Nitrogen Fertilization Creates New Niches for Ammonia-Oxidizing Microbial Communities in Soil

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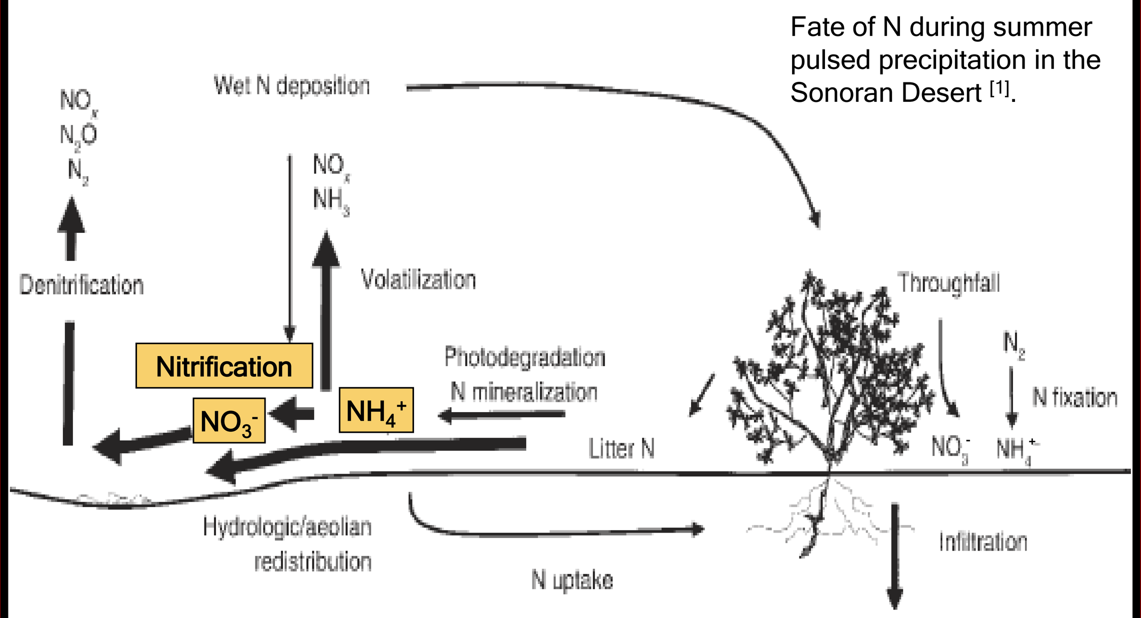
Introduction

• Soil archaea and bacteria oxidize ammonia to nitrite in a key step of nitrification.

• Various forms of nitrogen (N) are provided to soils from natural and anthropogenic sources.



• In general, N enrichment changes community composition of ammonia-oxidizers, increases population density, and elevates ammonia oxidation (AO) rates[2,3,4]. However, archaeal and bacterial groups (and subgroups) may respond differently to environmental changes. Any shifts or adaptations in enzymatic functions can lead to distinct ecosystem responses (nitrification rates).

RESEARCH QUESTION:

Does N fertilization affect ammonia oxidation through selective effects on particular microorganisms and their function at the physiological level?

Methods

• We measured AO rates and ammonia-oxidizing communities in N fertilized (NH_4NO_3; 60kg N ha^{-1} yr^{-1} since 2005) and unfertilized Sonoran Desert soils near Phoenix, AZ[1]. Soils were collected in common aridland patch types, away from plants and under the canopy of creosote bush shrubs.

• We used the nitrite-accumulation method (with sodium chlorate) to measure actual net AO rates using static incubations[5] and potential AO rates using shaken-slurries[2]. Rates were measured under a range of starting NH_4^+ concentrations for each method to evaluate the enzyme kinetics of ammonia-oxidizing communities in bulk soil[6].

• Ammonia-oxidizers in soil were quantified using real-time PCR and identified to the species level (97% nt) with clone libraries and pyrosequencing using *amoA* genes, a functional marker for AO[7].

