Shipley-Skinner Reserve – Riverside County Endowment Application

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The Impact of Atmospheric Nitrogen Deposition on the Microbial Nitrogen Cycle in Desert Soils

Project Summary: In desert systems, soil microbes are important drivers of biogeochemistry. Disturbance of microbial communities due to anthropogenic activities and climate change may be driving the expansion of desert ecosystems. Therefore, investigation into the drivers of desert soil microbial community composition and the mechanism of soil microbial activities are needed to understand how desert microbes will react to their changing habitat and impact ecosystem services. In this proposed research, I will investigate the impact of season and atmospheric nitrogen deposition on microbial mechanisms of nitrogen assimilation and emission during rewetting induced soil respiration. My field sites, including 2 UC reserves and 2 sites within Joshua Tree National Park, are arranged along a gradient of atmospheric nitrogen deposition in southern California, encompassing the edge of Los Angeles and extending to outlying desert ecosystems. My uniting research objective is to couple this regional experimental design to functional metagenomic and marker gene analysis of the soil microbiome in order to explicitly connect microscale dynamics of nitrogen to a larger theory of landscape ecosystem functioning.

RESEARCH STATEMENT

Background: In desert systems, where plant and animal biodiversity is limited by oppressive heat and infrequent rainfall, resilient soil microbes are important drivers of biogeochemistry. However, there has been little mechanistic investigation into the ecosystem processes carried out by desert adapted microbes. Disturbance of microbial communities due to anthropogenic activities and climate change may be driving the expansion of desert ecosystems, which already make up one fifth of Earth's surface area (Pointing and Belnap 2012; Makhalanyane et al. 2015). Therefore, investigation into the drivers of desert soil microbial community composition and function are needed to understand how the desert microbes will react to their changing habitat and impact ecosystem services.

Large pulses of soil respiration following rewetting of dry soils, know as the Birch effect have been established as an important driver of biogeochemistry (Unger et al. 2010). Following rewetting, organic matter is liberated from occluded soil particles and mineralized microbial biomass, providing a rush of labile carbon (C) & nitrogen (N) that spurs microbial respiration and community turnover. Higher availability of labile N & C in arid systems due to the accumulation of dessicated or UV degraded biomass creates high rewetting induced respiration in dry periods (Jenerette and Chatterjee 2012; Leitner et al. 2017). In arid systems, a single rewetting event can release up to 10% of annual productivity (Lee et al. 2004).

Research into rewetting induced microbial dynamics is also needed to inform understanding of desert nitrogen cycling. There is conflicting evidence on N availability in desert soils: N availability is thought to be limiting in arid ecosystems, but there is evidence that arid ecosystems may exhibit high N emission potential (Schaeffer et al. 2017; Homyak et al. 2017; Leitner et al. 2017). Preliminary research by the Aronson lab has shown rapid, large pulses of N2O following rewetting of desert soils in late summer (Fig. 1a), supporting the relationship between low ambient activity and high emission potentials associated with the Birch effect.

Furthermore, chronic exposure to atmospheric nitrogen deposition is a significant driver of N2O emissions. Downwind of urban ecosystems, N-dep in the form of emitted nitrogen oxides (N-dep) is a stress on arid ecosystems, and may increase invasive grasses and fires (Rao et al. 2009; Fenn et al. 2003). The impact of N-dep on the soil microbiome has been studied intensively, and established to have global patterns of decreased microbial biomass and respiration (Zhang, Chen, and Ruan 2018). A mechanistic understanding of how atmospheric deposited nitrogen oxides influence microbial biogeochemistry is needed. Research by the Aronson lab has shown that nitrogen deposition decreases rewetting induced emissions of N2O (Fig. 1a). This contradicts the theory that N-dep may increase N_2O emissions by relieving N limitation on biogeochemical processes (Aronson and Allison 2012). However, N-dep may decrease N2O emissions by increasing ambient activity, specifically by increasing microbial N assimilation and microbial community turnover (Fig. 1b).

The assimilation of nitrogen into organic molecules (i.e. nitrogen immobilization) is an important process with the potential to mitigate the emission of N. In general, it is understood that heterotrophic organisms prefer NH4+ as a source of N for biomass building. NH4+ represents the final nitrogenous product of organic matter decomposition, and is taken up by microbes via the mineralization-immobilization-transformation (MIT) route (Geisseler et al. 2010). However, microbial uptake of NO3 may also be an important process (Morrissey et al. 2018), especially in oligotrophic systems with slower rates of N mineralization (Davidson, Hart, and Firestone 1992; Myrold and Posavatz 2007). Although pure culture studies have established that assimilatory NO3 reduction is downregulated by the presence of NH4, in a heterogeneous soil environment NO3 uptake may be an important process for copiotrophic

organisms where labile C is readily available, in soil compartments where NO3 can diffuse more easily than NH4, or in complex microbial communities where high diversity of nitrogen uptake processes is beneficial for survival in periods of famine or drought.

Questions:

How does chronic exposure to nitrogen deposition impact the nitrogen assimilation strategies of desert soil microorganisms?

- Hypothesis 1.1: Chronic exposure to atmospheric N-dep selects for microbes capable of using NO3 as a source of assimilatory nitrogen.
- Hypothesis 1.2: Biogeochemical cycling of NO3 is carried out by a small, less diverse group of copiotrophic microorganisms compared to microbes able to assimilate mineralized nitrogen produced from organic matter decomposition.

How does N-deposition impact microbial functions responsible for rewetting induced pulses of N following rewetting?

- Hypothesis 2.1: Chronic exposure to atmospheric N-deposition downregulates the emission of N following rewetting by selecting for assimilation of the labile N that would otherwise be emitted as N2O.

Experimental Design:

Field Work: This field experiment will utilize 4 sites along an N-depositional gradient in Southern California: Boyd Deep Canyon Reserve, Oasis de los Osos Reserve, and two sites within Joshua Tree National Park. These sites are all dominated by creosote bush scrub and have a similar profile of soil pH, parent material, and texture. A solution of NH4, NO3, or water alone will be added to soils under creosote shrubs. Emissions of N2O will be quantified with an integrated system of dynamic flow-through closed chambers equipped with fast response analyzers for a period of 24 hours. 5cm soil cores will be sampled from under the shrubs before wetting, 15 minutes, 2 hours, 12 hours, and 24 hours post-wetting.

Soil Chemistry Analysis: Texture and pH will be established between soils at all sites. Gravimetric water content (GWC), total N (TN), total organic C (TOC), total microbial biomass (MB), NH4 concentration, and NO3 concentration will be measured in undisturbed soils and after rewetting experiments, described below.

SIP Incubations: To capture N assimilation by soil microbes after rewetting, soil cores will be taken back to the lab to perform qSIP. