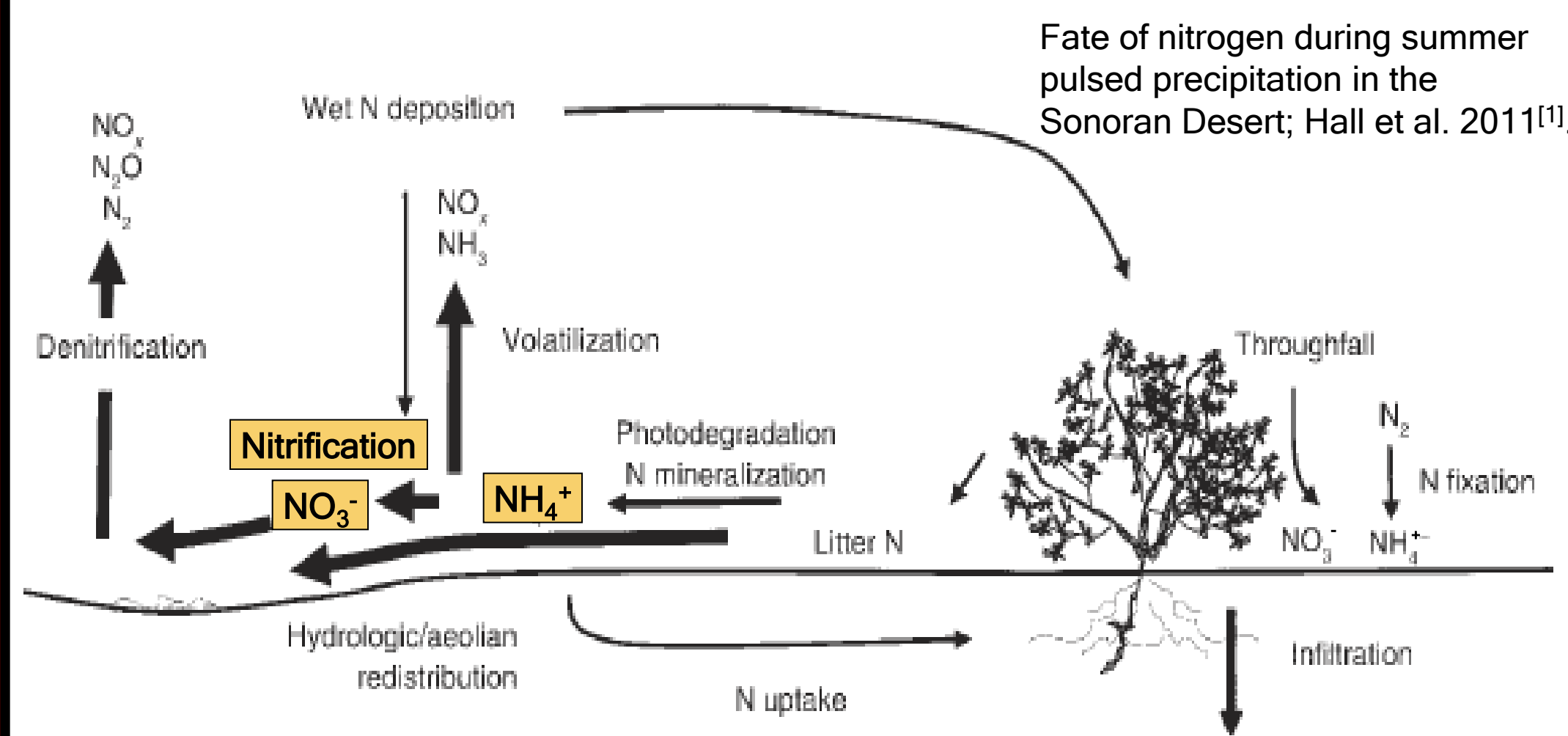


NICHE DIFFERENTIATION OF AMMONIA-OXIDIZING MICROBIAL COMMUNITIES AND THEIR FUNCTION IN SOIL

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 their subgroups) may respond differently to changes in the environment. Any shifts or adaptations in enzymatic functions can lead to distinct ecosystem responses.

RESEARCH QUESTION: Does N fertilization affect the ecology of AO through selective effects on particular microorganisms and their function at the physiological level?