Results

Nitrogen fertilization increases actual and potential rates of ammonia oxidation

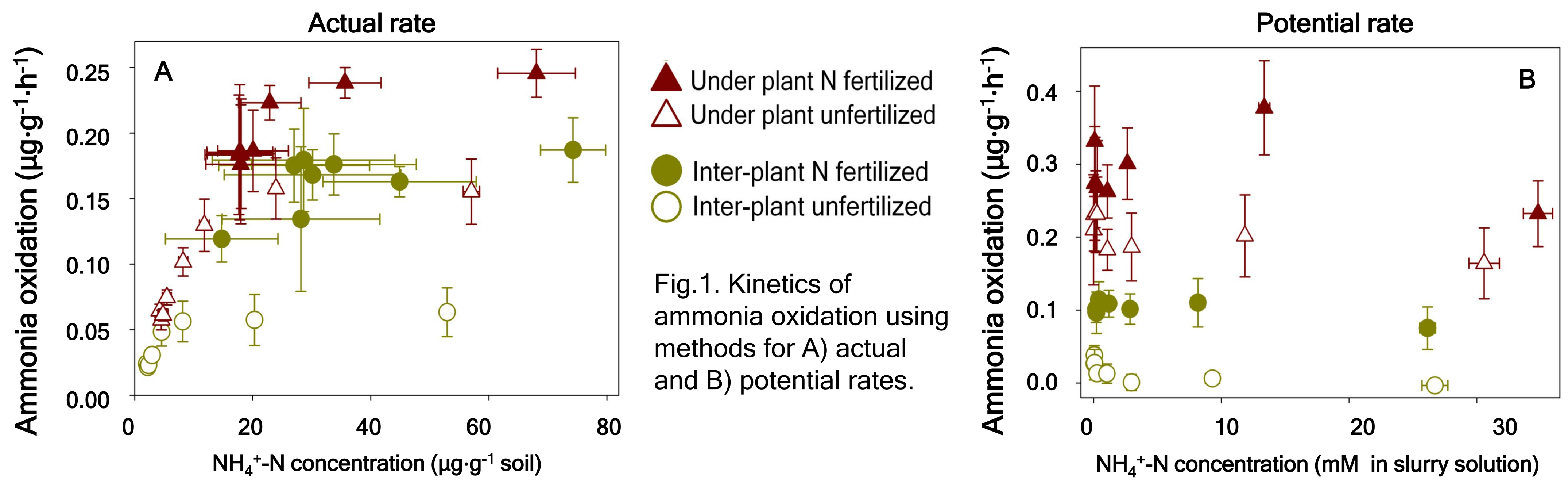


Fig.1. Kinetics of ammonia oxidation using methods for A) actual and B) potential rates.

• Long-term N fertilization increases rates of actual (Fig.1A) and potential (Fig.1B) AO in soils of both patch types, compared to the unfertilized control. In the actual rates for soil from unfertilized plots (conditions most similar to native desert soils), AO rates increased with supplemented NH_4^+ during the 48-h incubation.

Nitrogen fertilization increases total abundance and diversity of bacterial and archaeal ammonia oxidizers

