smith et al., 1998) and support the two water content levels used in our experimental design. Based on pilot experiments, we added soil to each jar at an amount that provided the maximum detectable levels of N_2O while minimizing total soil mass (to minimize respiration effects in a closed headspace), then standardized production rates per g of soil. In the high water incubations, we used 200 g fresh soil from the grassland NM and lawn sites and 50 g from the grassland AZ, remnant desert, and outlying desert sites. In the low water incubations, we used 150 g fresh soil from all sites.

Water containing the dissolved biocides (i.e. cycloheximide and streptomycin) was used to bring soils to low or high water content. All soil samples were stirred in their jars to homogenize the biocide solution within the soil matrix. Incubations were capped to prevent evaporation and allowed to sit for 24 h to ensure that biocides had taken effect. Jars were then uncapped for 1 min and re-sealed for the start of a 48 h incubation period (Castaldi and Smith, 1998; McLain and Martens, 2006). At the end of the 48 h period, gas samples were collected from the headspace of each jar using a Luer-Lok syringe and

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needle equipped with a stopcock (BD, New Jersey, USA). The needle was inserted into the septa of each lid, and 12 ml of headspace gas was extracted and injected into an N_2-purged and pre-evacuated 5 ml gas vial fitted with a grey butyl stopper septa (Kimble Chase, New Jersey, USA). Vials were over pressurized and sealed with silicone to maintain sample integrity and prevent leaks. Headspace concentrations of N_2O and carbon dioxide (CO_2) were analyzed within 48 h using a Combi PAL automated sampler (CTC Analytics, Minnesota, USA) connected to a modified Varian CP-3800 gas chromatograph (Agilent Technologies, California, USA) equipped with both TCD (CO_2) and ECD (N_2O) detectors.

4.3.4. Soil properties

Soil properties were measured on all samples to aid in the interpretation of potential nitrification and N_2O production patterns. Within 48 h of soil collection, soil samples were processed for moisture (% SM), organic matter content (% SOM), water-holding capacity (% WHC), extractable ammonium ($\mu g\ NH_4^+$-N g^{-1} dry soil) and extractable nitrate + nitrite (summed as $\mu g\ NO_3^-$-N g^{-1} dry soil) concentrations, and total C and N content (% C and % N; Table 1). Gravimetric SM was determined by drying 30 g of soil for 24 h in a 105°C oven (g water·100 g^{-1} dry soil). SOM was determined by the loss-on-ignition method (g organic matter·100 g^{-1} dry soil) as ash-free dry mass following combustion of oven-dried soils for 4 h at 550°C. Soil WHC was determined gravimetrically by saturating 20 g of soil with water in a leaching funnel and weighing

after 24 h of draining through a 55 mm GF-A filter (Whatman, Maidstone, England).

Extractable inorganic N concentrations were measured using 10 g of soil extracted in 50

mL of 2 M KCl by shaking for 1 h and filtering through pre-leached Whatman #42

ashless filters. The extracts were frozen until colorimetric analysis using a Lachat

autoanalyzer (Hach Company, Colorado, USA). Total inorganic N was calculated as the

sum of the concentrations of extractable NH_4^+-N and NO_3^--N concentrations. Total soil C

and N content were determined on dried soils using a PE 2400 elemental analyzer

(PerkinElmer, Massachusetts, USA). The content of total soil C and N was used to

calculate C:N ratios that may constrain the activity of microorganisms with different

stoichiometric requirements.

4.3.5. Data analysis

To explore the relative magnitude of 'background' rates of potential nitrification and N_2O

production across ecosystem types, we first compared rates using the water control

treatment only (no inhibitor). We then compared rates of N transformations following

inhibitor additions to rates from the water control treatment to interpret the fractional

contribution of each microbial group (fungi, bacteria, autotrophs) to each process using

the formula: ((1-[rate in inhibitor treatment/rate in water treatment]) x 100). Although we

expected that inhibitors would have no effect or would decrease rates (signifying no or

some contribution of the microbial group, respectively), the fractional contribution may

be underestimated if potential nitrification and N_2O production rates following biocides

increase due to elimination of competing microorganisms or the input of nutrients from

inhibited, dead cells to the remaining microbial groups (McLain and Martens, 2005,

2006; Rousk et al., 2008). Changes in N transformation rates following cycloheximide

additions were attributed to the absence of fungi, and changes in rates following

streptomycin additions were attributed to the absence of bacteria. Changes in potential

nitrification following acetylene additions were attributed to the absence of bacterial and

archaeal autotrophic NH_3 oxidizers (Mosier, 1980; Offre et al., 2009). To minimize

methodological bias that may have occurred towards any particular microbial group, we

estimated the microbial contribution to N transformations using multiple lines of

evidence with different inhibitors. For the N_2O production experiment, the fractional

contribution of N_2O by different microorganisms was assessed using data from the

August 2009 sampling event only (sections 3.2.1 - 3.2.3).

Statistical tests were carried out using SPSS (v. 20.0 for Windows). Since not all

ecosystem types contained soils for both patch types (under and away from plans),

separate ANOVAs were carried out instead of an all-factors inclusive ANOVA. For

potential nitrification, we used a one-way ANOVA with post-hoc Tukey's test (or Tukey-

Kramer test for instances of unequal sample size) on data from under-plant patches only

to analyze the effect of ecosystem type. We then used a two-way ANOVA test to assess

the independent effects of ecosystem and patch type, using only those ecosystems with

shrubs and interplant spaces (outlying desert, remnant desert, xeriscape). For N_2O

production, we used a two-way ANOVA test on data from under-plant patches only to

assess the independent effects of ecosystem type and water content. We then used a three-way ANOVA to test the effects of ecosystem type, patch, and water content using only those ecosystems with shrubs and interplant spaces (outlying desert and remnant desert). Results from the three-way ANOVA tests for N_2O production were compared to Bonferroni-corrected two-way ANOVA tests to confirm the independent effects of ecosystem and patch type (ecosystem x patch, separately for each water content) or the effects of ecosystem and water content (ecosystem x water, separately for each patch type). Across all tests, the dependent variables used were rates of NO_3^- or N_2O production from the water control only ('background rates'), and the fractional contributions of each microbial group (i.e. fungi, autotrophs, and bacteria). Significant interactions between main factors were interpreted based on profile plots produced by SPSS using estimated marginal means for each level of one factor across all levels of the second factor. Additionally, differences between groups within significant interactions were confirmed with post-hoc one-way ANOVA with multiple comparison tests. Linear model assumptions of normality and homoscedasticity were tested using Shapiro-Wilk and Levene's test, and observed visually using normal probability plots and plotting residuals against fitted values, respectively, and data were transformed (log_{10}, square root, or reciprocal square) when necessary to satisfy model assumptions. When assumptions were violated after transformation (only N_2O production rates in the water control; Levene's, $p < 0.001$), results from parametric tests were interpreted cautiously and compared to results from non-parametric Kruskal-Wallis ANOVA and Games-Howell post-hoc tests.

Due to high correlation between soil variables that prevented meaningful use of multiple regression techniques (Smith et al., 2009), we used bivariate Pearson correlation analysis in concert with canonical correspondence analysis (CCA; Palmer, 1993) in PAST (Hammer et al., 2001; Fu et al., 2006) to explore the effects of soil properties on N transformations across ecosystems and patch types (background N_2O production rates, fractional microbial contribution to N_2O production). CCA allows visualization of the multivariate relationships between dependent variables along environmental gradients. CCA was carried out separately using data from low and high water content incubations.

4.4. Results

4.4.1. Potential nitrification

4.4.1.1. Background rates of potential nitrification

Background rates of potential nitrification varied by plant patch type but were statistically similar across ecosystems (Figure 3, open bars, water control only; Table 2A, 'Background rates'). Rates were not significantly associated with ecosystem when analyzed under plants only (one-way ANOVA) or across patch types (two-way ANOVA). However, across all ecosystem types that contain both shrubs and interplant spaces, potential nitrification in the water control was higher under shrubs compared to away from plants. These analyses revealed neither direct effects of urbanization (i.e.

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land-cover change and management; remnant desert vs. lawn or xeriscape) nor indirect effects of urbanization (e.g., local climate, air quality; outlying desert vs. remnant desert) on NO_3^- production.

4.4.1.2. Fungal contribution to potential nitrification across ecosystem and patch type

Across all ecosystems, acetylene decreased rates of potential nitrification by ~81-100% in both patch types compared to the water controls (Figure 3, dark gray bars; Table 2A, 'Autotrophic contribution'), highlighting the potential importance of autotrophic nitrification in these soils. Considering the under-plant patch type only, the fractional contribution of autotrophs to nitrification was not significantly associated with ecosystem type. Similarly, in the three ecosystem types that contained both shrubs and interplant spaces, the fractional contribution of autotrophs to nitrification was substantial and similar across ecosystems and patch types. Cycloheximide did not significantly inhibit rates of potential nitrification compared to the control, in any combination of ecosystem and patch type (Figure 3, light gray bars; Table 2A, 'Fungal contribution'). In other words, results from both inhibitors suggest that autotrophs universally dominate rates of potential nitrification, and fungi are not significant contributors to NO_3^- production in the assays used and ecosystems sampled in this study.

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4.4.2. N_2O production

4.4.2.1. Background rates of N_2O production

Background rates of N_2O production varied by ecosystem type, patch type, and water content (Figure 4, open bars, water control only; Table 2B, 'Background rates'), highlighting microbial sensitivity to diverse environmental conditions. Two-way interactions across water levels were significant when ecosystems were grouped by the under-plant patch only (two-way ANOVA), and when grouped by the two ecosystem types that contained both shrub and interplant spaces (three-way ANOVA). In the two-way ANOVA analysis using under-plant patches only, N_2O production at high water content was lowest in the native grassland sites compared to the other ecosystem types (one-way ANOVA, $p < 0.001$), but was similar among native and urban ecosystem types (Figure 4d; no significant direct or indirect effects of urbanization; Games-Howell, $p > 0.26$). However, at low water content (ecosystem x water interaction), N_2O production was highest in remnant deserts, followed by outlying deserts, lawns, and native grassland ecosystems (Figure 4c; one-way ANOVA, $p < 0.001$). In other words, at low water content, the direct effects of urbanization (remnant desert vs. lawn; Games-Howell, $p = 0.004$) had a stronger effect on N_2O fluxes (i.e. larger mean difference) than the indirect effects (outlying vs. remnant desert; Games-Howell, $p = 0.007$), although both types of urban effects are statistically significant. When incubated under high water compared to the low water conditions, N_2O production rates were 10-fold higher in lawns, 6-fold

higher in deserts (remnant and outlying), and 2-fold higher in grasslands. In the three-way ANOVA with remnant and outlying deserts only, fluxes of N_2O were affected by patch type under most conditions tested (patch x water interaction) and by the indirect impacts of urbanization under certain conditions (ecosystem x water interaction). Specifically, rates were 4-fold higher in soils from under plants compared to away from plants at high water content in both ecosystem types, and remnant deserts at low water content. Additionally, background rates of N_2O were significantly larger in remnant desert compared to outlying desert when incubated at high water content in the interplant patch and at low water content under plants (Figure 4b, 4c, respectively). Together, these results show that instantaneous rates of N_2O production from formerly dry, native desert soil are highest under plants at high water content (e.g., simulating conditions immediately following rainfall), increase when soils are exposed to the urban environment with either ample water availability or presence of plants, and decline – at least as measured in our 48 h incubation – upon conversion to irrigated, managed lawn.

4.4.2.2. Fungal contribution to N_2O production across ecosystem, patch type, and water content

Cycloheximide decreased N_2O production by ~70-98% in most aridland soils across plant patch types and water conditions compared to the water controls (grassland NM, outlying desert, and remnant desert; Figure 4, light gray bars), suggesting that fungi are important contributors to N_2O production in these systems. This pattern is robust across seasons

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(three different sampling efforts) and years, showing that fungi are important contributors to N_2O production in desert soils across diverse environmental conditions (mean 79% ±15% standard deviation, n = 67; grassland NM, outlying desert, and remnant desert). In the clayey, native grassland AZ, the microbial contribution to N_2O production was fungal at low water content but switched to bacterial and/or archaeal at high water content (ecosystem x water interaction). In contrast, fungi did not significantly contribute to N_2O production in lawns, highlighting the direct effects of urbanization on the pathway of N_2O flux in managed soils compared to aridland systems.

When analyzed using a three-way ANOVA (outlying and remnant deserts only), the fractional contribution of fungi to N_2O production differed by ecosystem depending on both patch type and water content (three-way interaction, ecosystem x patch x water; Table 2B, 'Fungal contribution'). Contrary to predictions, fungi contributed significantly more to N_2O production in more fertile remnant deserts (93% of total) compared to less fertile outlying deserts (73%) under relatively high-resource soil conditions (high water content in both patch types; low water content under plants). However, in soils with the least resourceful conditions (low water content away from plants), fungi contributed more to N_2O flux in outlying deserts (84%) compared to remnant deserts (77%). Also contrary to our predictions, the importance of fungi to N_2O production in desert soils was not consistently affected by patch type. Similarly, the effect of water content on fungal N_2O production across both patch types was complex: in outlying deserts, higher water content decreased the fungal contribution (81% of N_2O produced by fungi at low water

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content, 72% at high water content), but in remnant deserts, high water content increased the fungal contribution (84% of N_2O produced by fungi at low water content, 95% at high water content). However, across all interactions between water content and patch type in native deserts, the differences in the fungal contribution were relatively small ($< 20\%$) compared to the overall importance of fungi to this biogeochemical pathway ($> 70\%$ minimum fungal contribution to N_2O production).

In support of the finding that fungi are the dominant N_2O producers, streptomycin did not inhibit N_2O production compared to the controls across ecosystem, patch type, and water content (Figure 4). In fact, streptomycin increased N_2O production compared to the water controls across most conditions tested. These results suggest that streptomycin removed bacterial competition with fungi or that substrates from the inhibited bacterial biomass became available for metabolism by fungi (McLain and Martens, 2005, 2006; Rousk et al., 2008).

4.4.2.3. Effects of soil properties on N_2O production

Background rates of N_2O production were significantly and positively correlated with total inorganic N, but were not significantly related to other soil variables tested at both low and high water contents (Table 3; soil variables include NH_4^+ and NO_3^- concentration, total C, total N, C:N ratio, SOM, field SM, and incubation water content). The fungal contribution to N_2O production was significantly and negatively correlated

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with field SM, incubation water content, total C, total N, C:N ratio, and SOM (Table 3). The bacterial contribution to N_2O production was significantly and negatively correlated only with total inorganic N. Consistent with predictions, CCA revealed positive relationships between background rates of N_2O production and NO_3^- concentrations, particularly in high resource conditions under plants (Figure 5; shown only for low water content for simplicity). Based on their position in ordination space, these patch effects are likely driven by a combination of soil N, C, and SOM. However, the fungal contribution to N_2O production generally appears to be more negatively related to SOM and incubation water content than bacteria across all sites (Figure 5), although the strength of the fungal contribution varies widely across patch and moisture (Table 2; Figure 4).

4.5. Discussion

4.5.1. Sources of NO_3^- and N_2O production in arid, semi-arid, and managed soils

Contrary to recent suggestions that fungi are important nitrifiers (McLain and Martens, 2005, 2006, Collins et al. 2008), we found that fungi do not contribute substantially to potential NO_3^- production in the arid, semi-arid, and managed ecosystems examined in this study. In contrast, our results reveal that fungi are significant sources of N_2O production in soils from semi-arid grasslands and deserts, expanding evidence in support

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of the TDND model that fungi play a vital role in the N cycle of aridlands (Collins et al., 2008).

In addition to fungi and bacteria, archaea play an important role in biogeochemical N cycling, as they contribute directly to ammonia oxidation (e.g., Tourna et al., 2011) and potentially to N_2O production (Santoro et al., 2011; Jung et al., 2011). Archaeal-specific inhibitors have not yet been identified for N cycling processes, but archaeal ammonia oxidizers are common globally (Leininger et al., 2006; Gubry-Rangin et al., 2011), and are present in high elevation semi-arid forests near our sites (Adair and Schwartz, 2008), as well as in nearby soils and desert biocrusts (Marusenko et al., in press). In the soils studied here, autotrophic ammonia oxidizers (bacterial or archaeal) were inhibited by acetylene, which nearly eliminated nitrification in most of our samples. Further development of inhibitor-based approaches may help differentiate the function of archaea, bacteria, and fungi, and their responses to environmental change.

While the background N cycling rates reported here are consistent with magnitudes found in studies from similar ecosystems (Kaye et al., 2004; Bremer, 2006; McLain and Martens, 2006; Hall et al., 2008; Davies and Hall, 2010), the contribution of fungi to N_2O production has not been synthesized across literature. Fungi contribute on average 84% of the total N_2O production in native semi-arid grasslands and desert soils examined here, which is higher than in other studies from different environments (56% in ephemeral wetland soil from Canada, Ma et al., 2008; 35% wetland sediment from Louisiana, USA,

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Seo and DeLaune, 2010; 18% in arable soil from eastern Scotland, Herold et al., 2012), but similar to the three studies that have quantified N fluxes from this metabolic pathway in soils from grassland or aridland ecosystems (89% fungal contribution to N_2O production in grassland soils from Northern Ireland, Laughlin and Stevens, 2002; 79% in riparian soils from AZ, McLain and Martens, 2006; 85% in grassland soils from NM, Crenshaw et al., 2008). This pattern suggests that the importance of fungal N_2O production may be generalizable to other environments worldwide, including dryland systems.

4.5.2. Factors controlling the magnitude and pathways of the nitrogen cycle in aridlands

Our study highlights the ecological relevance of fungi across a broad range of environments from dryland soils, including both urban and non-urban locations, in soil under and away from plants, and in different moisture regimes that are typical of the southwestern US. These soil conditions, both separately and in combination, affect total N_2O flux and the strength of the fungal contribution. Furthermore, we demonstrate that both the magnitude and source of N_2O production are affected by land-use change and other anthropogenic factors.

Microbial activity is significantly and positively influenced by 'islands of fertility' created by aridland perennial plants (e.g., high SOM content and inorganic N

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concentrations; Schlesinger et al., 1996; McCrackin et al., 2008). As predicted, production rates of soil NO_3^- and N_2O were highest in relatively resourceful soil conditions under desert shrubs. However, we expected that fertile, resource islands would support a smaller fungal contribution to N_2O flux than bacteria. Inconsistent with expectations, the fungal contribution to N_2O flux in desert soils was higher under vs. away from plants in one case (remnant desert at low water), was lower under vs. away from plants in two cases (remnant desert at high water, outlying desert at low water), and was not affected by patch type in the remaining case (outlying desert at high water). Although some fungi may be more tolerant of low-resource conditions compared to bacteria, their obligately heterotrophic metabolism and competition with other heterotrophs and ammonia oxidizers for inorganic N may lead to spatial variation in their abundance or activity within soils (Peterjohn and Schlesinger, 1991; McLain and Martens, 2006).