Methods

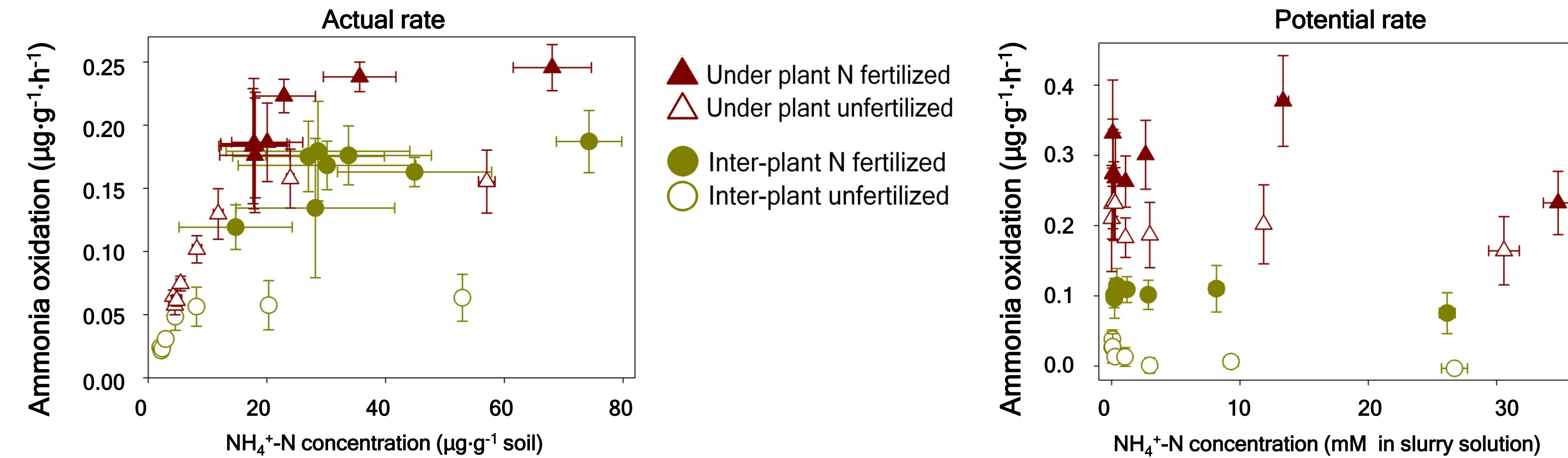
- We measured AO rates and ammonia-oxidizing communities in N fertilized (NH_4NO_3 added at $60\text{kg N ha}^{-1}\text{ yr}^{-1}$ since 2005) and unfertilized Sonoran Desert soils near Phoenix, Arizona^[1]. Soils were collected in common aridland patch types, away from plants and under the canopy of 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 using clone libraries and pyrosequencing based on *amoA*, a functional marker for AO^[7], from DNA.