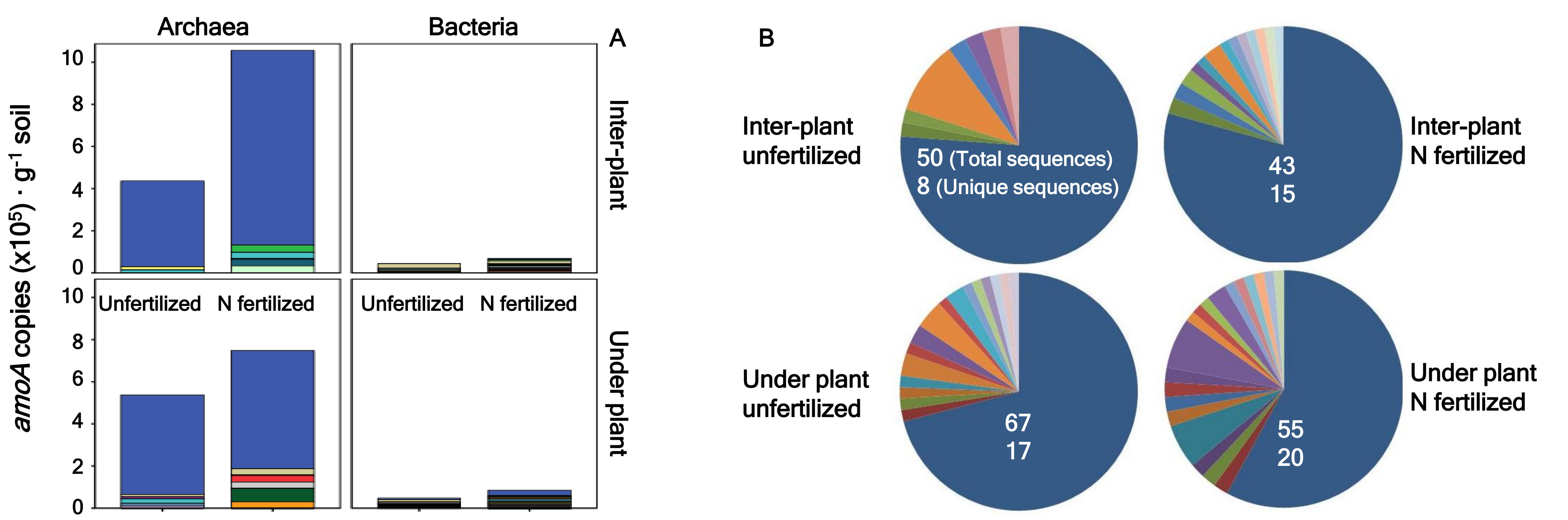


Fig. 2A. qPCR data for total abundance (*amoA* gene copy number·g^{-1}). Fig. 2B. Clone library for relative abundance (archaea and bacteria combined).

• N fertilization increases *amoA* abundance (Fig.2A) and community diversity (richness and evenness; Fig.2B). In contrast to many studies[e.g.,7], archaeal ammonia oxidizers were sensitive to N fertilization (positively). One type of archaeal population made up the bulk (74-95%) of ammonia-oxidizers across treatments and patch types.

Nitrogen fertilization changes ammonia oxidizers at the physiological level

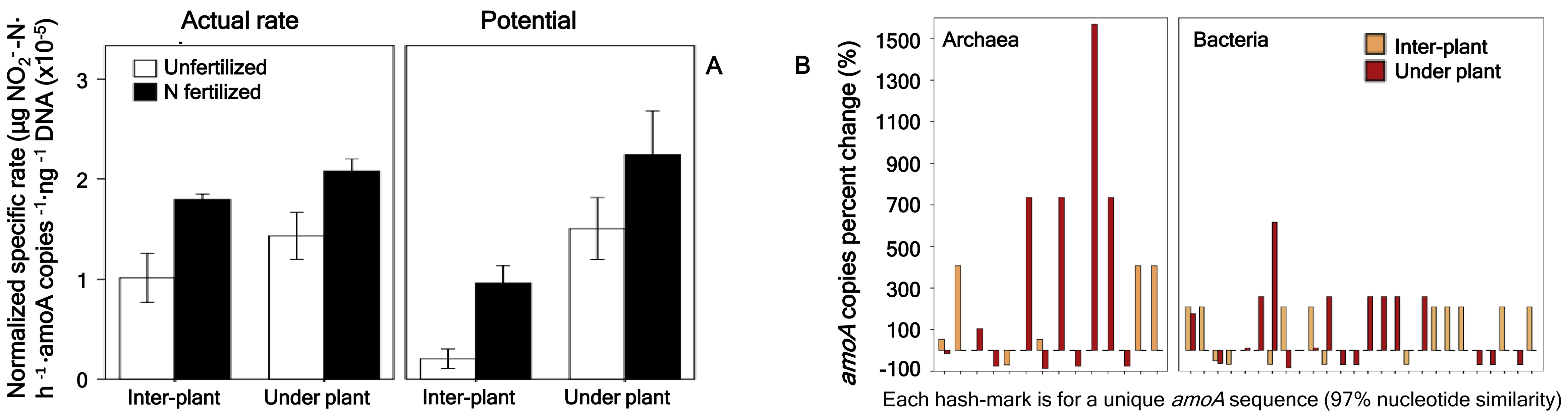


Fig. 3A. Specific rates. Since the AO rate of bulk soil depends on the total number of enzymes or cells carrying out the reaction, we accounted for population size differences between plots to evaluate N treatment effects. Fig. 3B. Percent change in *amoA* from unfertilized to N fertilized soils.