The dominance of fungal N_2O production across variable moisture regimes suggests that some eukaryotic denitrifiers are adapted to the pulsed nature of water supply in aridland systems, including high water content after rain events and low water potentials experienced throughout desiccation and drought (Strickland and Rousk, 2010; Parker and Schimel, 2011; Sullivan et al., 2012). Optimal soil conditions for denitrification occur heterogeneously in micro-patches, aggregates, or horizons following temporal changes in respiration, aeration and soil water content (Linn and Doran, 1984; Peterjohn and Schlesinger, 1991; Wrage et al., 2001). Thus, high rates of precipitation inputs and

infiltration combined with key geomorphic conditions (e.g., soils with subsurface clay horizons; Kramer and Boyer, 1995) are likely to promote low-O_2 conditions that will maximize fungal denitrification, while total anoxia will support bacterial denitrification (Shoun et al., 2012; Seo and DeLaune 2010). For example, in the grassland AZ site, the pathway of N_2O production switched from fungal to non-fungal in low water compared to high water incubations, likely because the clayey grassland soils were incubated under slightly more anoxic conditions (~62% WHC for the 'high' water content treatment) than the sandy aridisols from the desert sites (~51% WHC). However, in the other aridland sites, N_2O flux increased with saturation and was consistently produced by fungi.

We infer that the N_2O produced from dryland soils was from fungal denitrification, although we did not directly investigate the mechanism or species of denitrifiers. Nitrous oxide is also produced from bacterial processes such as heterotrophic nitrification, autotrophic ammonia and nitrite oxidation, autotrophic nitrifier denitrification, and denitrification (Prosser, 1989; Wrage et al. 2001; Klotz and Stein 2008; Hayatsu et al., 2008). However, because 70-98% of the N_2O produced in the dryland soils was inhibited by the fungicide but not the bactericide, a significant autotrophic or bacterial contribution to N_2O emissions is less likely. Aridland fungal communities are typically dominated by a diversity of species within the groups Ascomycota and Basidiomycota (many of which are denitrifiers; Shoun et al., 1992; Prendergast-Miller et al., 2011; Shoun et al., 2012), and Glomeromycota (Cousins et al., 2003; Porras-Alfaro et al., 2011; Bates et al., 2012). The most common subgroups include dark septate endophytes, known to survive in high

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temperatures and dry conditions, and arbuscular mycorrhizal fungi, which are widespread and associated with desert-adapted plants (Cousins et al., 2003; Porras-Alfaro et al., 2008; Blackwell, 2011). Results from this study clearly show that variation in aridland N_2O production is related to spatial and temporal heterogeneity in resource supply for denitrification (organic C, NO_3^-, O_2 availability affected by water content). However, the diversity of fungal groups in dryland soils also suggests that N_2O flux may be constrained by the physiological capacity of different denitrifying species across heterogeneous environmental conditions (Singh et al., 2010).

The combined effects of soil C, inorganic N, and moisture on the magnitude and importance of autotrophic nitrification and fungal N_2O production likely underlie the patterns found across ecosystems, including those influenced by urban processes. For instance, we measured higher rates of N_2O production in soil from remnant deserts compared to outlying deserts (indirect effects of urbanization), likely due in part to elevated C and N pools in urban open-space parks from atmospheric deposition, altered microclimates, or modified floral and faunal assemblages associated with the built environment (Table 1; Lohse et al., 2008; Kaye et al., 2011; Hall et al., 2009; Bang et al., 2012). However, the fractional contribution of fungi to N_2O emissions was similar in the desert soils, regardless of proximity to the city.

In contrast to the indirect effects of urbanization, direct conversion of native land to irrigated, fertilized turfgrass altered the source of N_2O from fungi to prokaryotes.

Together, these patterns support conventional understanding of the dominance of bacterial denitrification in soils where organic C and NO_3^- are readily available and conditions are primarily anaerobic (Firestone et al., 1980; Wrage et al., 2001). Although denitrification potential is high in managed lawns, and turfgrass contributes significantly to the annual budget of N_2O emissions in urban landscapes (Kaye et al., 2004; Hall et al., 2008; Raciti et al., 2011a), the total magnitude of N_2O produced during the 48 h incubation was lower in lawn compared to desert soil. This trend may be explained by the high rates of internal N cycling (e.g., N immobilization, N assimilation; Raciti et al., 2011b) that contribute to the surprisingly low availability of NO_3^- for denitrifiers in managed lawns compared to desert soil. Alternatively, since our desert soils were collected following a long, dry period between the winter and summer rainy seasons, incubation water additions likely facilitated rapid microbial utilization of inorganic N that accumulated since the previous rain event. This rapid spin-up of microbial activity may have contributed to high rates of fungal N_2O production over the short incubation period (Peterjohn and Schlesinger, 1991; Mummey et al., 1994), but may have been followed by lower rates of production as the inorganic N pool diminished and soils dried. In contrast, conditions for bacterial denitrification are likely to be optimal year-round in lawns, and would contribute to high annual N_2O emissions from this managed landscape type. Together, our results show that both the source of denitrification and magnitude of N_2O flux are affected by anthropogenic factors, highlighting the sensitivity of fungal denitrifiers to urbanization.

4.5.3. Conclusion

Using inhibitors for specific microbial groups and metabolic pathways, we found that prokaryotic autotrophs are the main sources of potential NO_3^- production in current and former dryland soils from the Sonoran and Chihuahuan Deserts, while fungi dominate N_2O production in all soils examined except highly managed urban lawns. These results refine predictions of the TDND model, which posits that fungi contribute substantially to N cycling in arid and semi-arid ecosystems (Collins et al., 2008). Further, this research quantifies fundamental ecological differences between the indirect effects of the city environment (alters *rates* of N_2O flux) and direct effects of land cover and land-use change (alters *source* of N_2O flux). In addition to urbanization, extensive environmental changes in aridlands are also expected from climate variability, grazing, and biological invasions, with consequences for microbial functioning, ecosystem-level biogeochemical cycling, and biosphere-atmosphere exchange. Due to the different adaptations of eukaryotes and prokaryotes, and the different metabolic requirements of heterotrophs and autotrophs, future changes to climate and soil resources are likely to affect aridland biogeochemistry in ways not currently accounted for in ecosystem models that assume N transformations are controlled only by bacteria. Predictions of nutrient cycling following environmental change will benefit from inclusion of fungi as key mediators of aridland ecological processes.

Acknowledgements

4.6. Tables/Figures

Figure 1. Map of study sites in the CAP LTER, the Agua Fria National Monument (AFNM) in Arizona, and within the boundaries of the Sevilleta National Wildlife Refuge (SNWR) in New Mexico.
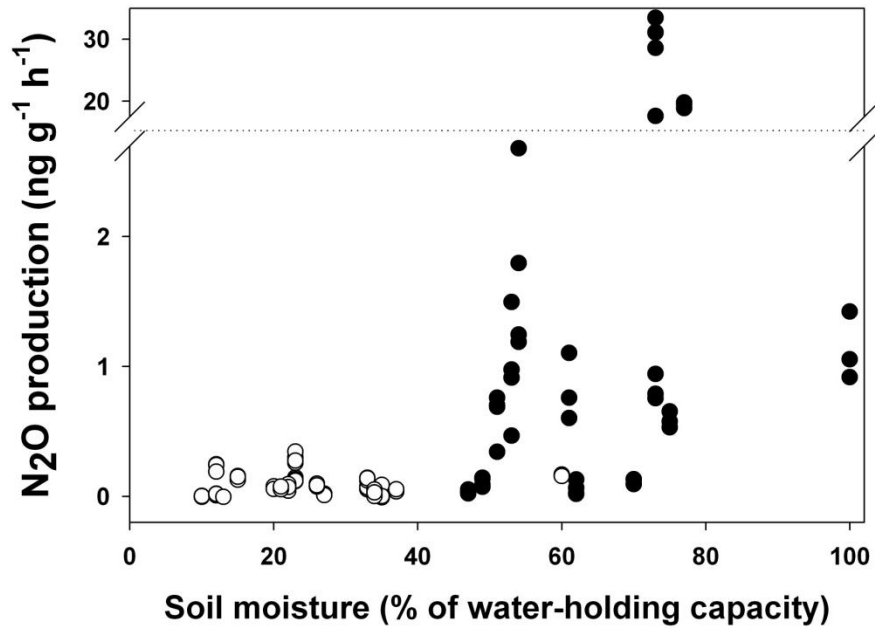
Figure 2. Rate of N$_2$O production in the water control treatment measured from soils incubated at a range of moisture levels across ecosystems, patch types, and seasons (n = 114). Open circles: 'low water'; closed circles: 'high water'.
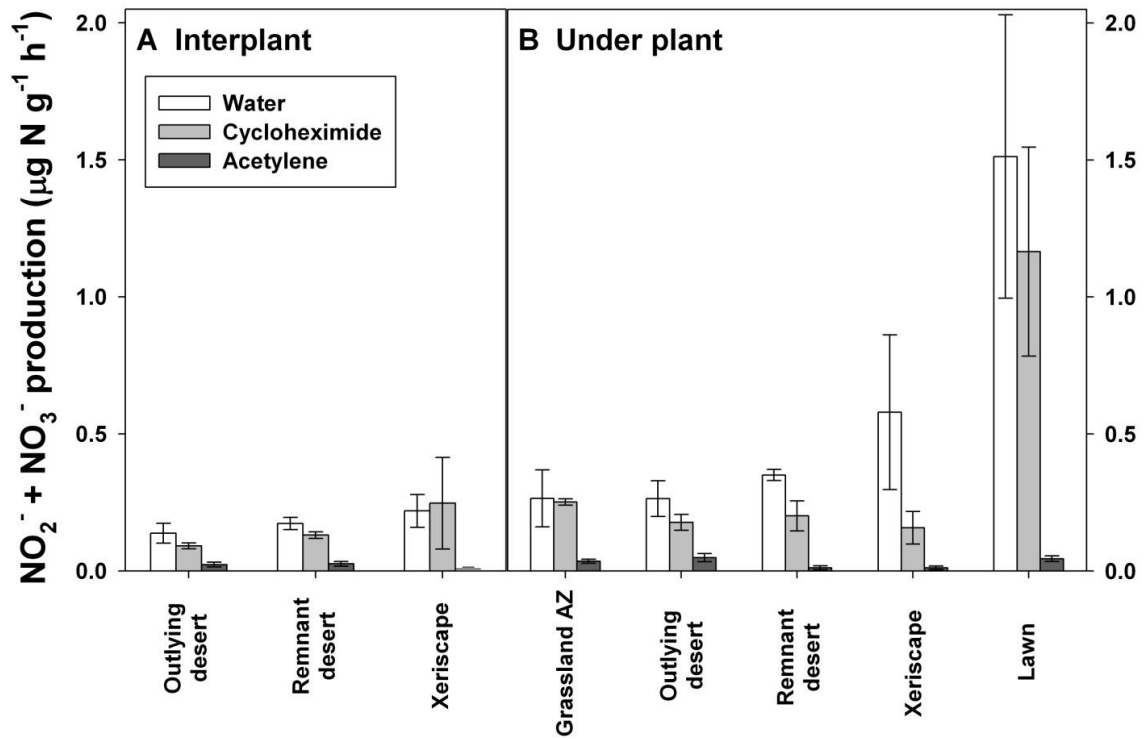
Figure 3. Rates of potential nitrification measured in soils collected from A) interplant spaces and B) under plant canopies across a range of ecosystems.
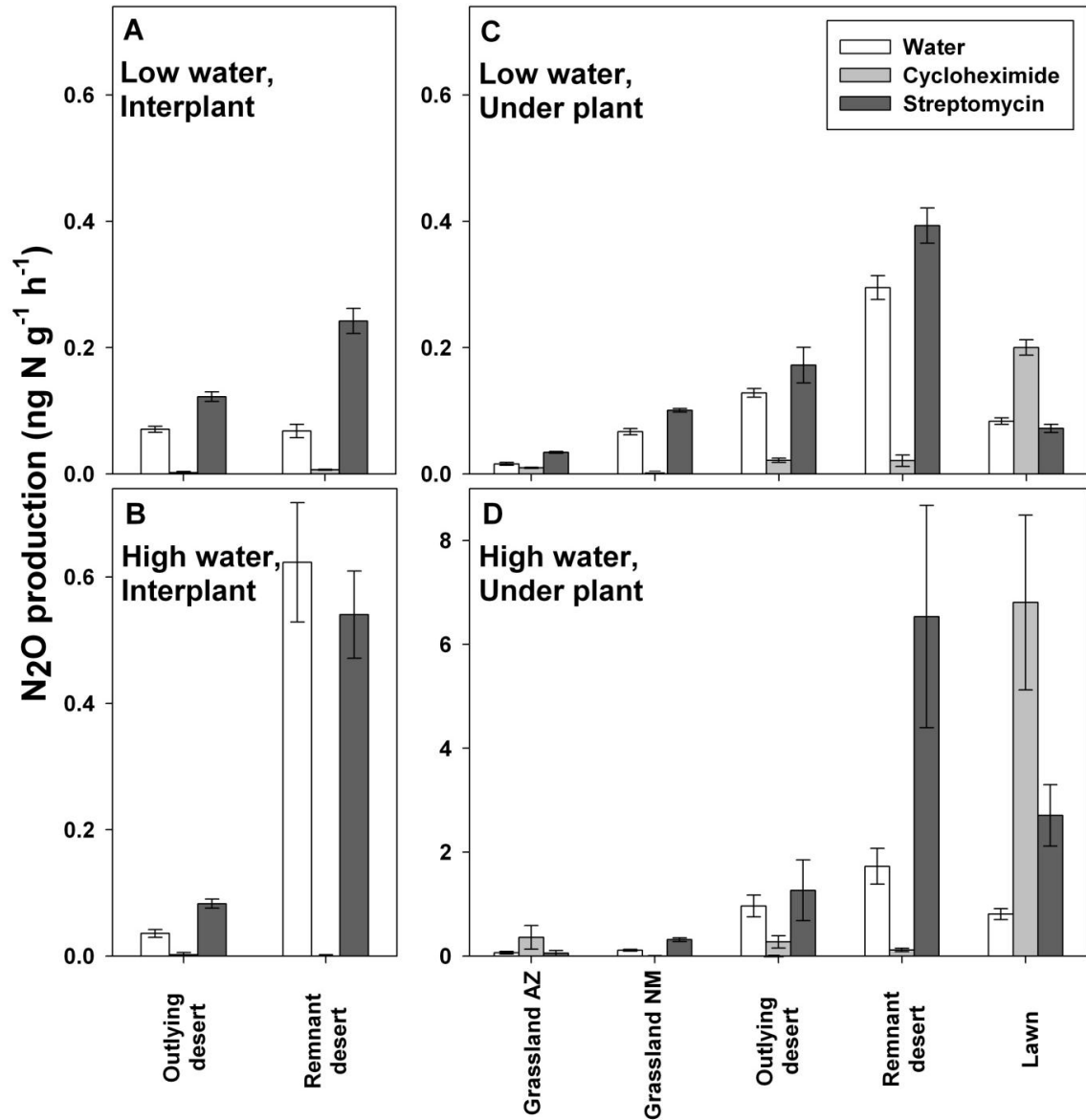
Figure 4. N$_2$O production measured in soils across ecosystems collected in August 2009 from A) interplant patch and incubated at low water content, B) interplant patch and high water, C) under plant patch and low water, and D) under plant patch and high water. Note larger scale of Y axis in panel D.
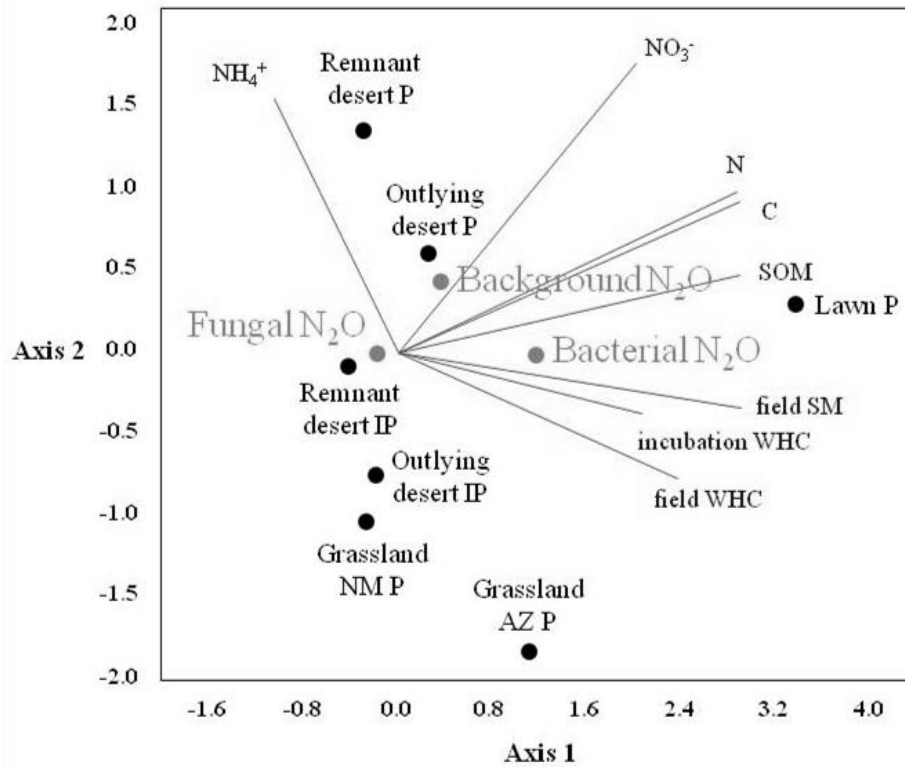
Figure 5. Canonical correspondence analysis of environmental factors (NH_4^+ and NO_3^- concentration, total N, total C, SOM, field soil moisture, incubation water content, field water holding capacity) across all ecosystems and patch types ('IP' interplant space; 'P' under plants). These environmental variables drive the placement of ecosystem types and N transformation variables in ordination space, including background rates of N_2O production ('Background N_2O'); fungal contribution to N_2O production ('Fungal N_2O'); and bacterial contribution to N_2O production ('Bacterial N_2O').

Table 1. Site characteristics and soil properties across a range of ecosystem types in the US Southwest.