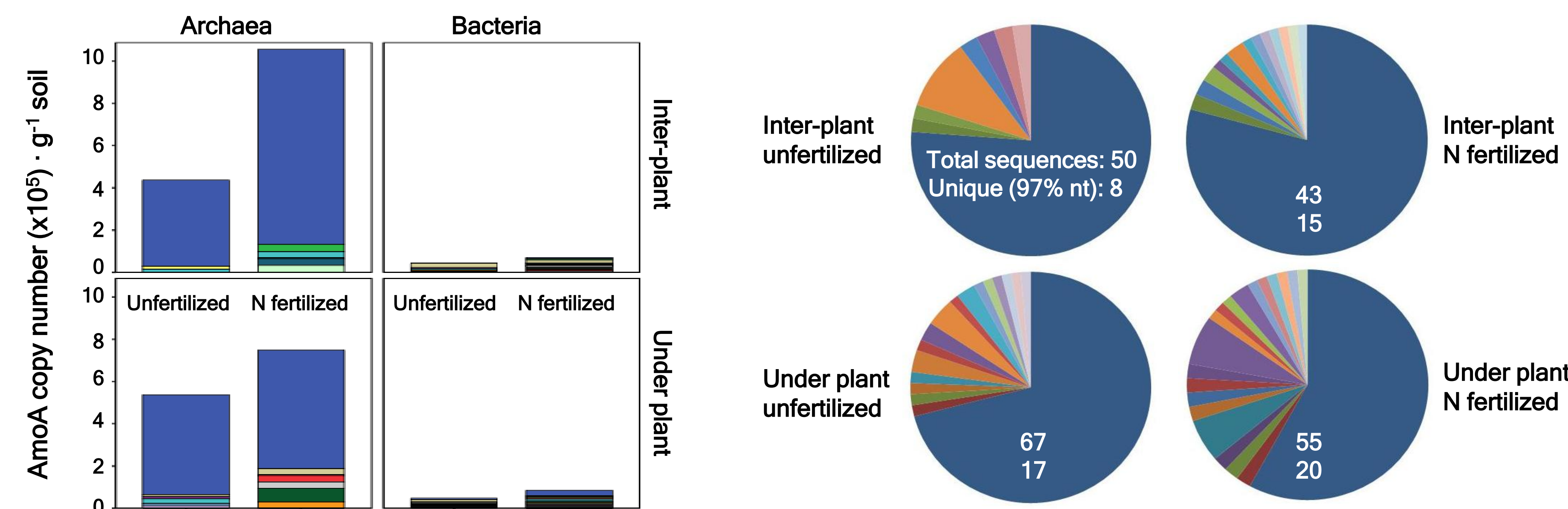
Results

Nitrogen fertilization increases ammonia oxidation rates over the short-term (days) and long-term (years)



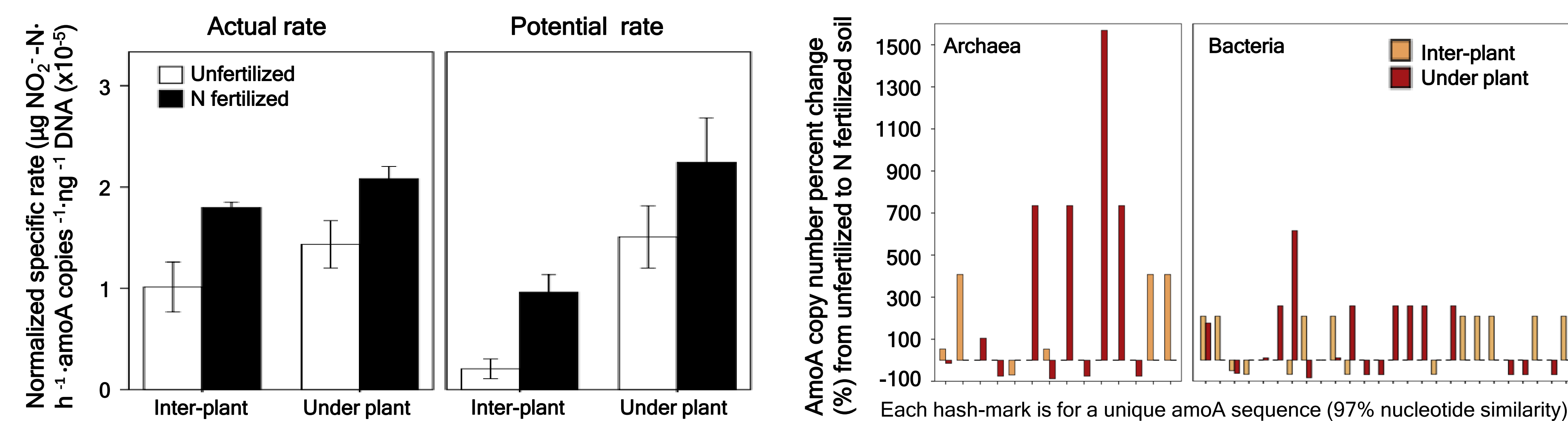
- Long-term N fertilization increases rates of actual and potential AO in soils of both patch types, compared to the unfertilized control. In the actual rates for soil from unfertilized plots (conditions most similar to native desert soils), AO rates increased with supplemented NH_4^+ during the 48-h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

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



- N fertilization increases population abundance (numbers of *amoA* copies) and community diversity (richness and evenness) of the ammonia oxidizers (archaea and bacteria combined). In contrast to many studies^[7], we found that archaeal ammonia oxidizers were sensitive to N fertilization. Furthermore, one 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