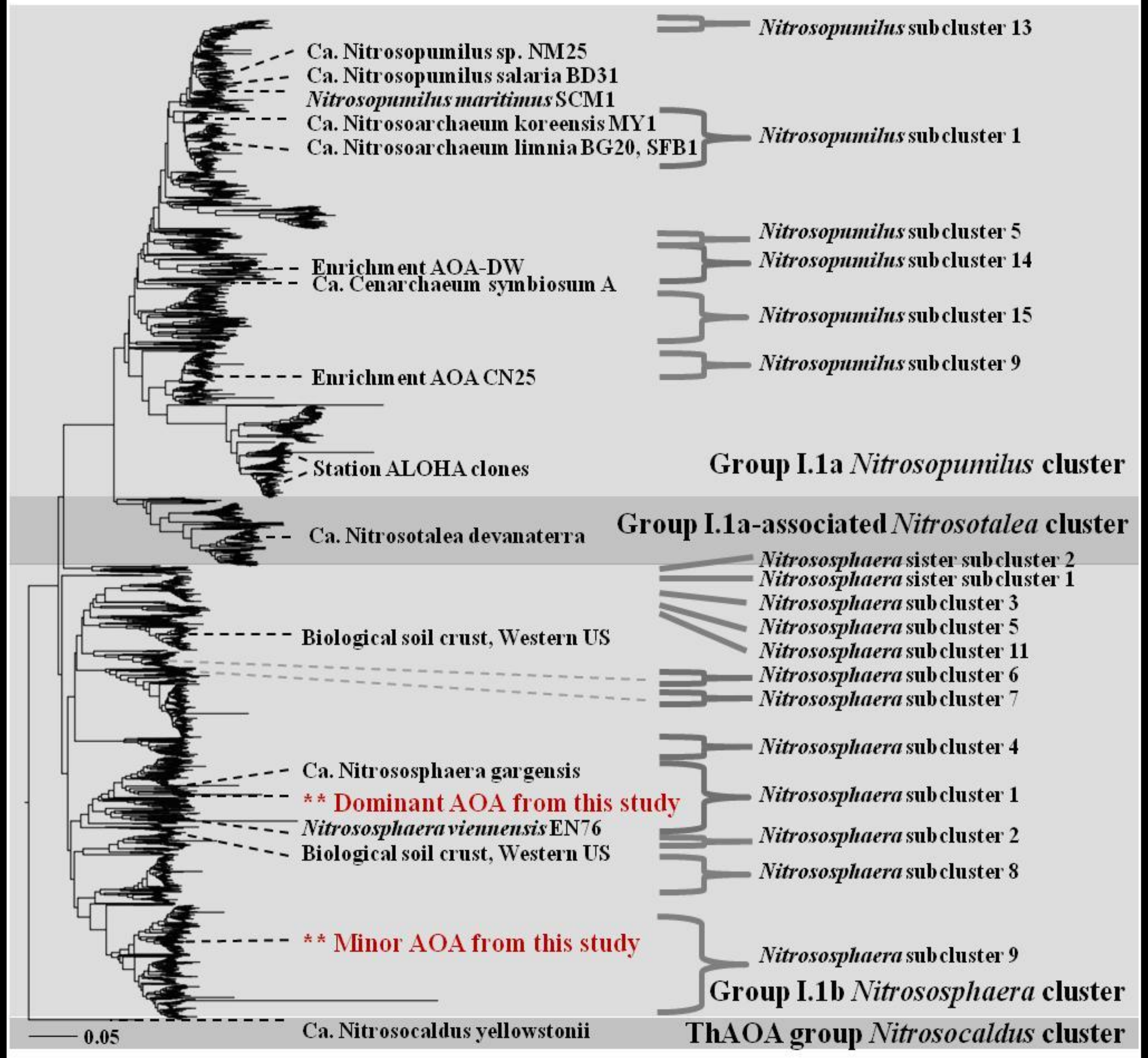
• N fertilization increased specific AO rates (i.e., AO efficiency) per *amoA* quantified relative to DNA (Fig.3A). Also, species-level phylogenetic resolution shows that individual populations respond differently to N treatment (Fig.3B).

Discussion/Conclusions

• **N-fertilizing processes have the potential to affect soil function in the Sonoran Desert.**

• **Community structure is altered through changes within the bacterial and, surprisingly, archaeal subgroups.** Only few studies have discovered any association between archaeal ammonia oxidizers and soil NH_4^+ content or AO rates[2]. We found that phylogenetically distinct ammonia oxidizers are present in this soil and respond differently to N input.

• **The diversity of archaeal ammonia oxidizers found here form only two separate clusters compared to sequences globally[8]** (Figure below; Phylogeny of ammonia-oxidizing archaea; the *Thaumarchaeota*), while bacteria are represented in 7+ distinct clades (not shown). These results suggest that archaea adapt more selectively than bacteria to their specific ecosystem, such as Sonoran Desert conditions (e.g., high temperature, desiccation, high salinity, infrequent and pulsed precipitation, alkaline soils).



• **Long-term environmental N addition in aridlands changes ammonia-oxidizing communities at the population level through shifts in abundnace and community structure, resulting in higher nutrient cycling rates at the ecosystem scale.** N effects at the population and community levels are confirmed at the functional level: N fertilization increases specific AO rates, suggesting that the treatment selects for the type of *amoA* and population being active.

Acknowledgements

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