Ecosystem type	Patch type[a]	Experiment[b]	Dominant plant species	Climate			Soil properties[f]												
				Elevation (m)	MAP (mm)[c]	MAT (°C)[d]	Soil organic matter (%)		NO_3^- ($\mu g \cdot g^{-1}$)		NH_4^+ ($\mu g \cdot g^{-1}$)		Total inorganic N ($\mu g \cdot g^{-1}$)		Soil WHC (%)[e]		C (%)		N (%)		C:N	
				Mean	Mean	Mean	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Grassland AZ	P	NO_3^-, N_2O	P.mutica	1350	412	16	4.2	(0.3)	5.6	(0.5)	2.8	(0.3)	8.3	(0.7)	75.4	(1.0)	1.17	(0.03)	0.11	(0.003)	10.3	(0.1)
Grassland NM	P	N_2O	B.gracilis	1630	255	13	1.5	(0.0)	1.6	(0.3)	3.0	(0.4)	4.6	(0.6)	28.0	(0.7)	0.56	(0.10)	0.05	(0.005)	11.5	(0.7)
Outlying desert	P	NO_3^-, N_2O	L.tridentata	504	201	22	3.1	(0.1)	4.7	(0.3)	6.5	(0.1)	11.1	(0.4)	32.6	(1.1)	1.41	(0.06)	0.12	(0.004)	11.5	(0.2)
	IP	NO_3^-, N_2O	none	504	201	22	1.8	(0.1)	2.8	(0.2)	3.6	(0.1)	6.4	(0.3)	24.7	(1.2)	0.58	(0.02)	0.06	(0.002)	10.4	(0.2)
Remnant desert	P	NO_3^-, N_2O	L.tridentata	416	183	22	3.5	(0.1)	9.5	(0.6)	8.9	(0.7)	18.3	(1.3)	36.6	(0.4)	1.35	(0.01)	0.13	(0.001)	10.3	(0.1)
	IP	NO_3^-, N_2O	none	416	183	22	2.0	(0.1)	3.6	(0.1)	3.9	(0.0)	7.5	(0.1)	29.0	(0.1)	0.56	(0.01)	0.06	(0.001)	10.2	(0.2)
Xeriscape	P	NO_3^-	E.farinosa	362	193	23	6.1	(2.4)	71.4	(44.2)	4.3	(1.4)	75.7	(45.4)	46.8	(3.0)	na	na	na	na	na	na
	IP	NO_3^-	none	362	193	23	3.9	(0.2)	24.5	(10.9)	4.6	(1.4)	29.0	(12.2)	50.0	(3.2)	na	na	na	na	na	na
Lawn	P	NO_3^-, N_2O	C.dactylon	365	193	20	8.0	(0.1)	11.9	(1.8)	6.6	(0.1)	18.5	(2.0)	72.3	(1.8)	4.74	(0.11)	0.36	(0.005)	13.3	(0.1)

[a] P = under plants; IP = interplant space. [b] NO_3^- = potential nitrification; N_2O = N_2O production. [c] MAP = mean annual precipitation: NOAA, 2009. [d] MAT = mean annual temperature: Briggs et al., 2007; Collins et al., 2008; Buyantuyev and Wu, 2010; Martin, 2008. [e] WHC = water-holding capacity (% gravimetric moisture after 24 h drain time). [f] Soil organic matter, inorganic N, WHC, and total C and N were measured from samples collected in August 2009 from all sites except xeriscapes. Values for the xeriscape sites are from samples collected previously in January 2008 (Hall et al., 2009) and May-June 2007 (Davies and Hall, 2010). na = not available.

Table 2. Statistical analyses for nitrogen transformation experiments.

Factors in ANOVA	Ecosystem type[a]	Patch	Water content	Dependent variable used in ANOVA			
				p-values			
				Background rates	Fungal contribution	Autotrophic contribution	Bacterial contribution
A. Potential nitrification experiment							
Ecosystem	GA, OD, RD, XE, LA	P	na	0.147	0.851	0.188	na
Patch				0.010*	0.634	0.697	na
Ecosystem	OD, RD, XE	P + IP	na	0.296	0.578	0.253	na
Ecos.× Patch				0.932	0.661	0.050	na
B. N₂O production experiment							
Water	GA, GN, OD, RD, LA	P	Low + High	<0.001*	<0.001*	na	0.054
Ecosystem				<0.001*	<0.001*	na	0.601
Ecos.× Water				<0.001*	<0.001*	na	0.216
Ecosystem	OD, RD	P + IP	Low + High	0.001*	<0.001*	na	0.244
Patch				<0.001*	0.166	na	0.847
Water				<0.001*	0.010*	na	0.873
Ecos.× Patch				0.414	0.388	na	0.367
Ecos.× Water				0.009*	0.002*	na	0.631
Patch × Water				<0.001*	0.017*	na	0.032*
Ecos.× Patch × Water				1.000	0.002*	na	0.019*

[a] GA: grassland AZ; GN: grassland NM; OD: outlying desert; RD: remnant desert; XE: xeriscape; LA: lawn. na = not applicable.
*Significance at $\alpha = < 0.05$.

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Table 3. Pearson correlation statistics between N_2O production and soil variables.

Soil variable	Statistic	Background N_2O		Fungal contribution to N_2O		Bacterial contribution to N_2O	
		Low water	High water	Low water	High water	Low water	High water
Total inorganic N	R	0.78	0.75	-0.03	-0.31	-0.81	0.71
	p	0.04*	0.05*	0.96	0.50	0.03*	0.07
Field soil moisture	R	-0.07	-0.29	-0.85	-0.98	-0.35	0.34
	p	0.89	0.53	0.01*	<0.01*	0.44	0.45
Soil organic matter	R	0.28	-0.04	-0.70	-0.96	-0.42	0.43
	p	0.55	0.93	0.08	<0.01*	0.34	0.33
Incubation water content	R	0.15	-0.15	-0.90	-0.70	-0.31	0.15
	p	0.75	0.75	0.01*	0.08	0.49	0.74
Total C	R	0.30	0.04	-0.53	-0.94	-0.42	0.53
	p	0.51	0.94	0.22	<0.01*	0.35	0.22
Total N	R	0.33	0.08	-0.55	-0.93	-0.44	0.54
	p	0.47	0.86	0.20	<0.01*	0.32	0.21
C:N	R	0.10	-0.10	-0.21	-0.81	-0.29	0.62
	p	0.83	0.83	0.65	0.03*	0.54	0.14
NO_3^- concentration	R	0.70	0.56	-0.34	-0.63	-0.73	0.65
	p	0.08	0.19	0.45	0.13	0.06	0.11
NH_4^+ concentration	R	0.55	0.74	0.55	0.41	-0.56	0.48
	p	0.20	0.06	0.20	0.36	0.19	0.28

* Significance at $\alpha = < 0.05$.

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5. Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands

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5.1. Abstract

Biological soil crusts (BSCs) can dominate surface cover in dry lands worldwide, playing an integral role in arid land biogeochemistry, particularly in N fertilization through fixation and cycling. Nitrification is a characteristic and universal N transformation in BSCs that becomes important for the export of N beyond the microscopic bounds of the crust itself. The contribution of ammonia-oxidizing bacteria (AOB) in BSCs has been shown, but the role and extent of the recently discovered ammonia-oxidizing archaea (AOA) have not. We sampled various types of crusts in four desert regions across the western United States and characterized the composition and size of ammonia-oxidizing communities using clone libraries and quantitative PCR targeting the *amoA* gene, which codes for the ammonia monooxygenase enzyme, universally present in ammonia-oxidizing microbes. All archaeal *amoA* sequences retrieved from BSCs belonged to the *Thaumarchaeota* (*Nitrososphaera* associated Group I.1b). Sequences from the Sonoran Desert, Colorado Plateau, and Great Basin were indistinguishable from each other but distinct from those of the Chihuahuan Desert. Based on *amoA* gene abundances, archaeal and bacterial ammonia oxidizers were ubiquitous in our survey, but the ratios of archaeal to bacterial ammonia oxidizers shifted from bacterially dominated in northern, cooler deserts to archaeally dominated in southern, warmer deserts. Archaea are shown to be potentially important biogeochemical agents of biological soil crust N cycling. Conditions associated with different types of BSCs and biogeographical factors reveal a niche differentiation between AOA and AOB, possibly driven by temperature.

5.2. Introduction

Plant inter-spaces in arid lands are typically colonized by biological soil crusts (BSCs), which in some areas can cover large portions of the landscape (Belnap 1995; Pointing and Belnap 2012). Pioneering cyanobacteria, such as *Microcoleus vaginatus*, initiate the formation of BSCs by stabilizing loose soils (Garcia-Pichel and Wojciechowski 2009), allowing a succession that involves other bacteria (Garcia-Pichel et al. 2001; Gundlapally and Garcia-Pichel 2006; Nagy et al. 2005), archaea (Soule et al. 2009), and fungi (Bates and Garcia-Pichel 2009; Bates et al. 2012), as well as lichens and mosses in well-developed crusts (Belnap and Lange 2003). Functional roles for the majority of nonphototrophic microbes inhabiting BSCs are not known, however, and remain to be established experimentally.

BSC topsoil assemblages are considered "mantles of fertility" (Garcia-Pichel et al. 2003) as they play important roles in biogeochemical processes within arid ecosystems (Belnap and Lange 2003; Evans and Johansen 1999; Strauss et al. 2012), fixing an estimated 0.1 Pg of C and 10 Tg of N annually across the globe (Elbert et al. 2012). Nitrification (with ammonia oxidation as its rate-limiting step) mediated by BSC microbes is another important component of arid land nutrient cycling that directly impacts soil fertility and rivals N-fixation in its magnitude (Johnson et al. 2005). Nitrifiers, such as *Nitrosospira*, have been recovered from BSCs in molecular surveys, and most probable number assessments suggest ammonia-oxidizing bacteria (AOB) are numerically abundant in

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BSCs (Gundlapally and Garcia-Pichel 2006; Johnson et al. 2005). Although archaea are likely important soil ammonia oxidizers (Leininger et al. 2006; Zhang et al. 2010; Bates et al. 2011; Stahl and de la Torre 2012) and sizable archaeal populations have been reported from BSCs (Soule et al. 2009), little is known about the role of archaea in the arid land N-cycle.

Recent research has improved our understanding of factors that drive the dynamics between archaeal and bacterial ammonia oxidizers in soils. For example, alkaline soils (Gubry-Rangin et al. 2011) and lower NH_4^+ concentrations (Martens-Habbena et al. 2009) may favor some ammonia-oxidizing archaea (AOA) over their bacterial counterparts. Although desert soils are typically characterized by these conditions, few studies, have specifically examined AOA in BSCs or the factors that structure communities of N-cycling microbes in arid lands (e.g., Johnson et al. 2005, 2007; Marusenko et al. 2013). As approximately one-third of the terrestrial surface is arid or semi-arid land, and BSC cover can be substantial, understanding the dynamics of nitrification mediated by crust microbes has relevance to the global N-cycle. Considering the potential for archaea to play an important role in arid land nitrification as ammonia oxidizers, we assessed the diversity and abundance of archaeal *amoA* genes in BSCs within four biogeographically distinct deserts in the western United States. For comparison, we quantified the abundance of bacterial *amoA* genes and estimated the portion of AOA and AOB in these BSC microbial communities using published determinations of archaeal 16S rRNA genes from the same samples (Soule et al. 2009).

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We also examined the role of environmental factors, particularly those associated with latitudinal gradients, in structuring these communities of crust ammonia oxidizers.

5.3. Methods

5.3.1. Sampling and DNA extraction

We sampled different crust types (e.g., those dominated by cyanobacteria, lichen, or moss) in a variety of geographically dispersed sites (Figure 1, Additional file 1) across four distinct desert systems in the western United States, including the Great Basin, Colorado Plateau, Chihuahuan Desert, and Sonoran Desert (listed in order of increasing temperature; Belnap and Lange 2003). The mean annual temperature across the four desert regions sampled ranged from 4°C to 22°C. These crust samples were previously characterized for archaeal diversity (Soule et al. 2009) based on 16S rRNA genes. For sampling, the bottom portion of a 55 mm Petri plate was used to excise a circular portion of the crust matrix (to a depth of ~ 1 cm) after wetting with a mist of sterile ultra-pure milli-Q water in order to make the crust supple to facilitate collection. All samples were allowed to air-dry, given a unique identification number, sealed in Zip-lock plastic bags for transport, and then stored dry in the lab's repository at room temperature, as recommended for arid land soil samples (Campbell et al. 2009), until DNA extraction. Approximately 1 g of the crust matrix was aseptically transferred to microcentrifuge vials of the Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA),

and standard protocols were used in DNA extraction following the kit instructions. All extracts were stored at −80°C until use in downstream applications.

5.3.2. Clone libraries and phylogeny of *amoA* genes

Clones were obtained from PCR products ofcommunity DNA using the *amoA* primers (position 4–23 and 619–638) and protocol described by Francis et al. (2005). Primers designed to hybridize at the ends of *amoA* gene are good options for general assays and have been successfully used for soils (Chen et al. 2008; Mao et al. 2011; Nicol and Prosser 2011). One clone library was constructed for each desert region by pooling extracts from at least 10 individual sites. All PCR products were checked for quality against an EZ Load Precision Molecular Mass Ruler (Bio-Rad Laboratories, Hercules, CA, USA) on 1% agarose gels (with a TAE buffer base) by standard gel electrophoresis, followed by ethidium bromide staining and imaging using the Fluor-S MultiImager system (Bio-Rad Laboratories). Products were then purified for ligation using the QIAquick PCR Purification Kit (Qiagen Sample and Assay Technologies, Valencia, CA, USA) prior to constructing the libraries using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's specifications. Cloning and transformation success were verified through PCR with 1 µl of clone-containing media as the template DNA. The clones obtained were then sequenced in the forward and reverse directions at a commercial laboratory.

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Phylogenetic analyses were carried out on a single alignment file that included *amoA*

gene sequences from our clone libraries (one representative sequence from each *amoA*

phylotype that was recovered from the four deserts) as well as reference groups of AOA

retrieved from GenBank and originating from previous studies (Venter et al. 2004;

Konneke et al. 2005; Hallam et al. 2006; Hatzenpichler et al. 2008; de la Torre et al.

2008; Walker et al. 2010; Park et al. 2010; Tourna et al. 2011; Matsutani et al. 2011; Jung

et al. 2011; Blainey et al. 2011; Lehtovirta-Morley et al. 2011; Santoro and Casciotti

2011; French et al. 2012; Mosier et al. 2012). Additionally we included 3,619 high-

quality sequences from the Dryad data repository (see sequence quality filtering by

Fernandez-Guerra and Casamayor 2012a, 2012b). All sequences were combined and

realigned using MAFFT (Katoh et al. 2002) and analyzed with the phylogenetic tree

building module of the MEGA 5 software package with the following parameters:

neighbor-joining statistical method, Jukes-Cantor nucleotide substitution model,

bootstrapping for 100 replicates, uniform rates, and complete deletion of gaps/missing

data (Tamura et al. 2011). Representative sequences of novel archaeal *amoA* genes from

each desert have been submitted to GenBank (NCBI accession #: Sonoran, EU439775;

Great Basin, EU439776; Colorado Plateau, EU439777; Chihuahuan, EU439778).

5.3.3. Quantitative PCR of archaeal and bacterial *amoA* genes

For use in quantitative PCR (qPCR), we developed archaeal-specific *amoA* primers based

on sequences obtained from our clone library work (those known to be present in our

BSC samples) as well as those available in public databases. These primers, amoA310f (5'-TGGATACCBTCWGCAATG-3') and amoA529r (5'-GCAACMGGACTATTGTAGAA-3'), were designed to yield PCR products of approximately 220 bp, of optimal length for qPCR. This primer set was then used for qPCR in 20 μl reactions that contained the following: 10 μl iTaq SYBR Green Master Mix (Bio-Rad Laboratories), 300 nM amoA310f/amoA529r, and 10 ng of environmental DNA. The reaction conditions had an initial denaturation step of 2.5 min at 95°C followed by 55 cycles of 15 s at 95°C and 1 min at 54°C, and a final dissociation step later used to check the fidelity of the qPCR results. For quantification, a standard curve (log-linear $R^2 > 0.97$) was generated using a purified, linearized, and quantified archaeal *amo*A clone plasmid in a dilution series that spanned from 10^1 to 10^9 gene copies per reaction.

Bacterial *amoA* qPCR was carried out in 20 μl reactions using primers amoA-1f and amoA-2r (Rotthauwe et al. 1997) at a final concentration of 500 nM, along with 10 μl iTaq SYBR Green Master Mix and 10 ng of environmental DNA. The reaction conditions were as follows: initial denaturation for 2.5 min at 95°C followed by 45 cycles of 15 s at 95°C and 1 min at 55°C, and a final dissociation step. A standard curve for quantification (log-linear $R^2 > 0.95$) was generated using genomic DNA from *Nitrosomonas europaea* ATCC 19718 (Chain et al. 2003) in a dilution series that spanned from 10^1 to 10^9 gene copies per reaction.

Multiple measures were taken to ensure quality qPCR data. All reactions for both archaeal and bacterial *amoA* qPCR runs were carried out in triplicate to account for analytical variability. Triplicates were averaged prior to any data calculations reported in the results. Melting curves obtained in the denaturation step were visually inspected to verify the quality of each reaction and to insure the absence of primer-dimers. Reported results contain only determinations for which C_t values could be interpolated within our standard curves, and failed or suspect reactions (those with questionable melting curves) were excluded from the data set.

5.3.4. Data analyses

We downloaded environmental data from the Commission for Environmental Cooperation for elevation and climate (years 1950–2000; CEC, http://www.cec.org) and used ArcGIS version 10.1 to extract exact values (for environmental data) corresponding to coordinates from the sites used in this study (Additional file 1). Canonical correspondence analysis (CCA; Ter Braak 1986; Palmer 1993) was used in the Palaeontological Statistics (PAST; Hammer et al. 2001; Fu et al. 2006) software package to explore associations between environmental factors [e.g., mean annual temperature (MAT) and mean annual precipitation (MAP)] and the dependent variables. The three dependent variables used were *amoA* gene abundances (archaeal, bacterial) and the archaeal to bacterial *amoA* ratio.

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Statistical tests were carried out using SPSS (version 20.0 for Windows). Linear model

assumptions were tested using Shapiro-Wilk and SPSS normal probability plots (for

normality) and Levene's test (for homoscedasticity), and data were transformed (natural

log) when necessary. Bivariate Pearson correlation and linear regression analyses were

performed to assess significance of relationships between *amoA* data and environmental

factors throughout the four desert regions (Hocking 1976). Significant differences of

regression slopes across environmental gradients in replicated data for gene copy

numbers were analyzed as a multiple linear regression (multiple dependent variables, one

independent variable). Due to co-correlation of external factors (latitude positively

correlated with elevation and MAP, and negatively with MAT), regressions were carried

out separately for each independent variable to assess the potential effect of each variable

without being able to interpret the combined effects of multiple variables (Smith et al.