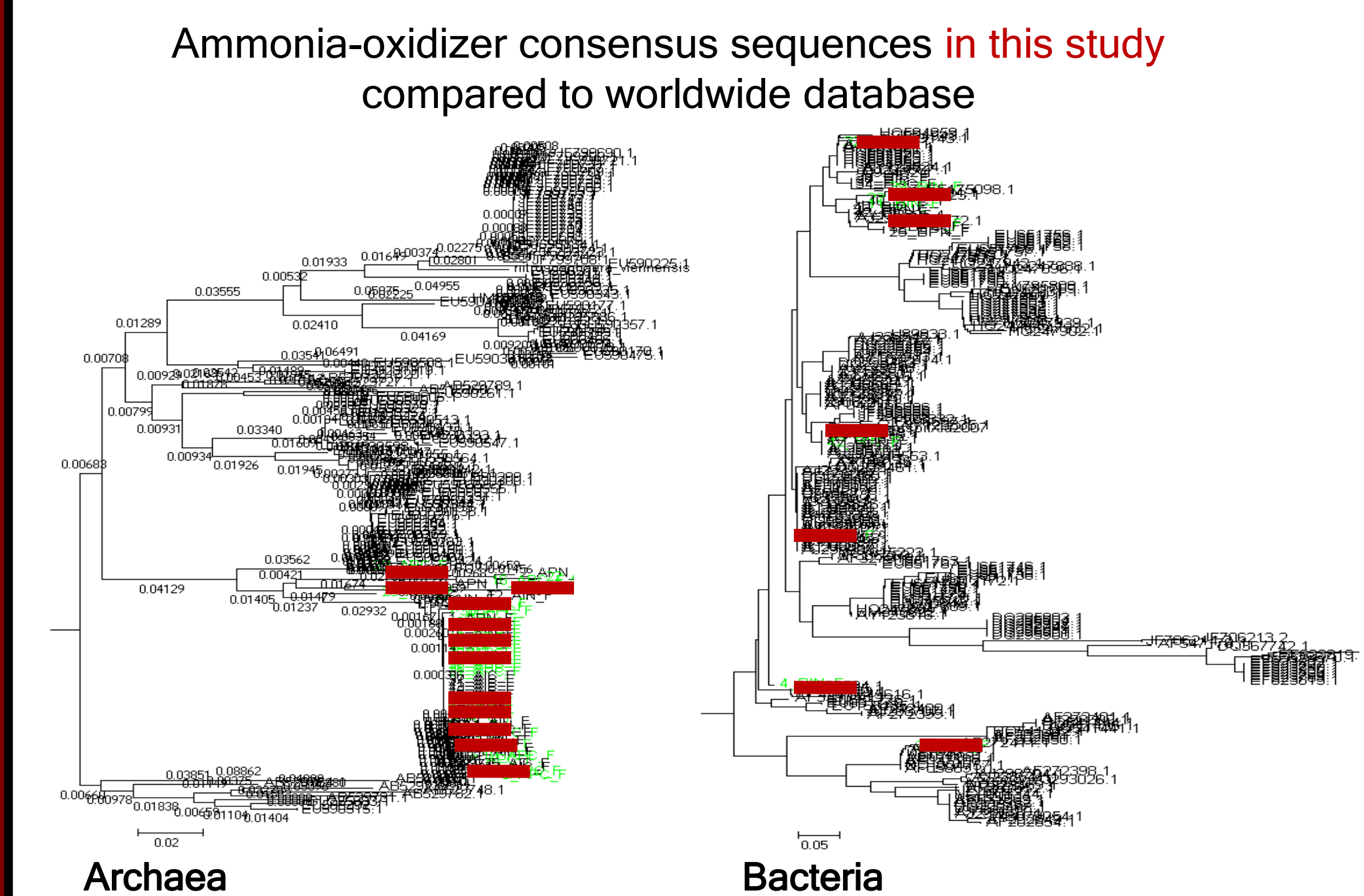
- Since the AO rate of bulk soil depends on the total number of enzymes or cells carrying out the reaction, we accounted for population size differences between plots to evaluate N treatment effects: N fertilization increased specific AO rates (i.e., AO efficiency) per *amoA* quantified relative to DNA. Finally, inspection at a fine level of phylogenetic resolution shows that individual populations respond differently to N treatment.

Discussion/Conclusions

- Human activities, including N fertilization, have the potential to affect soil function in the Sonoran Desert.

- **Community structure is altered through compositional 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]. Here, species-level resolution reveals that phylogenetically distinct and relatively diverse ammonia oxidizers are present in this soil and the populations respond differently to N input.

- The diversity of archaeal ammonia oxidizers found in this desert form a separate cluster compared to sequences globally. Meanwhile, the bacteria are represented in clades that are found in environments all over the world. **These results suggest that archaea adapt more selectively than bacteria to their specific ecosystem, such as conditions in the Sonoran Desert** (e.g., high temperature, infrequent and pulsed precipitation, alkaline soils, high salinity, desiccation).



- Long-term environmental N addition in aridland soils changes ammonia-oxidizing communities at the population level through shifts in population size and community structure, resulting in higher nutrient cycling rates at the ecosystem scale. The 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 enzyme or population being active.

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