2009). The sets of dependent variables were archaeal *amoA*, bacterial *amoA,* ratio of

archaeal to bacterial *amoA*, separately for the simple linear regression, and archaeal *amoA*

vs. bacterial *amoA* in the multiple regression.

5.4. Results

5.4.1. Diversity and phylogeny of archaeal *amoA* genes of BSCs

To obtain a geographically integrated survey of the diversity in archaeal *amoA* genes, we

analyzed clone libraries representing each of the four distinct desert systems across the

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western U.S. (Chihuahuan, Great Basin, Colorado Plateau, and Sonoran). Analysis of clone libraries, with more than 40 clones in each desert, revealed that monophyletic *amoA* gene populations corresponding to each desert region exist in arid land BSCs: only a single archaeal *amoA* phylotype was recovered from each desert (98.5–100% nucleotide similarity). Phylogeny (Figure 2) of the four representative sequences revealed that phylotypes from three of the deserts (Great Basin, Sonoran, and Colorado Plateau) were indistinguishable, grouping in a well-defined clade (>95% similarity, 76% bootstrap support), while a lone sequence type (representing the Chihuahuan Desert) was distinct (only 81% similarity to other *amoA* gene sequences obtained from BSCs). All four phylotypes, however, are part of the Group I.1b *Nitrososphaera* cluster as identified by Pester et al. (2012). The Chihuahuan Desert sequence grouped near sequences representing *Nitrososphaera* subcluster 2, closely related to the *amoA* gene of *N. viennensis* (Tourna et al. 2011; the only pure thaumarchaeotal isolate from soil), while the other three desert phylotypes were most closely related to *Nitrososphaera* subclusters 6 and 11.

5.4.2. Abundance of archaeal and bacterial *amoA* genes of BSCs

Population densities of putative ammonia oxidizers were assayed through qPCR of *amoA* genes from the four arid regions. Archaeal *amoA* copy numbers average around 7×10^5 per gram of soil (332 copies per ng DNA) across all desert sites (Table 1), whereas their bacterial counterparts averaged only slightly (and nonsignificantly) below this value at 3

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$\times 10^5$ (154 copies per ng DNA). The sizes of the AOA populations in BSCs were more variable than those of AOB, with densities of archaeal *amoA* genes ranging over three orders of magnitude in individual sites within each desert (ca. five orders of magnitude across all deserts), and bacterial *amoA* genes ranging over only one to two orders of magnitude (ca. three orders of magnitude across all deserts). More notably, the average of the ratios for archaeal to bacterial *amoA* gene copy number for each desert (Table 2) were highest in the southernmost, warmer deserts (54.7 and 3.5 for Sonoran and Chihuahuan, respectively) as compared to the cold deserts of more northern latitudes (1.3 and 0.01 for Colorado Plateau and Great Basin, respectively).

CCA revealed patterns between environmental factors and ammonia oxidizers across geographical provinces encompassed by the survey (trace *p* value < 0.01; Figure 3). Generally, the Sonoran Desert sites are associated with higher MAT, while those of the Great Basin and Colorado Plateau sites are more positively associated with elevation and MAP. Conditions associated with lower latitudes in our dataset (i.e., higher temperatures, lower elevation, lower precipitation) were negatively related to the size of the bacterial *amoA* population and positively related to both archaeal *amoA* and the ratio of archaeal to bacterial *amoA*.

Correlation and regression approaches clearly confirmed distinct relationships between archaeal and bacterial *amoA* gene abundances of BSCs and specific environmental factors across the four deserts (Table 3). For example, archaeal *amoA* abundance was

most strongly correlated with latitude ($r = -0.53$, $p = 0.001$). Bacterial *amoA* abundance, on the other hand, was most strongly correlated with elevation ($r = 0.78$, $p < 0.001$). Most revealing were the strong, significant correlations between the archaeal to bacterial *amoA* gene abundance ratio and a range of environmental factors: latitude, elevation, MAT, and MAP. These relationships translated into the general geographic trend of archaeal *amoA* gene abundance dominating over that of AOB in the warmer, more southern deserts, with AOB dominating in the more northern, colder deserts (Figure 4).

5.5. Discussion

Our results show that archaeal *amoA* genes are conspicuously and widely represented in BSCs from several arid regions of North America, suggesting that archaea are potentially involved in the process of ammonia oxidation of these soil communities. In general, average AOA abundance in BSCs was 10- to 20-fold lower than in most other types of soils, with some overlap in range (He et al. 2007; Leininger et al. 2006), and less than 10-fold smaller as reported in few other environments (Chen et al. 2008; Gleeson et al. 2010; Zeglin et al. 2011). Judged by the counts of *amoA* gene copies, AOA in fact outnumbered AOB when averaged over all sites.

The populations of *amoA*-bearing archaea are, however, of low diversity based on initial surveying in these systems using clone libraries, which mirrors the findings that archaeal diversity is also generally restricted in both bulk soils (Auguet et al. 2010; Fernandez-

Guerra and Casamayor 2012) and in BSCs (Nagy et al. 2005; Soule et al. 2009). We note that most publicly available sequences that had high similarity (>97% at the nucleotide level) to those of BSCs from this study originated from terrestrial environments with source soils of relatively alkaline character (Leininger et al. 2006; Shen et al. 2008; Zhang et al. 2009, 2011; Liu et al. 2010; Glaser et al. 2010; Fan et al. 2011). Because the number of samples analyzed is not exhaustive, we cannot assert with confidence that the two main *amoA* phylotypes found in BSCs in this study represent crust-specific lineages, although this remains a possibility to be explored further. Other studies have shown that certain AOA lineages have adapted to specific levels of pH (Gubry-Rangin et al. 2010, 2011). Arid lands are characteristically extreme environments exposed to intense UV radiation, limited availability of nutrients, alkaline soils, as well as distinct seasonal changes of long desiccation periods punctuated by pulsed precipitation events (Schlesinger 1997; Safriel et al. 2005), all of which may help carve separate niches for soil organisms (Wall and Virginia 1999). AOA dynamics may be distinct and depend on the range of a certain environmental variable (e.g., temperature gradient in only alkaline soils) in different types of BSCs and other local conditions that affect crusts (Garcia-Pichel et al. 2003; Pointing and Belnap 2012). What seems clear is that crust-dwelling AOA are part of a broader consortium of archaea more related to the group I.1b *Nitrososphaera* cluster than to any other *Thaumarchaeota* group. All BSC *amoA* archaeal phylotypes were nested within a larger group that holds the sequence from the only pure culture isolate from soil capable of chemolithoautotrophic ammonia oxidation, *Nitrososphaera viennensis* (Tourna et al. 2011).

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Unexpected patterns of distribution emerged when AOA and AOB population size was related to geography. Variables that are associated with latitude become important predictors of *amoA* abundance. Since an AOA/AOB ratio of >10 (accounting for cell sizes, specific growth rates; Prosser and Nicol 2012) suggests archaea outcompete bacteria in ammonia-oxidizing activities, such latitudinal factors likely structure communities and soil function across the dry lands surveyed here. Temperature was positively associated with AOA abundance and with the ratio of AOA/AOB, in support of other studies showing that AOA respond preferentially to elevated temperature in enrichment cultures (Kim et al. 2012) and in soil microcosms (Tourna et al. 2008), and correlate well with environmental temperature gradients (Bates et al. 2011; Cao et al. 2011), while some studies show negative or no response to temperature (Adair and Schwartz 2008; Jung et al. 2011). Together with our results, these findings suggest that temperature may be an important driver of niche separation for AOA, potentially leading to diverse ecosystem function responses that will depend on the magnitude of temperature change in the environment.

Based on previously reported qPCR determinations of 16S rRNA gene copy numbers for archaea from the same sample set (Soule et al. 2009), AOA can account but for a small proportion of the extant total population of BSC archaea. Assuming the number of copies per cell for *amo*A (1 for archaea, 2.5 for bacteria) and 16S rRNA genes (1 for both archaea and bacteria) as can be inferred from known genome studies (Klappenbach et al.

2001; Norton et al. 2002; Hallam et al. 2006; Blainey et al. 2011), putative AOA represent only about ~5% of the archaeal populations present in BSCs across all deserts and as little as 0.03% on average for the Great Basin samples. Even when considering possible uncertainties in these estimates stemming from primer bias (Baker et al. 2003; Agogue et al. 2008), our results are consistent with other soil environments globally (Lehtovirta et al. 2009; Ochsenreiter et al. 2003; Schleper and Nicol 2010). This clearly implies that the bulk of archaeal populations in BSCs (particularly the few dominant crust phylotypes documented by Soule et al. 2009) cannot be identified as AOA, leaving the functional role for the bulk of BSC archaea as undetermined. While archaea are potentially important for nitrification processes in arid lands, AOA must then be found among the rarer, possibly as yet to be detected, members of BSC microbial communities.

In conclusion, microbial involvement in regulating the availability of usable forms of N and controlling productivity in pristine systems is critical for the ecosystem (Schimel and Bennett 2004; van der Heijden et al. 2008; Nannipieri and Eldor 2009). BSCs of arid lands contain *Thaumarchaeota* that lose dominance to AOB with increasing latitude, from southern, warmer deserts to northern, colder climates. In some BSCs, AOB outnumber AOA by 100-fold, which to our knowledge is greater than any current report for soil environments where AOB dominate (e.g., Di et al. 2009; Hallin et al. 2009; Gleeson et al. 2010), more closely resembling other types of ecosystems and conditions (estuary, Mosier and Francis 2008; wastewater treatment bioreactor, Ye and Zhang 2011). Niche differentiation plays a role amongst AOA and AOB communities in general

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(Gubry-Rangin et al. 2011; Hatzenpichler 2012), and the same may also be true for BSCs. The BSC system can be used in further research to elucidate novel aspects of ammonia oxidation and AOA, such as AOA capacity for mixotrophic growth and the potential for denitrifying ability (Bartossek et al. 2010; Tourna et al. 2011; Xu et al. 2012). For example, phototrophic contributions in surface crusts create unique temporal and spatial gradients of pH, nitrogen, oxygen, and carbon availability (Garcia-Pichel and Belnap 1996; Johnson et al. 2007), which can be used to test AOA response at the microscale as well as across biogeographical regions. Overall, arid lands may provide further insight into environmental drivers of ammonia oxidation and community shifts of ammonia oxidizers, which has important implications for understanding nitrogen cycling at the global scale.

Acknowledgements

Abbreviations

BSC, Biological soil crust; AOA, Ammonia-oxidizing archaea; AOB, Ammonia-oxidizing bacteria; MAT, Mean annual temperature; MAP, Mean annual precipitation

Author contributions

IA, SJ, SB, TS, and YM participated in lab bench and/or phylogenetic work. FGP and SB designed the experimental approach. YM, SB, TS and FGP contributed to the drafting of the manuscript. All authors contributed intellectually to the ideas and data interpretations in this work. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

5.6. Tables/Figures

Table 1 Population densities of ammonia-oxidizing microbes in biological soil crusts estimated by qPCR of *amoA* genes

Province	Archaeal[a] *amoA*		Bacterial[b] *amoA*	
	Range	Average (±SD)	Range	Average (±SD)
Sonoran	2.8×10^3–5.5×10^6	$0.75 \pm 1.6 \times 10^6$	3.9×10^3–3.7×10^5	$0.47 \pm 1.0 \times 10^5$
Chihuahuan	1.5×10^3–6.7×10^6	$1.1 \pm 2.0 \times 10^6$	8.7×10^4–5.0×10^5	$2.5 \pm 1.9 \times 10^5$
Colorado Plateau	2.0×10^3–5.8×10^6	$0.59 \pm 1.8 \times 10^6$	9.2×10^4–5.9×10^5	$3.4 \pm 1.6 \times 10^5$
Great Basin	5.4×10^1–4.8×10^4	$1.7 \pm 2.3 \times 10^4$	2.2×10^4–5.0×10^6	$1.1 \pm 2.0 \times 10^6$
All deserts	5.4×10^1–6.7×10^6	$0.69 \pm 1.6 \times 10^6$	3.9×10^3–5.0×10^6	$3.4 \pm 8.4 \times 10^5$

[a]In descending order n = 12, 10, 11, 6, 39. [b]In descending order n = 12, 8, 12, 6, 38. All qPCR data are for *amoA* gene copies per gram of crusted soil.

Table 2 Relative densities for ammonia-oxidizing microbial communities in biological soil crusts (*amoA* or 16S genes)

Province	Ratio of archaeal to bacterial[a] *amoA*		Ratio of archaeal[b] *amoA* to archaeal 16S[c]	
	Range	Average (±SD)[d]	Range	Average (±SD)[d]
Sonoran	0.073–257.995	54.733 ± 83.924	5.0×10^{-5}–1.0×10^{-1}	$3.9 \pm 6.8 \times 10^{-2}$
Chihuahuan	0.020–15.844	3.459 ± 5.165	6.9×10^{-4}–2.6×10^{-1}	$6.8 \pm 9.9 \times 10^{-2}$
Colorado Plateau	0.003–12.664	1.279 ± 3.794	2.6×10^{-5}–7.4×10^{-1}	$7.4 \pm 23.2 \times 10^{-2}$
Great Basin	0.003–0.009	0.006 ± 0.003	9.9×10^{-6}–7.0×10^{-4}	$2.5 \pm 3.9 \times 10^{-4}$
All deserts	0.003–257.995	19.510 ± 53.405	9.9×10^{-6}–7.4×10^{-1}	$5.3 \pm 14.8 \times 10^{-2}$

[a]In descending order $n = 11, 8, 11, 3, 33$. [b]In descending order $n = 9, 6, 10, 3, 28$. [c]Determinations for general archaeal 16S rRNA gene copies per gram from Soule et al. 2009. [d]Average of all ratios for the designated province. All data here are for qPCR determinations of *amoA* or 16S genes as copies per gram of crusted soil.

Table 3 Results from correlation and multiple linear regression analyses for microbial communities in biological soil crusts

Independent variable	Bivariate correlation (Pearson r coefficients)				Regression (p values)
	Archaeal $amoA$ (n = 39)	Bacterial $amoA$ (n = 38)	Archaeal to bacterial $amoA$ (n = 33)	Archaeal $amoA$ to 16S (n = 28)	Archaeal $amoA$ vs. bacterial $amoA$
Latitude	-0.53^{**}	0.40^{*}	-0.70^{**}	-0.55^{**}	<0.001
Elevation	-0.22	0.78^{**}	-0.61^{**}	-0.25	<0.001
MAP	-0.13	0.67^{**}	-0.48^{**}	-0.12	0.004
MAT	0.39^{*}	-0.65^{**}	0.69^{**}	0.40^{*}	<0.001

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level. MAP, mean annual precipitation; MAP, mean annual temperature.
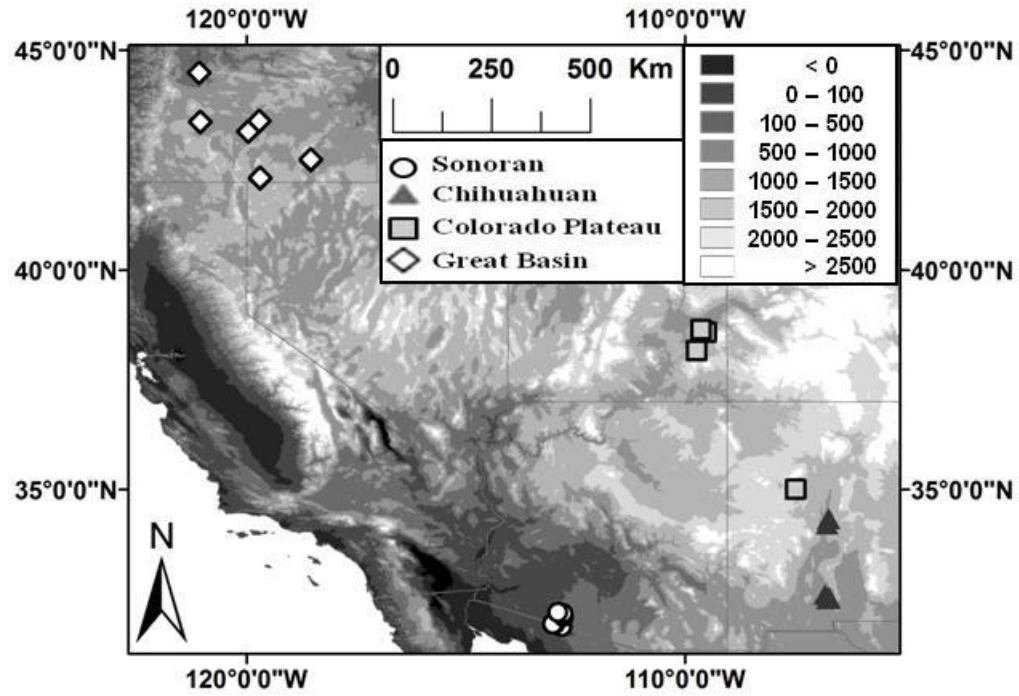
Figure 1 Map of study sites in arid lands of western North America. Legend shows elevation of the terrain (meters).
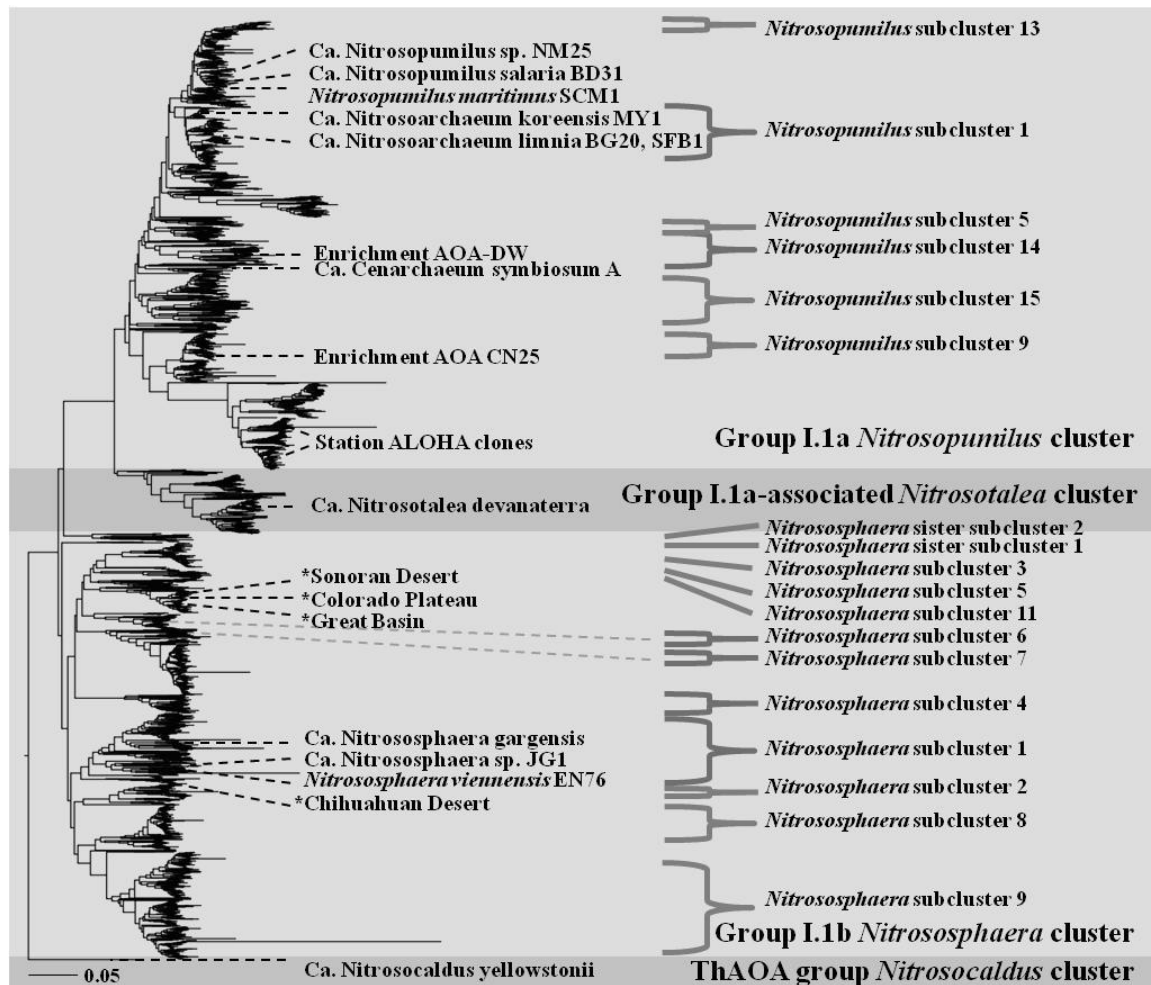
Figure 2 Phylogenetic tree of archaeal *amoA* gene sequences. Data were obtained from clone libraries of genomic community DNA isolated from biological soil crust samples in this study (indicated by an asterisk *), as well as reference sequences from GenBank for species of *amoA*-encoding archaea that have been characterized. Without clustering, these sequences were combined and realigned with 3,619 high-quality sequences from GenBank (Fernandez-Guerra and Casamayor 2012). Dashed lines connect the identified sequences to the position in the tree. Bracketed clustering groups are based on designations at multiple phylogenetic levels within the *Thaumarchaeota* as proposed by Pester et al. (2012).

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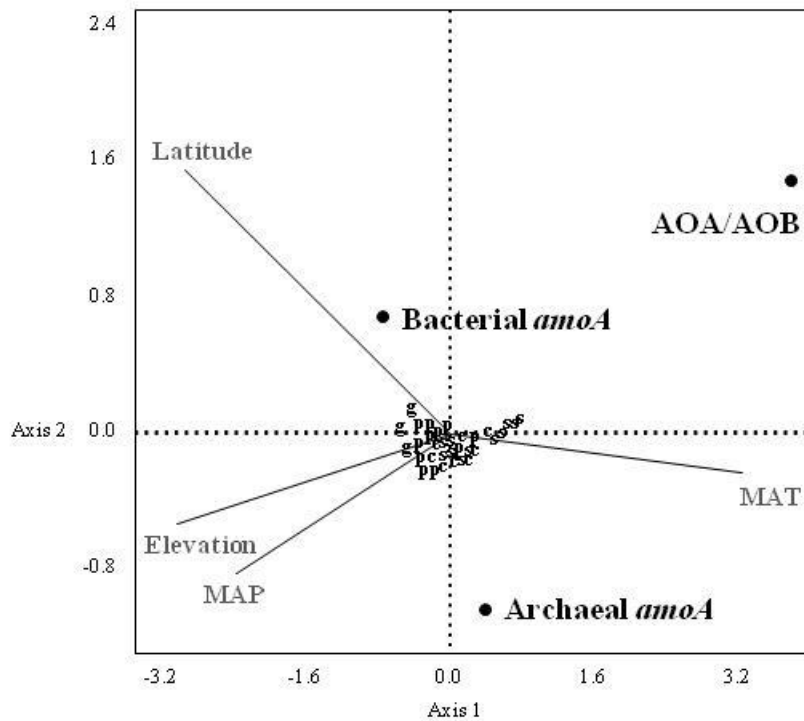
Figure 3 Canonical correspondence analysis (CCA) across all study sites. The dependent variables (ammonia-oxidizing abundance and community data) ordinate along a gradient that is driven by vectors related to the independent variables (environmental factors). AOA/AOB, ratio of archaeal to bacterial *amoA*; MAT, mean annual temperature; MAP, mean annual precipitation; c, Chihuahuan Desert; p, Colorado Plateau; g, Great Basin; and s, Sonoran Desert.
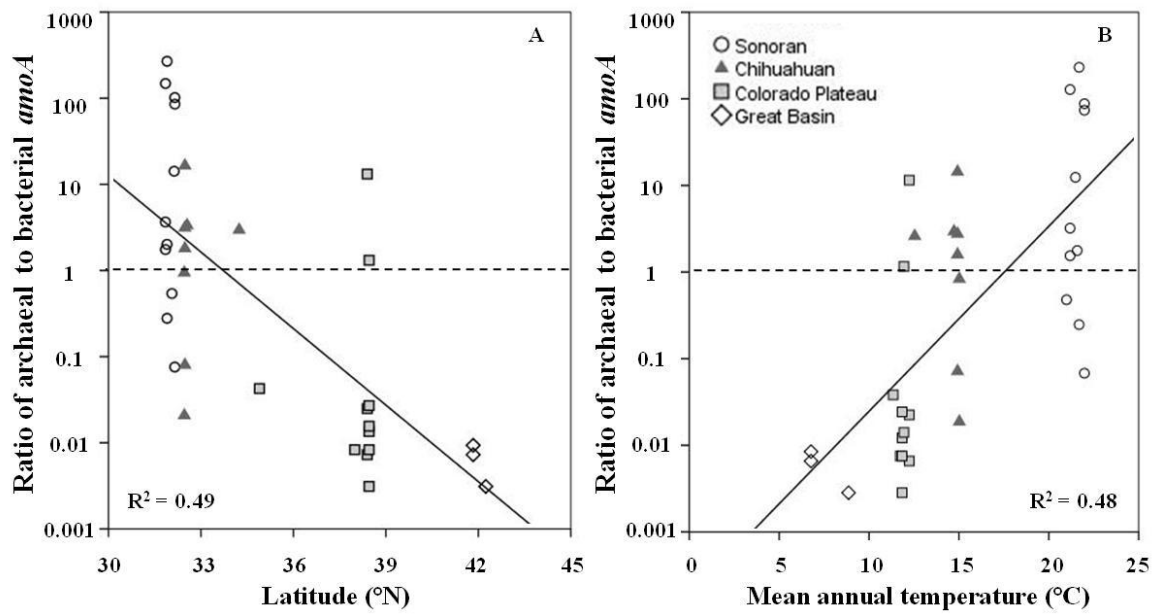
Figure 4 Relationship of archaeal to bacterial *amoA* ratio across all geographical locations. Ammonia-oxidizing community is analyzed with (a) latitude and (b) mean annual temperature. Dotted line at y = 1 indicates division between archaeal vs. bacterial *amoA* dominance.

5.7. Supplementary materials

Supplementary Table 1. Origin and type of biological soil crusts used in this study

Biogeographical Province Site	ID	Type of crust[a]	Latitude N	Longitude W	MAT	MAP
Sonoran						
Alamo wash	2 [bcd]	Lichen	32° 06.220	112° 46.150	21.0	239
Kuakatch wash	11 [bd]	Light	32° 10.610	112° 46.587	21.5	220
Bate's Well Road	23 [bcd]	Light	32° 11.930	112° 54.151	22.0	194
	43 [bcd]	Light	32° 12.003	112° 54.133	22.0	195
	51 [bcd]	Light	32° 11.898	112° 54.133	22.0	195
Camino de Dos Republicas	63 [bcd]	Light	31° 53.305	112° 48.437	21.2	208
	67 [bc]	Dark	31° 53.267	112° 48.400	21.2	208
	75 [bcd]	Light	31° 53.239	112° 48.322	21.2	208
Quitobaquito Spring	87 [bcd]	Lichen	31° 56.519	113° 01.133	21.7	173
	99 [bcd]	Lichen	31° 56.492	113° 00.963	21.7	173
Puerto Blanco Drive	103 [cd]	Dark	31° 56.359	112° 59.602	21.6	180
	105 [b]	Dark	31° 56.380	112° 59.591	21.6	180
	109 [bc]	Dark	31° 56.429	112° 59.000	21.6	181
Colorado Plateau						
Slick Rock	123	Dark	38° 34.802	109° 31.652	12.2	244

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Location	Sample	Type	Lat	Long		
			38°	109°		
	125 [d]	Light	34.822	31.631	12.2	244
	129 [bcd]	Light	38° 34.861	109° 31.584	12.2	244
	133 [bcd]	Light	38° 34.902	109° 31.541	12.2	242
	135 [bcd]	Light	38° 34.922	109° 31.518	12.2	242
Sunday Churt			38°	109°		
	143 [bc]	Dark	38.636	38.919	11.9	235
	153 [bcd]	Dark	38° 38.557	109° 38.910	11.9	235
	155 [bcd]	Dark	38° 38.541	109° 38.883	11.8	238
	161 [bcd]	Dark	38° 38.491	109° 38.799	11.8	238
	163 [bcd]	Dark	38° 38.480	109° 38.771	11.8	238
	165 [bcd]	Dark	38° 38.457	109° 38.753	11.8	238
Canyonlands NP, Needles District	173 [bcd]	Dark	38° 09.945	109° 44.458	11.7	237
	187 [cd]	Dark	38° 09.806	109° 44.614	11.7	237
Acoma Tribal Land	568 [bcd]	Dark	35° 00.380	107° 29.240	11.3	243
NW Great Basin						
Culver Road			44°	121°		
	302 [d]	Mossy	29.603	04.730	7.9	290
	304 [d]	Mossy	44° 29.623	121° 04.706	7.9	290
	306 [bcd]	Mossy	44° 29.557	121° 04.769	8.0	285
Egli Well Road			43°	119°		
	316 [d]	Lichen	23.399	42.669	7.5	258
	318 [b]	Light	43° 23.385	119° 42.698	7.5	258
Christmas Valley Road			43°	119°		
	322 [d]	Lichen	09.410	57.406	7.8	239
	324 [bd]	Lichen	43° 09.386	119° 57.424	7.8	239
	328	Light	43° 09.361	119° 57.460	7.8	239
Blizzard Gap Valley	338 [b]	Light	42°	119°	6.7	326

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Site	Sample	Type[a]				
			05.576	41.494		
			42°	119°		
	340	Lichen	05.561	41.462	6.7	326
			42°	119°		
	342 [bc]	Light	05.552	41.430	6.7	326
	346 [bcd]		42°	119°		
		Dark	05.530	41.363	6.7	326
Alvord Desert			42°	118°		
	360 [d]	Lichen	30.744	31.989	8.8	233
			42°	118°		
	362 [c]	Mossy	30.716	31.993	8.8	233
	364 [bcd]		42°	118°		
		Lichen	30.687	31.998	8.8	233
			42°	118°		
	368 [cd]	Lichen	30.634	32.004	8.8	233
Fort Rock			43°	121°		
	386 [d]	Dark	22.379	03.479	7.4	280
Chihuahuan (LTER sites)						
Jornada del Muerto Dune System	418 [bcd]	Light	32° 36.453	106° 48.300	14.7	257
Jornada del Muerto	448 [bc]	Lichen	32° 30.793	106° 44.623	15.0	253
Jornada del Muerto	478 [bcd]	Lichen	32° 30.770	106° 44.542	15.0	253
Jornada del Muerto Range 7 Rd.	484 [bcd]	Light	32° 32.015	106° 44.397	14.9	253
Jornada del Muerto Grassland	498 [bcd]	Lichen	32° 32.033	106° 43.725	14.9	253
Jornada del Muerto Sandy Soil	516 [bcd]	Light	32° 31.995	106° 42.728	14.9	252
	518 [bc]	Lichen	32° 32.002	106° 42.712	14.9	252
Sevilleta, Gypsum outcrops	520 [bd]	Lichen	34° 12.695	106° 45.567	12.6	258
Sevilleta, 5-Points Grassland	556 [bc]	Light	34° 19.968	106° 43.398	12.5	255
	560 [b]	Light	34° 20.067	106° 43.363	12.5	255
	562	Light	34° 20.095	106° 43.393	12.5	255

[a] Light: typically light colored, no lichen, smooth appearance and very cryptic; Dark: typically dark colored, no lichen, abundant surface cyanobacteria, typically

rugose or pedicelled;

Lichen: typically dark colored, with significant lichen cover, typically rugose or pedicelled;

Mossy: typically dark colored, with significant moss cover, typically rugose or pedicelled.

[bcd] Identification of sites where high-quality reaction was obtained in quantitative PCR for determining archaeal *amoA*[b] and bacterial *amoA*[c] gene densities in this study, and archaeal 16S rRNA abundance from Soule et al. 2009[d].

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6. Ammonia oxidation: a synthesis including arid lands

6.1. Overview

Research on the ecology of ammonia oxidizing microorganisms (AOM) has been lacking from arid lands compared to other ecosystems. In this chapter, I review some of the important environmental factors that structure ammonia oxidizing communities in soils globally. For each environmental variable, I include the main findings from research in arid land ecosystems. In addition, I synthesize the work from my dissertation in the context of previous knowledge about the ecology of AOM. I also carry out a meta-analysis at a global scale to characterize how potentially-important environmental parameters predict the abundance of ammonia oxidizing archaea (AOA) and bacteria (AOB).

6.2. Ammonia oxidation

Microorganisms that oxidize ammonia (NH_3) to nitrite (NO_2^-) play a significant role in controlling the amounts and type of nitrogenous compounds in terrestrial and aquatic habitats. In part to this reason, microbial communities have been researched in managed environments, where nitrogen (N) cycling is fast (Hayatsu et al. 2008; Galloway et al. 2013). Much effort has been placed into inhibiting the activities of AOM in economically relevant soils to minimize N release into the environment coming from agricultural fields.

Consequently, our knowledge about microorganisms carrying out ammonia oxidation (AO) in terrestrial systems comes from farmed lands, typically with acidic soils and heavy irrigation or high inputs of precipitation (He et al. 2012). However, other ecosystem types have also revealed novel aspects of the N cycle. For example, ammonium oxidation as an anaerobic process was discovered in wastewater treatment reactors (Mulder et al. 1995). The first pure isolate of an ammonia oxidizing archaeon originated from coastal waters (Konneke et al. 2005). AO is known to occur at temperatures as high as 97°C from hot springs where AOA signatures have been detected (Reigstad et al. 2008). Here we review how recent studies on arid land systems also reveal novel characteristics of AOM that can help understand the global N cycle.

6.3. Arid lands

Over 35% of terrestrial surface is covered by arid land soils (hyper-arid, arid, semi-arid, and Mediterranean arid; Koohafkan and Stewart 2008; Pointing and Belnap 2012). About 70% of this area is used as pasture or for agriculture, indicating that 25% of global land is used for farming practices specifically in arid lands (Koohafkan and Stewart 2008; World Bank 2011). Arid lands are also home to some of the fastest growing cities, which alter the biogeochemistry of the surrounding regions (Kaye et al. 2006; Grimm et al. 2008a; Grimm et al. 2008b). For example, physiological response of soil communities is affected by changes in magnitude and composition of atmospheric pollutants that deposit on the soil surface (Kaye et al. 2011; Riddell et al. 2012). An important contribution to arid land

fertility and management is driven by biological soil crusts (BSCs) that are widely distributed in deserts globally (Johnson et al. 2005; Pointing and Belnap 2012; Elbert et al. 2012). Additionally, native and managed arid lands play a key role in the global N cycle (Chapter 4; Schlesinger et al. 1990; Austin et al. 2004; Hall et al. 2008), in part due to intense pulses of microbial activity after water inputs. The extent of arid lands, and their increasing importance in urban sites, makes them a growing component of the global economy (e.g. Net benefit of $45 billion in Australia, $1.5 billion from Mojave Desert in California; Kroeger and Manalo 2007; Rola-Rubzen and McGregor 2009; Maestre et al. 2012). Despite the relevance of arid lands, they have been understudied compared to other ecosystem types (Adams 2003; Martin et al. 2012).

6.4. Using arid lands to test microbial ecology theories relevant for ammonia oxidizers

Niche differentiation can be used as an underlying theme to test hypotheses about the ecology of AOM. Fundamental understanding of the interactions between organisms and their environment started from the study of plant ecophysiology but subsequently has benefitted microbial ecologists as well (Hutchinson 1959; Tiedje 1999; Fierer et al. 2007). This review covers some of the important environmental factors that determine the distribution, dominance, and function of AOM. AOM communities are structured spatially both at broad geographical scales (Fierer et al. 2009; Bates et al. 2011) and also at the micro-level within soil micro-sites (Aakra et al. 2000; Jiang et al. 2011). Distinct

AOM also respond in time to factors like seasonality in temperature and precipitation (Gleeson et al. 2008; Bouskill et al. 2011) as well as to short-term fluctuations that occur within seasons or to daily events (Placella and Firestone 2013).

Studying the ecology of AOM in arid lands may prove to be useful for understanding the N cycle. Compared to other ecosystems, arid lands represent the extreme end of the continuum in numerous environmental conditions, including high summer temperatures, high solar irradiation, high-pH soils, low productivity away from vegetation and biocrusts, occasional massive dust storms altering nutrient pools, and low precipitation. These extreme conditions may uncover novel aspects of microbial ecology that may otherwise be inconspicuous in more mesic settings (Wall and Virginia 1999; Fierer et al. 2012). In this review, we aim to expand our knowledge of the AOA and highlight the unique aspects of AO and AOM in respect to arid land soils.

6.5. Predictors of ammonia-oxidizing community parameters

Numerous reviews have described the ecology and genetics of AOB (Prosser 1989; De Boer and Kowalchuk 2001; Arp et al. 2007), more recently of the AOA (Erguder et al. 2009; Schleper and Nicol 2010; Stahl and de la Torre 2012; He et al. 2012; Hatzenpichler 2012; Zhalnina et al. 2012; Offre et al. 2013), and current synthesis about the AOM in general (Klotz and Stein 2011; Prosser and Nicol 2012; Fernandez-Guerra and Casamayor 2012). This literature is heavily biased against findings and synthesis from

arid land ecosystems (Figure 1). We present some of this traditional work below and compare it to findings from dryland soils. As we review literature on AOM in arid land environments, we highlight unique aspects that are relevant in expanding general understanding of AO processes and communities of AOM globally.

6.5.1. Temperature

Microbial communities are known to respond to changes in temperature through stimulation of activity at certain temperatures and through selective effects on particular populations (Stark and Firestone 1996; Garcia-Pichel et al. 2013). Temperature is an important factor structuring the AOA (Tourna et al. 2008) and AOB (Avrahami et al. 2003; Avrahami and Bohannan 2007; Fierer et al. 2009) communities as well. The AOA appear to have a wider habitat range in relation to temperature than the AOB, with extreme conditions likely favoring the archaea (Valentine 2007; Stahl and de la Torre 2012). From a limited number of pure and enrichment cultures, optimal temperature for AOA is in the range of 32-40°C from near neutral soils (Tourna et al. 2011; Kim et al. 2012) and at 25°C from an acidic soil (Lehtovirta-Morley et al. 2011). These values are generally higher than for the AOB (typically 20-30°C; Holt et al. 1994), suggesting that it would be expected to detect AOA dominance in soils exceeding 30°C (i.e. hot deserts).

Some AOA in fact may prefer warm temperatures. In agricultural soils from a warm, humid climate, abundance of AOA (and AOB) was higher in summer than winter months

(He et al. 2007). In Negev Desert soils, AOA were dominant in the hot summers but were outcompeted by AOB in the winter (Sher et al. 2012; Sher et al. 2013). This seasonal trend is supported at the regional scale in BSCs across the Western US, with the ratio of AOA/AOB being positively related to temperature (Chapter 5). In contrast to this result, AOA/AOB ratio decreased due to warming in acidic soils from colder climates (Szukics et al. 2010; Jung et al. 2011). One explanation for the diverse effects of temperature is that AOA and AOB have distinct physiology among and within the groups. Additionally, other environmental parameters may be co-factors in driving the distribution and relative abundance of AOM.

6.5.2. Moisture

Water availability can influence AO directly through effects on cell physiology, metabolic regulation, and substrate accessibility (Stark and Firestone 1995; Stark and Firestone 1996), which controls abundance of AOA and AOB (Avrahami and Bohannan 2007; Szukics et al. 2012). Microbial activities may shift from nitrification dominated in aerated soils to nitrifier-denitrification and denitrification-dominated once NO_2^- reaches toxic concentrations and oxygen diffusion is limited in saturated soils (Wrage et al. 2001; Avrahami and Bohannan 2009; Szukics et al. 2010). Moisture content is considered in studies that are typically from soils receiving high inputs of water, such as agricultural systems, and research focusing on the relative magnitude of N-cycling processes and gas emissions predominantly from anaerobic conditions in flooded/non-aerated soils (Ishii et

al. 2011; Akiyama et al. 2013). However, microorganisms in arid land systems often experience stresses associated with limitation rather than an excess of water.

Fluctuations in water availability and the frequency of wetting/drying events affects the dynamics of which ammonia oxidizers are active and any consequences on AO rates in soil (Fierer and Schimel 2002; Saetre and Stark 2005; Placella and Firestone 2013). For example, water availability was positively correlated with potential nitrification activity and affected AOB community structure in semi-arid Australian soils (Gleeson et al. 2008). In hyper-arid soils, bloom events after precipitation increased the diversity of AOB (Orlando et al. 2010). Moisture availability at larger temporal scales may also affect the ammonia oxidizers. In the Negev Desert, communities shifted between AOB-dominated in wet, winter soils to AOA-dominated in dry, summer soils (Sher et al. 2013). This pattern is consistent with the significant negative correlation between the AOA/AOB ratio and precipitation in BSCs across arid lands (Chapter 5). Metagenomic analyses reveal that microbial communities in environments from deserts and non-deserts have different proportions of functional genes associated with water stress response (Fierer et al. 2012), highlighting that the moisture regimes in arid lands are in part contributing to the unique and extreme conditions experienced by ammonia oxidizers.

6.5.3. Ammonia

The source and concentration of ammonia have been important factors in selecting for distinct niches within and among the AOA and AOB (Prosser and Nicol 2012). The substrate for the active site of the ammonia monooxygenase (AMO) enzyme in AOB is NH_3, and not ammonium (NH_4^+; Suzuki et al. 1974). This specificity is currently unknown for the AOA. Since a decrease in pH shifts the NH_3/NH_4^+ equilibrium favoring protonation ($pK_a = 9.25$ at 25°C), the NH_3 availability in acidic soils would be below the known thresholds for AOB (He et al. 2012). However, the widespread findings of AO occurrence in acidic soils were surprising (De Boer and Kowalchuk 2001). The question was answered with the discovery of AOA that have a higher affinity for NH_3 than AOB in culture (Martens-Habbena et al. 2009) – which would allow growth in low-pH/low-NH_3 conditions – and with the cultivation of an acidophilic AOA (Lehtovirta-Morley et al. 2011). Environmental data from studies of acidic and neutral pH soils have confirmed that the AOA outcompete AOB in 'oligotrophic' conditions (i.e., low NH_3 content) and that N-rich soils favor AOB (reviewed in Hatzenpichler 2012). In a literature review, He et al. concluded that the source of N may also create separate niches for the AOA and AOB (2012). We have categorized these and other studies, showing that the AOA respond positively only to organic sources of N (e.g. manure, urea) and AOB generally to inorganic sources (Table 1). However, this pattern is partially challenged by our recent results from arid lands where some AOA also favor N inputs from inorganic sources and may be as tolerant as AOB of high NH_3 concentrations (Chapters 2 and 3).

Microbial activity in arid lands is controlled by the high spatial and temporal variability in environmental conditions (i.e. fertility islands and pulses of precipitation; Noy-Meir 1973; Schlesinger et al. 1996; Cable and Huxman 2004). For example, in the Sonoran Desert soil away from vegetation and biocrust patches, AOA abundance was positively related to availability of N from an inorganic source (fertilization with NH_4NO_3 or $(NH_4)_2SO_4$)) in both long-term (Chapter 2; Figure 2) and short-term experiments (Chapter 3). However, long-term N fertilization did not significantly increase AOA abundance in soils under shrubs (Chapter 2). We also found that potential AO rates were higher in soils under N fixing plants compared to soil enriched with reduced inorganic N (unpublished data; Figure 3). Additionally, it is known that fertile soils near vegetation and biocrusts have higher nitrification rates than interplant spaces, with NO_3^- production rates that can equate to N inputs from N fixation (Johnson et al. 2005; Schade and Hobbie 2005; Strauss et al. 2012).

Together, results from arid land soils suggest that AOA have the ability to use NH_3 for energy generation from various environmental sources, including directly from reduced inorganic N originating from anthropogenic fertilization, from N fixers, and from organic N sources in fertile soils. The only pure AOA isolate from soil, *Nitrososphaera viennensis*, also grows on organic and inorganic N sources (Tourna et al. 2011). Some AOA may have the capacity to switch metabolism, depending on the amount and type of

N available, as soil conditions change (Tourna et al. 2011; He et al. 2012; Spang et al. 2012).

6.5.4. Soil organic matter

Some authors suggest that AOA may be mixotrophic by switching to heterotrophy for their carbon (C) demand (Jia and Conrad 2009; Tourna et al. 2011; Spang et al. 2012), but the extent and distribution of this metabolic plasticity is unclear. This idea is supported by studies in relatively productive soils where AOA abundance and AO rates are positively associated with availability of organics (Chen et al. 2008; Kelly et al. 2011). Similarly, AO rates are positively correlated with SOM across plant patch types and N treatments in arid lands (Figure 3). In addition, Chapter 2 shows that AO rates and *amoA* gene copy-specific AO rates were higher in soils under plants than away from plants. Abundances of particular groups within the AOA were also correlated with SOM in soil patches under plants. However, these patterns may simply be indicators of optimal conditions for growth due to more nutrients, water retention, and shade in soil near vegetation than in interplant spaces. The complete aspect of C metabolism in AOA remains unanswered (Offre et al. 2013).

6.5.5. pH

Soil pH has been one of the most discussed factors of niche differentiation among and within the AOA and AOB (reviewed in Hatzenpichler 2012; He et al. 2012; Zhalnina et

al. 2012). The consensus is that the relative importance of AOA to AOB increases with decreasing pH (Yao et al. 2011), which can be explained by particular AOA ecotypes being enriched in acidic conditions (Gubry-Rangin et al. 2011). A decrease in pH is expected to lower NH_3 availability, creating an environmental pressure for most microorganisms, including ammonia oxidizers, in acidic soils (Frijlink et al. 1992; Arp et al. 2002). In arid lands, a one-time N pulse was followed by an expected decrease of NH_4^+ concentration, pH, and AO rates (Chapter 3). However, these soil changes were surprisingly associated with a decrease in AOA/AOB ratio. In a long-term experiment, again soil pH was unexpectedly negatively related to AO rates and abundance of ammonia-oxidizers (Chapter 2). In soil biological crusts, cyanobacterial activities drive pH changes that in some biocrusts shift >1 pH unit, coinciding with depths where ammonia oxidizers are present (Garcia-Pichel and Belnap 1996; Johnson et al. 2005; Johnson et al. 2007). Even though the archaea are better suited than bacteria to withstand direct effects of pH fluctuations (e.g. cell homeostasis; Valentine 2007), increasing pH in alkaline desert soils (pH 7-10) will favor deprotonation and may completely alleviate NH_3-limitation for ammonia oxidizers. These results suggest that pH is relevant in controlling AO dynamics, but distinctly in alkaline soils compared to acidic soils.

6.6. Synthesis

6.6.1. Meta-analysis

6.6.1.1. Background

Niche differentiation has been described among the AOA and AOB, as well as within these groups, due to factors such as pH (Gubry-Rangin et al. 2011; Hu et al. 2013), NO_2^- concentration (Auguet and Casamayor 2013), and habitat types (Cao et al. 2013). Data from arid lands also show that different phylogenetic groups of AOA have distinct contributions to AO and varied responses to changes in the soil environment (Figure 4; Supplementary Chapter, Figure 1). Research has been focused on characterizing the relationships between ammonia oxidizing communities and ecosystem properties spanning from the micro scale up to broader levels of regional areas. However, comprehensive studies on AOM at continental and global scales have been few in number (Bates et al. 2011; Gubry-Rangin et al. 2011; Fierer et al. 2012; Fernandez-Guerra and Casamayor 2012; Cao et al. 2013). We gathered literature data to explore relationships between ecosystem characteristics (climate and soil properties) and AOM communities (total and relative abundance) at the global scale. Of course this meta-analysis can only be used to search for general trends and to create hypotheses that would need to be tested further.

6.6.1.2. Data collection

We used the following three sets of search terms: 1) amoa; 2) soil* or terrestrial; 3) aoa or thaumarch* or "ammoni* oxidi*ing arch*" or "arch* ammoni* oxidi*er*". The keyword search rules include use of * for wildcard characters and " " to capture words next to each other (http://images.webofknowledge.com/WOK46/help/WOS/ht_search_rules.html). The searches were carried out in the *topic* search box in Web of Science (Web of Knowledge; Thomson Reuters) and within the *full text* in ScienceDirect (Elsevier), producing ~500 article hits. The search results were manually checked to ensure the article is on topic, excluding unidisciplinary studies that are unlikely to report environmental data, before looking further into each article.

We collected data for soil pH (in H_2O), concentrations of NH_4^+, NO_2^-, and NO_3^- (standardized to µg N per g soil), *amoA* abundance of AOA and AOB (gene copy number per g soil), ratio of AOA:AOB, and depth of soil collection. Measures of C were recorded, but produced limited sample size since studies used diverse metrics (i.e. SOM, total organic C, total C, dissolved organic C, water extractable organic C). Total organic C was multiplied by the conversion factor of 1.72 to obtain estimates of SOM (Nelson et al. 1996). Analyses were carried out on combined SOM and estimated SOM data. Functional data (i.e. AO rates) were recorded, but produced limited sample size. Analyses were restricted to potential nitrification rates measured from the shaken slurry method

(Hart et al. 1994). Mean annual temperature (MAT) and precipitation (MAP) were recorded if reported in the study, otherwise data were collected for the nearest city, geographic coordinate, or weather station available from National Oceanic and Atmospheric Administration (NOAA; www.weather.gov) and www.worldweatheronline.com. If data was not presented in a table or within the text, we estimated average values from figures. Each datum is the mean of analytical qPCR replicates and biological replicates from a particular sample type in each study. Data were log-transformed, if necessary, to fit data assumptions of statistical tests. Segmented-regression analysis was conducted on the full data set using SegReg software (http://www.waterlog.info/segreg.htm). All other statistical analyses (single linear regression, multiple linear regression, and Pearson correlations) were conducted in SPSS (v. 20).

6.6.1.3. Data considerations

The meta-data search produced 223 rows of data from 35 studies. To enable multiple regression analysis, 171 of those data points include soil pH, NH_4^+ concentration, and MAT as independent variables, and *amoA* gene abundance (AOA, AOB, or AOA/AOB) as the dependent variable. As a positive control to ensure validity of data interpretations and quality of data collection methods, a significant relationship was observed between MAP and soil pH (Figure 5; $P = 0.001$, $R^2 = 0.344$), as would be predicted at the global scale for these well-studied variables (Brady and Weil 2001). Therefore, we expect that

any relationship found between environmental and *amoA*-based variables at the global scale will also have ecological relevance.

We followed protocol to minimize bias if possible (Thompson and Higgins 2002; Walker et al. 2008). Interpretations from this work must be conservative due to several assumptions: 1) Depth of soil collection used by each study is chosen where microbial activity is the highest for that particular ecosystem. We checked to ensure that soil depth was not a good predictor of any other measured variables (all R^2 values ≤ 0.03). 2) To obtain the maximum power in the analysis, we treat each sample (out of the 223 total data rows) independently regardless of the number of data points presented by each study (i.e. each sample from a multi-sample study weighed the same as a one-sample study). Inclusion of studies with different sample size may be advantageous by increasing the generalizability of the conclusions (Berlin and Colditz 1999). 3) Efficiency was the same across studies for each method (e.g. DNA extraction, *amoA* PCR amplification, primer bias targeting particular groups, NH_4^+ extraction). Studies included in the meta-analysis were checked to ensure that their methods are previously published, to minimize potential methods bias, although variability in methods may still occur. Considering that many of these assumptions are quite strong (e.g. it is unlikely that methods were carried out identically by different researchers across studies), it would be fascinating if any global patterns are found at all.

6.6.1.4. Observations and patterns

Segmented-regression revealed that AOM community parameters were significantly predicted by soil pH across the entire data set. For the population size of AOA (Figure 6) and AOB (Figure 7), each analysis found a breakpoint for where the direction of the relationship between pH and abundance changed. The pH value of the breakpoint (including range of 90% CI) was higher for the AOA than the AOB. For the AOB, multiple linear regression (using pH, NH_4^+, and MAT as independent variables) showed that soil pH was the only significant factor predicting abundance in the model, which was positively in soils pH < 6.11 and negatively in soils pH > 6.11 (Table 2). The AOA were also predicted significantly only by pH, but weakly in soils pH < 8.41 and based on a limited number of samples in soils pH > 8.41. The relative abundance of AOA to AOB was also dependent on pH, with a breakpoint at pH 6.22 (Figure 8). The AOA/AOB ratio was higher at pH > 6.22 than at pH < 6.22. This breakpoint may have a physiological or ecological role since multiple linear regression revealed only one significant model, in which NH_4^+ was the sole factor negatively related to the ratio of AOA/AOB in soils pH > 6.22 (but not in pH < 6.22).

6.6.1.5. Data interpretations

The meta-analysis revealed interesting patterns about the relationship between ammonia oxidizers and soil pH at the global scale. Consistent with our results, previous studies at

the local scale have shown a significant positive correlation between pH in acidic-neutral soils and abundance of both the AOB (He et al. 2007; Yao et al. 2011) and AOA (He et al. 2007; Wessen et al. 2010). This pattern has also been confirmed at a broader landscape scale (Bru et al. 2011; Hu et al. 2013). In addition to seeing this trend at the global scale for soils with relatively low pH, we discovered that the abundance of both the AOA and AOB begins to diminish after soil pH reaches a certain level (i.e. breakpoint). These relationships were not detected simply by linear correlations across the entire data set (Table 3). Future studies and management of soils will benefit from considering that ammonia oxidizers may respond differently (+ or -) to environmental changes depending if conditions are relatively acidic or alkaline, and that the tipping point of a distinct response to pH change is higher for the AOA compared to the AOB. Fluctuations in environmental conditions may change the cytoplasmic pH between 6-8 (in AOB; Kumar and Nicholas 1983), which is normally around 6.8-7.2 (Schmidt et al. 2004). However, the archaea are able to withstand pH-dependent changes better than the bacteria (Valentine 2007; Slonczewski et al. 2009).

Low activity and abundance of AOB in acidic soils has been typically explained by low NH_3 availability, which is lowered with decreasing pH (Frijlink et al. 1992). Consequently, higher affinity for NH_3 of AOA compared to AOB has been shown to increase the ratio of AOA/AOB in low pH soils (Yao et al. 2011). However, we did not detect any significant relationships between NH_4^+ concentration and total abundance of AOA and AOB. Additionally, the capacity of AOA to dominate over AOB in NH_3-

depleted, low-pH soils was not observed at the global scale here. It is not uncommon for a particular factor to have varying levels of influence on the ammonia oxidizing community that depends on the spatial scale (Bru et al. 2011; Martiny et al. 2011). Additionally, unmeasured confounding factors across studies and environments – which often limit meta-analyses – may mask relationships (Thompson and Higgins 2002). Another explanation is that some factors are more important than pH-driven NH_3 availability in structuring ammonia oxidizing communities at a coarse level of biogeographical resolution. This idea is plausible since ammonia oxidizers depend on regulation of genes for many functions, such as for C fixation and nitrite reduction (Wei et al. 2004; Cho et al. 2006), and that many cellular processes are interlinked and pH dependent (Whittaker et al. 2000; Weidinger et al. 2007).

Ammonia oxidizing communities are known to have particular phylogenetic groups that dominate depending on pH of the environment (Nicol et al. 2008; Gubry-Rangin et al. 2011; Hu et al. 2013; Tripathi et al. 2013). This niche specialization may be occurring at the global scale as we saw a higher ratio of AOA/AOB in soils pH > 6.22 compared to pH < 6.22. There was a clear absence of dominance by AOB (AOA/AOB ratio < 1) at only around neutral pH (pH 6-8). These patterns may be indicators of where AOA outcompete AOB, leading to a higher contribution to AO rates by AOA than their bacterial counterparts (Prosser and Nicol 2012).

6.7. Review chapter conclusion

Considering the extent of arid lands and their importance ecologically and economically, much can be gained from research in these systems. This work highlights that ammonia-oxidizers in deserts are able to fill many distinct niches that are created in heterogeneous spatial and temporal changes, in some cases shifting between dominance of AOA or AOB. The unique positive response of AOA to reduced inorganic N addition in arid soils reveals that changes in soil due anthropogenic sources (i.e. increased atmospheric deposition of N) may stimulate nitrification that otherwise would not occur in other ecosystem types. However, environmental changes that shift soil pH closer to neutral conditions are more than likely to increase abundance of ammonia oxidizers at broad spatial scales, with likely consequences for the fate of N in the ecosystem.

6.8. Tables/Figures

Table 1. Literature review of studies measuring response of AOA after N inputs from various sources.

	Treatment type			
	Inorganic N (no/low mineralization)	Inorganic N (high mineralization)	Organic N	No N addition (mineralization effect)
+AOA response	Tourna et al. 2011*	Treusch et al. 2005;** Chen et al. 2008;** Xia et al. 2011;** Verhamme et al. 2011**	Schauss et al. 2009; Lu et al. 2012; Levicnik-Hofferle et al. 2012; Lu and Jia 2013	Offre et al. 2009; Jia and Conrad 2009; Stopnisek et al. 2010; Verhamme et al. 2011
- or no AOA response		Jia and Conrad 2009; Stopnisek et al. 2010; Levicnik-Hofferle et al. 2012; Zhang et al. 2012	Shen et al. 2008; Di et al. 2009; Di et al. 2010; Wertz et al. 2012	Many studies; various confounding factors

* Laboratory experiment with cultures. ** These are the only studies that have reported a positive response by AOA after inorganic N addition. However, these studies are from environments that likely have high rates of N mineralization and/or high inputs of organics into the system. All other categories without * or ** list only several of many examples. AOA response indicates either increase in *amoA* or 16s (specific for ammonia oxidizers) gene abundance in comparison to soil mass, total DNA, or bacterial ammonia oxidizers.

Table 2. Results from multiple linear regression analysis.

Dependent variable[a]	< Breakpoint[b]					> Breakpoint[b]				
	Factor[c]	Direction[d]	P	R^2	Data points	Factor[c]	Direction[d]	P	R^2	Data points
AOB abundance	pH	+	<0.001	0.295	85	pH	-	<0.001	0.082	77
AOA abundance	pH	+	<0.001	0.102	162	pH	-	<0.001	0.855	10[e]
AOA/AOB	none				96	NH_4^+	-	<0.001	0.232	65

[a] One dependeent variables is included in each multiple linear regression analysis. [b] Multiple linear regression analysis was repeated for each set of data < and > breakpoint value. The breakpoint was determined from segmented-regression, with pH (but not NH_4^+ and temperature) as the only variable with significant breakpoints across the entire data set (pH breakpoint values- AOB, 6.11; AOA, 8.41; AOA/AOB, 6.22). [c] Only the significant factor is shown from a model that includes pH, NH_4^+, and temperature as independent variables. [d] Positive or negative direction of relationship between dependent variable and significant factor. e To evaluate the relationship between pH and AOA abundance further with a larger sample size, we conducted single linear regression (using pH only) for all data at pH > 8.21 (based on 90% confidence interval). This analysis returned P < 0.001 and R^2 = 0.398 from 33 data points, confirming the trend found in the multiple linear regression.

Table 3. Bivariate Pearson correlation analysis between ammonia oxidizing community parameters, soil properties and processes, and climate at the global scale across all studies.

	Statistics[a]	Ratio of AOA/AOB	AOA amoA genes/g soil	AOB amoA genes/g soil	pH	Mean annual temperature	NH4+-N ug/g soil	Mean annual precipitation	NO3--N ug/g soil	Soil organic matter
AOA amoA genes/g soil	R	.454**								
	P	.000								
	N	212								
AOB amoA genes/g soil	R	-.389**	.631**							
	P	.000	.000							
	N	212	214							
pH	R	.090	.096	.092						
	P	.204	.164	.193						
	N	200	211	202						
Mean annual temperature	R	.188**	.185**	-.016	-.269**					
	P	.006	.006	.818	.000					
	N	212	223	214	211					
NH4+-N ug/g soil	R	.051	-.124	-.145	-.051	.075				
	P	.507	.095	.057	.506	.313				
	N	173	183	174	171	183				
Mean annual precipitation	R	.018	-.110	-.171*	-.586**	.271**	.179*			
	P	.803	.107	.014	.000	.000	.015			
	N	204	214	205	202	214	183			
NO3--N ug/g soil	R	-.231**	-.178*	.005	-.308**	.096	.209**	.206**		
	P	.003	.020	.945	.000	.214	.007	.007		
	N	159	169	160	157	169	164	169		
Soil organic matter (%)	R	.201	.419**	.335**	.011	.326**	.216	-.150	-.164	
	P	.093	.000	.004	.924	.005	.084	.215	.194	
	N	71	73	73	73	73	65	70	64	
Potential ammonia oxidation rate (ug/g/h)	R	.132	.471**	.417**	.243	.009	-.297*	.037	-.122	-.073
	P	.338	.000	.002	.074	.947	.039	.790	.430	.681
	N	55	55	55	55	55	49	55	44	34

[a] R, Pearson correlation; P, significance value; N, sample size. Correlation is significant at 0.01** and 0.05*.
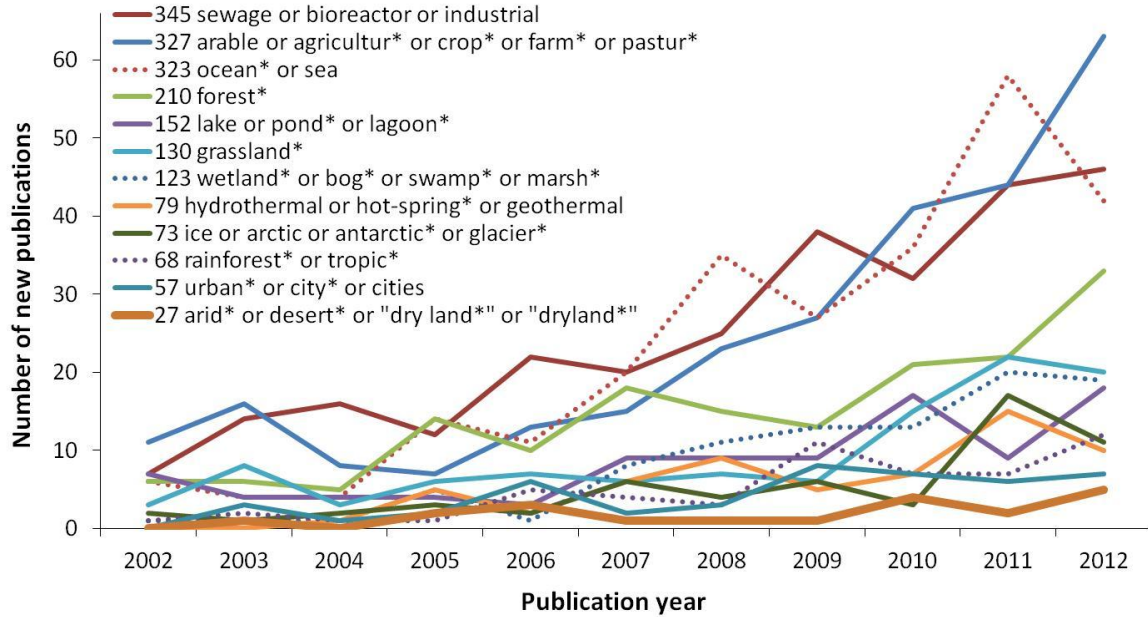
Figure 1. Number of publication search results per year in Web of Science using keywords related to ecosystem type. Each search was also paired with the following one set of terms: *amoa or aoa or aob or "ammonia oxidi*"*. Number in legend indicates total number of search results in decreasing order (top to bottom). The text after the number indicates the exact string of characters used for the search. Older data were excluded from the bar chart. The top set of terms (345 results) had an additional 575 results (that were excluded for simplicity) if the following terms would have been included: sludge or wastewater. A study may have overlap between multiple ecosystem types. * is used as a wildcard. Quotes are used to keep words together in a search.

Figure 2. Quantification of *amoA* gene copy numbers for AOB and AOA from Sonoran Desert soil in N addition and control plots. Error bars are standard errors of independent field triplicates. After 7 years of N addition, only AOB were positively affected under the canopy of shrubs, as expected in relatively fertile soils with moderate N mineralization (He et al. 2012). However, in soils away from plants, where N mineralization is typically low, both AOA and AOB responded positively to inorganic N addition.
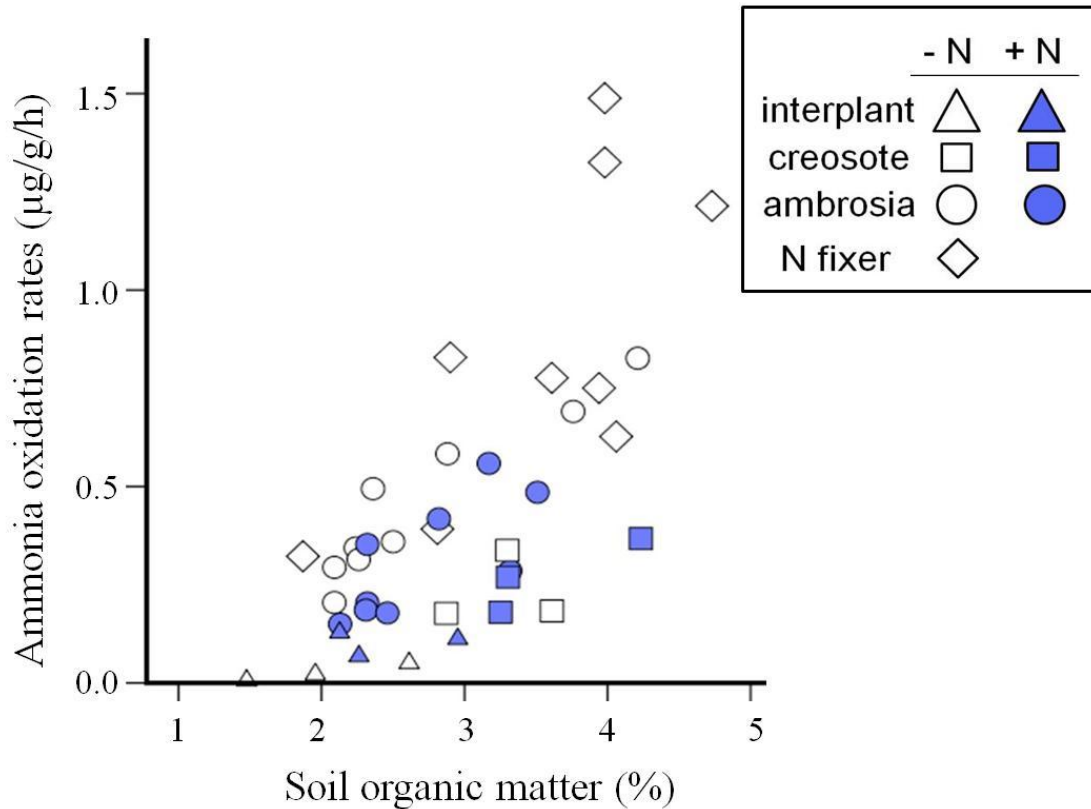
Figure 3. Soil organic matter vs. with ammonia oxidation rates in various plant patch types and nitrogen treatment plots in Sonoran Desert soils. - N = unfertilized control; + N = fertilized with NH_4NO_3 at 60 kg N ha^{-1} yr^{-1} for 7 years. We tested the effects of N source from different plant patch types and N treatment on AO rates in several soils across the Sonoran Desert. We used soils from 1) interplant spaces (between plants), 2) under creosote bush, 3) under ambrosia bush, and 4) under legume plants (N fixer). All patches, except those with the N-fixing plants, were also sampled in nearby plots fertilized with NH_4NO_3 (60 kg/ha/y for 7 years). Multiple linear regression analysis revealed that AO rates across all patches and treatments were most strongly predicted by soil organic matter (SOM) content (positively correlated; shown in figure), followed by pH (negatively) and NH_4^+ concentration (positively), highlighting the importance of multiple co-occurring factors that potentially affect AO processes. Ramirez et al. unpublished.
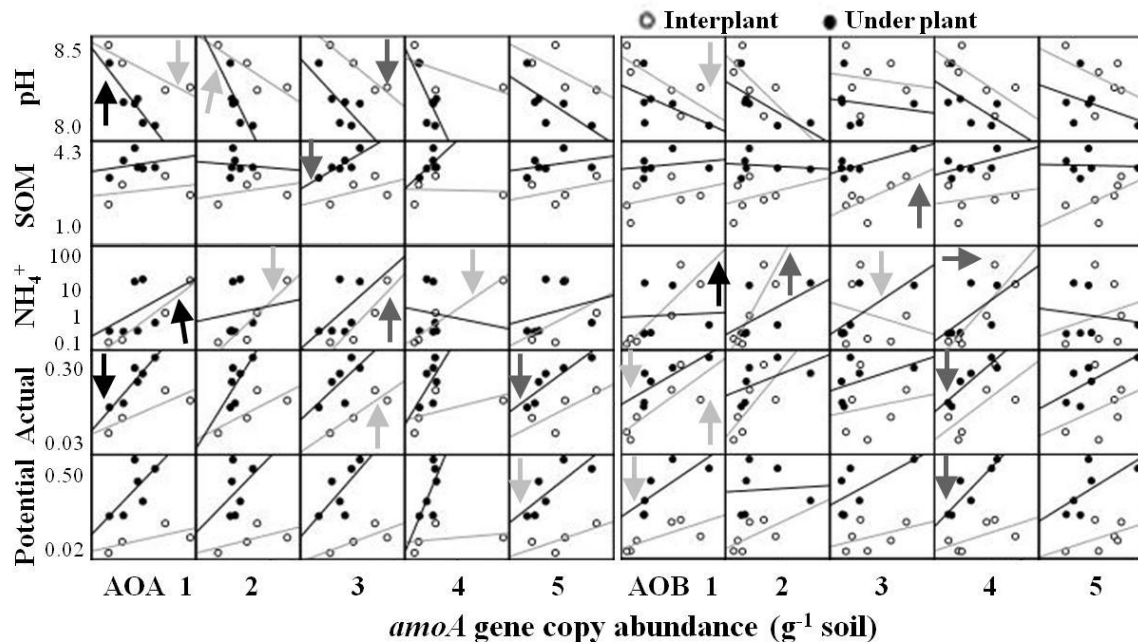
Figure 4. Correlation of *amoA* gene copies for individual OTUs of dominant AOA and AOB versus maximum ammonia oxidation rates and soil properties. Trend lines are plotted across control and N addition plots, separately for plant patch types (Interplant and Under plant). Arrows point at regressions with P-values of 0.01 (black), 0.05 (dark gray), and 0.10 (light gray). All AOA are *Nitrososphaera* subcluster 1.1-related. Top four AOB are *Nitrosomonas*-related. AOB #5 is related to *Nitrosospira* from US Southwest biocrust. These ten OTUs (five most abundant OTUs from each domain) comprise 85% of the *amoA*-containing community. Figure from Chapter 2.

225
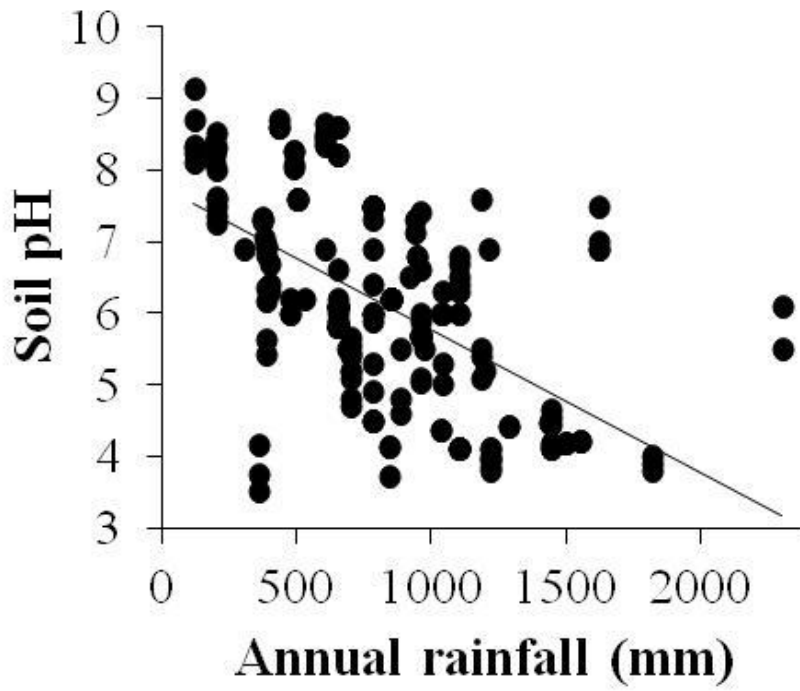
Figure 5. Negative correlation between mean annual precipitation and pH data gathered from global meta-analysis. P = 0.001, R^2 = 0.344.
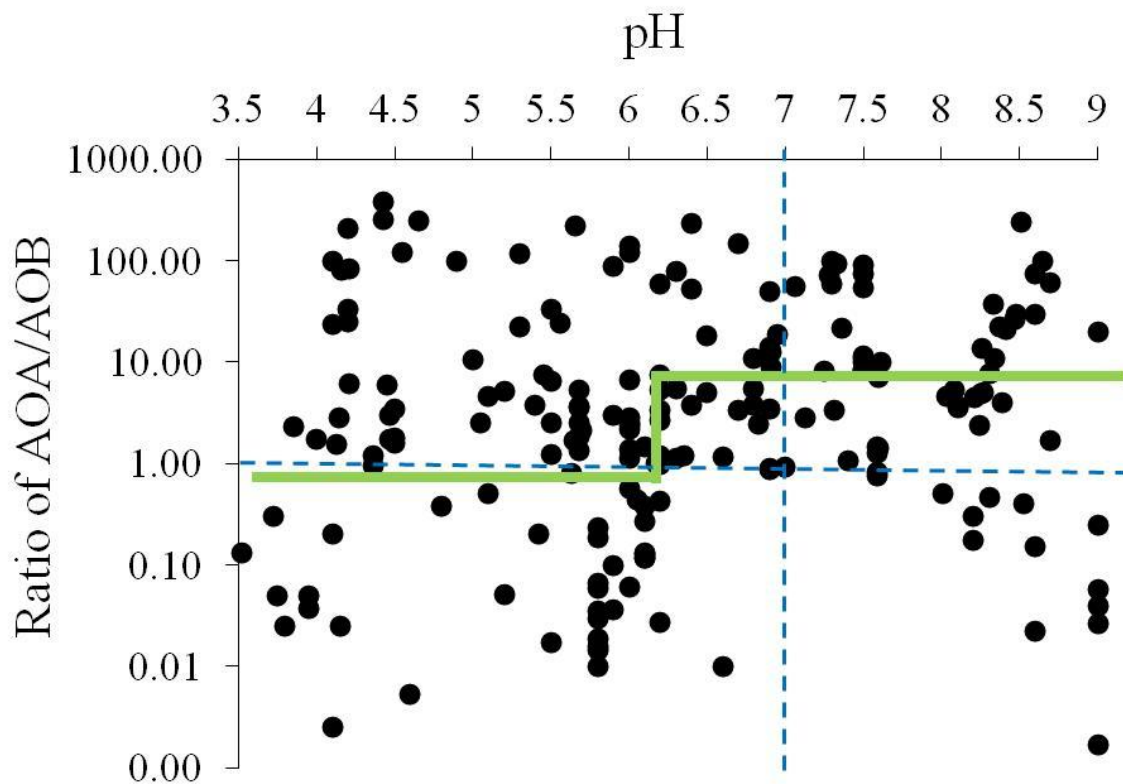
Figure 6. Soil pH vs. AOA/AOB ratio across all data points in the analysis. Dotted line at y = 1 indicates division between archaeal (above line) vs. bacterial (below line) *amoA* dominance. Dotted line at x = 1 indicates neutral pH. Green lines indicate segmented-regression results with optimum breakpoint (6.22). NH_4^+ is the only significant variable in multiple linear regression, using pH, NH_4^+, and temperature as independent variables: pH > 6.22, P = 0.001, R^2 = 0.232 (negative relationship). Only pH is shown as a factor in the figure.
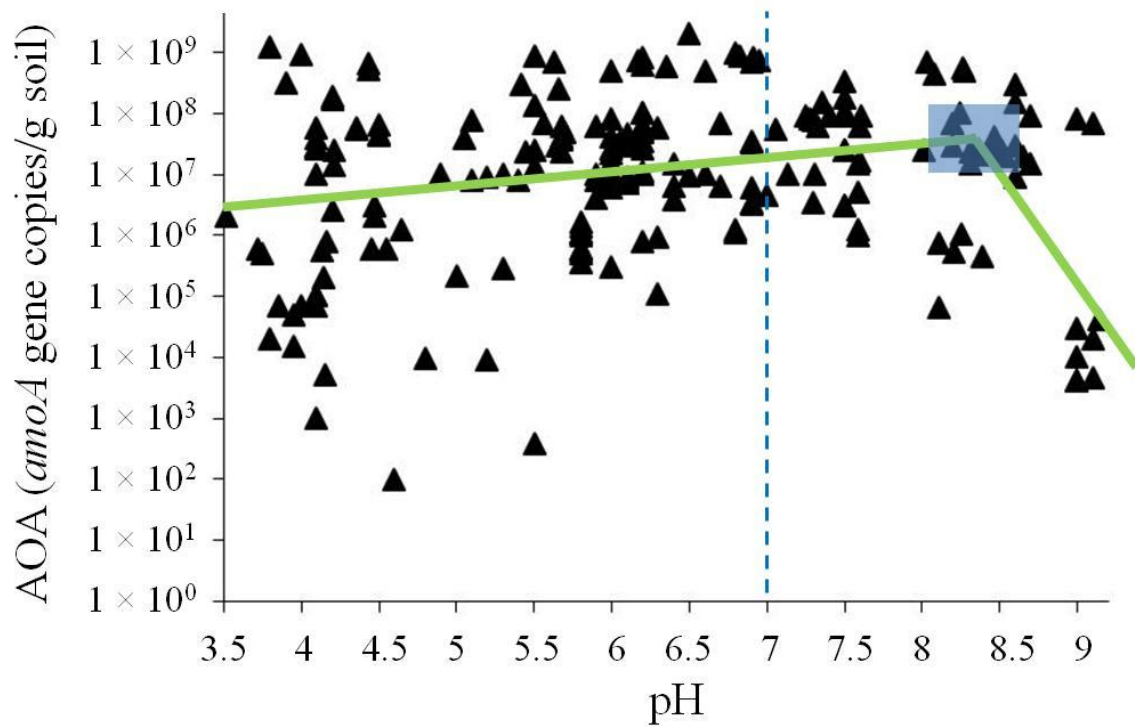
Figure 7. Soil pH vs. AOA abundance across all data points in the analysis. Dotted line at x = 1 indicates neutral pH. Green lines indicate segmented-regression results with optimum breakpoint (8.41). Blue box indicates 90% confidence block of breakpoint. Multiple regression from data in soils pH < 8.41 (P = 0.001, R^2 = 0.102) and in soils pH > 8.41 (P = 0.001, R^2= 0.855, from only 10 points; reanalyzed with 22 points from 5 studies, R^2 = 0.517, pH only in model), using pH, NH_4^+, and temperature as independent variables. Only pH variable is significant. Only pH is shown as a factor in the figure.
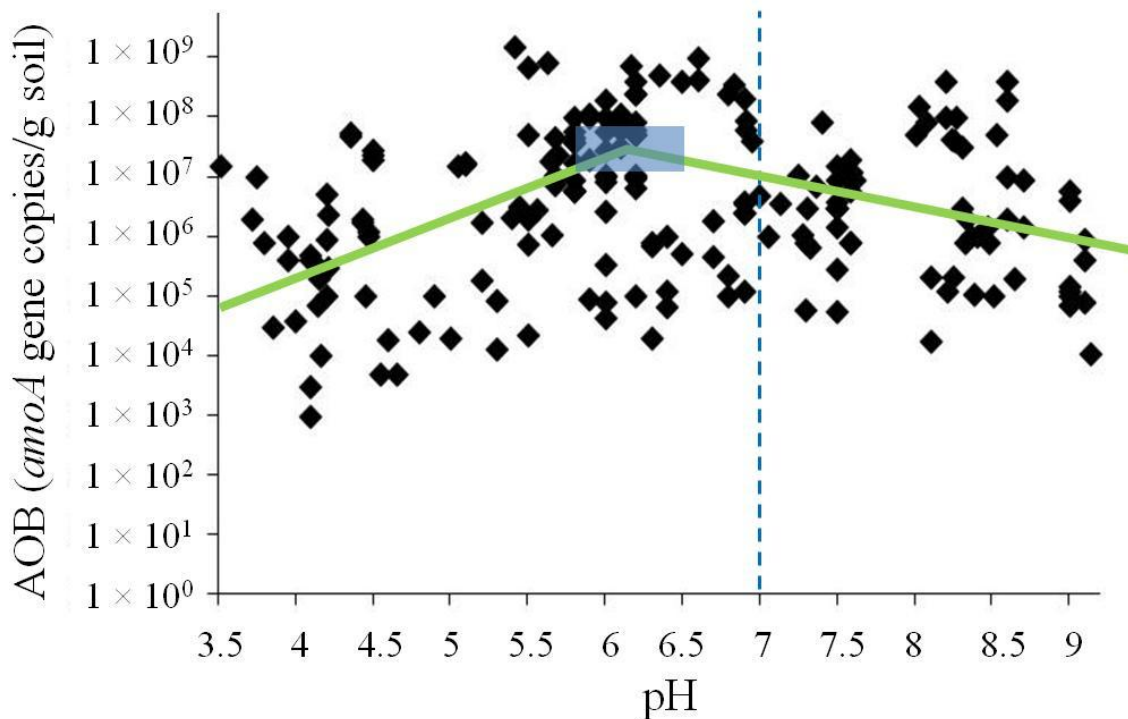
Figure 8. Soil pH vs. AOB abundance across all data points in the analysis. Dotted line at x = 1 indicates neutral pH. Green lines indicate segmented-regression results with optimum breakpoint (6.11). Blue box indicates 90% confidence block of breakpoint. Multiple regression from data in soils pH < 6.11 (P = 0.001, R^2 = 0.295) and in soils pH > 6.11 (P = 0.012, R^2 = 0.082), using pH, NH_4^+, and temperature as independent variables. Only pH variable is significant in both pH < 7 and pH > 7 models. Only pH is shown as a factor in the figure.

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7. Concluding remarks

7.1. Main point(s) from each Chapter

Chapter 2- We discovered that the responses of AOM to changes in soil conditions is unique in desert soils compared to relatively more mesic ecosystems. AOA responded positively to inorganic N fertilization in soils away from vegetation. N availability, among other factors like pH and SOM, is controlling the magnitude of AO primarily through changes in AOM population sizes, and to a lesser extent through compositional or functional changes.

Chapter 3- The relative abundance of AOA to AOB declined as conditions changed from the beginning of a N pulse (higher pH, higher NH_4^+) to the later stages (lower pH, lower NH_4^+). In other ecosystem types, literature shows a contrasting pattern in which soil acidification leads to AOA outcompeting AOB. The work from our short-term experiment supports the conclusion from the long-term experiment (Chapter 2) that there is a positive relationship between inorganic N availability and the AOA.

Chapter 4- In support of recent evidence, we show that fungi are dominant contributors to N_2O production. We found this pattern in a wider array of land-use types than previously recognized, including arid and semi-arid soils. N_2O production shifted from fungal-dominated in arid lands to bacterial/archaeal-dominated in lawn soils, indicating a role of niche differentiation due to factors associated with ecosystem type (e.g. aridity,

urbanization). However, potential nitrification is mainly controlled by autotrophs (i.e. AOB and AOA) rather than by heterotrophs (i.e. fungi) in the arid lands that we tested.

Chapter 5- Temperature was the strongest factor in structuring AOM communities in biocrusts of the western US. The relative abundance of AOA:AOB was higher in warmer deserts, while the AOB dominated in the colder deserts. The ratio of AOA:AOB were lower than any values previously reported from soil environments, highlighting the unique conditions created for microbial communities in arid land biocrusts.

Chapter 6- The review highlights that research in arid land systems has made a significant contribution to understanding the phylogeny, physiology, and ecology of AOM. However, the number of studies in these environments is limited. Our meta-analysis reveals that the abundance of AOM at a global scale may be dependent on soil pH more so than NH_4^+ availability or mean annual temperature.

7.2. Dissertation contribution

The contributions I made through my dissertation can be summarized in two major categories:

1) Linking genes and ecosystems through a multidisciplinary microbial ecology approach to understand ammonia oxidation processes. Using molecular and ecological methods, we have filled in many gaps of knowledge about the distribution of

ammonia oxidizing microorganisms, how environmental factors affect these communities, and how these dynamics translate to process changes at the ecosystem scale (i.e. rates of ammonia oxidation). Information gained here expands our understanding about niche differentiation within and among the AOA and AOB, which can be tested further in other ecosystems that experience extreme and fluctuating conditions.

2) Importance of research in arid lands. The unique findings here create opportunities for continuing to ask questions about the ecology of ammonia oxidizing microorganisms in arid land ecosystems.

One of the current major enigmas is to figure out the occurrence and mechanism of "mixotrophic" AOA that exhibit a heterotrophic metabolism in some conditions (Hatzenpichler 2012; Stahl and de la Torre 2012). Understanding of the N cycle will change if AOM, thought to be autotrophic, are affected by C substrates through anthropogenic enrichment and environmental management. This puzzle becomes fascinating to investigate when considering the system of biological soil crusts. The microbial communities in these soils are in many ways controlled by cyanobacteria that may have associations with ammonia oxidizing communities. For instance, separate studies have shown a clear biogeography where latitudinal factors (mainly mean annual temperature) select for AOA over AOB in warmer deserts (Chapter 5), coinciding with particular cyanobacterial population replacements in these conditions (Garcia-Pichel et al. 2013). The phototrophs affect the availability of resources that are relevant for ammonia

oxidizers, such as NH_4^+ (from N fixation) and oxygen at different depths of the biocrust (Johnson et al. 2005). It is unclear if there is a separate niche for ammonia oxidizers, such as mixotrophic AOA, that is associated with C inputs and unique conditions in biocrusts of arid lands.

Since literature shows that inorganic N addition favors AOB over AOA in relatively mesic systems (He et al. 2012), the response of ammonia oxidation after anthropogenic N enrichment is believed to depend on the AOB. However, we now know that consideration of the AOA is crucial to fully understand the consequences of N addition on N cycling. In conditions where organic N inputs are low, AOA populations may be selected for that take advantage of additional sources of N (e.g. inorganic N deposition in deserts). These examples highlight that the study field of ammonia oxidation can benefit from carrying out research in arid lands.

7.3. References

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APPENDIX A

PUBLICATION CITATIONS

Publications used in this dissertation

1. **Marusenko, Y.,** F. Garcia-Pichel, and S.J. Hall. Ammonia oxidizing archaea respond positively to inorganic N addition in desert soils. In review.

2. **Marusenko, Y.,** S.J. Hall., and F. Garcia-Pichel. Temporal dynamics of ammonia oxidizing communities after a nitrogen pulse in desert soil. In prep.

3. **Marusenko, Y.,** B. Ramirez, J. Shaw, S.J. Hall, and F. Garcia-Pichel. A synthesis of ammonia oxidizing microorganisms in arid land environments. In prep.

4. 2013 **Marusenko, Y.,** D. Huber, and S.J. Hall. Fungi mediate nitrous oxide production but not ammonia oxidation in aridland soils of the southwestern US. *Soil Biology and Biochemistry (3.65 impact factor)*. 63, 24-36.

5. 2013 **Marusenko, Y.,** S.T. Bates, I. Anderson, S.L. Johnson, T. Soule, and F. Garcia-Pichel. Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands. *Ecological Processes (new journal, no impact factor)*. 2:9. <u>* Special issue</u>

Other publications

6. R. Potrafka, **Y. Marusenko**, and F. Garcia-Pichel. Continental-scale distribution of bacterial communities in desert biological soil crusts. In prep. <u>* Special issue</u>

7. 2013 Romolini, M., R. Garvoille, S. Geiger, **Y. Marusenko**, and S. Record. The next generation of scientists: Examining the experiences of graduate students in network-level socio-ecological science. *Ecology & Society (2.83 impact factor)*. 18(3): 42.

8. 2013 Garcia-Pichel, F., V. Loza, **Y. Marusenko**, P. Mateo, and R. Potrafka. Temperature determines the continental-scale distribution of keystone species in topsoil microbial communities. *Science (31.2 impact factor)*. 340 (6140), 1574-1577. <u>* Cover issue</u>

9. 2013 **Marusenko, Y.,** J. Shipp, J.L.L. Morgan, J. Hutchings, G.A. Hamilton, M. Keebaugh, H.Hill, A. Dutta, X. Zhuo, N. Upadhya, E.Shock, H. Hartnett, P. Herckes, and A. Anbar. Bioavailability of nanoparticulate hematite to *Arabidopsis thaliana*. *Environmental Pollution (3.7 impact factor)*. 174, 150-6.

10. 2011 **Marusenko, Y**., P. Herckes, and S.J. Hall. Distribution of polycyclic aromatic hydrocarbons in soils of an arid urban ecosystem. *Water, Air, and Soil Pollution (1.6 impact factor)*. 219 (1-4), 473-487.

11. 2008 Sabo, J.L., K.E. McCluney, **Y. Marusenko**, A. Keller, and C.U. Soykan. Greenfall links groundwater to aboveground food webs in desert river floodplains. *Ecological Monographs (7.4 impact factor)*. 78 (4), 615-631.

APPENDIX B

SUPPLEMENTARY FIGURE
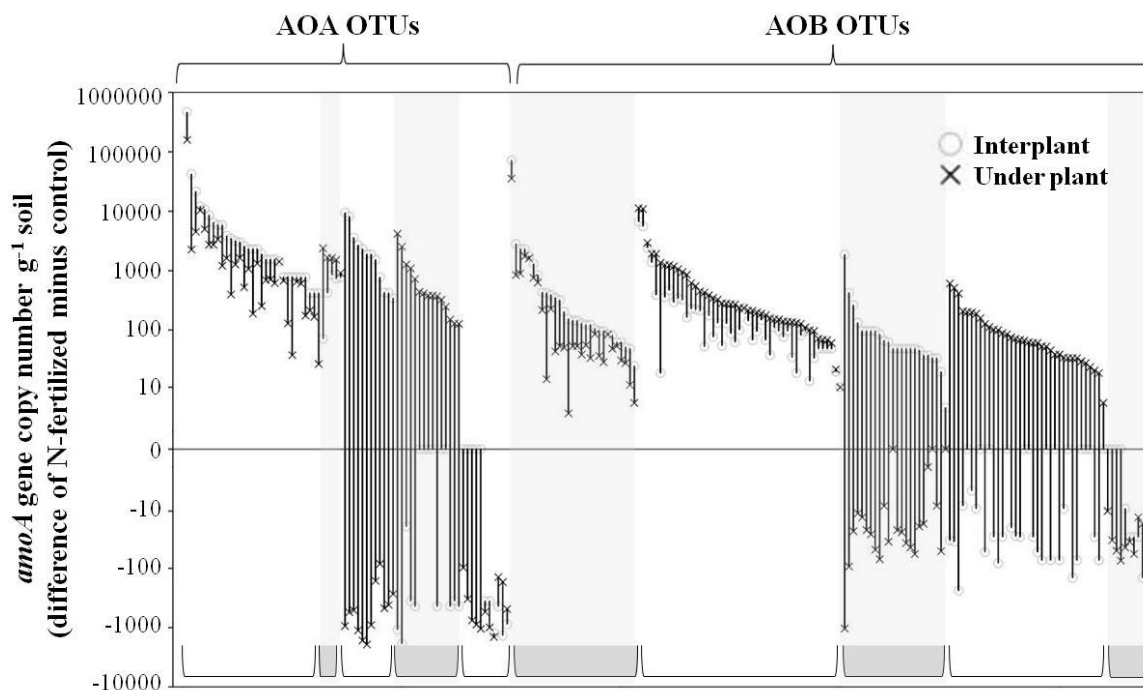
Supplementary Figure 1. Change in abundance of *amoA* gene copies for each OTU due to N-fertilization. Data from Chapter 2. The x-axis contains each unique OTU at 97% nucleotide similarity for the AOA and AOB. The y-axis contains the *amoA* abundance net change. Gene copy difference due to treatment for each unique OTU is plotted separately for interplant and under plant patches, connecting the same unique OTU across patch types with a vertical line. Bottom brackets and white/gray shading alternate for clarity to separate different groups of response types. The OTUs are grouped based on similar types of positive, mixed, or negative responses due to N-fertilization, and also grouped whether the abundance change is stronger in interplant or under plant patch. Horizontal line at zero indicates either absence of OTU or that the OTU *amoA* gene copy number was identical in the control and N-fertilized treatment. The value for each OTU is an average of independent field, pyrosequencing, and qPCR replicates.

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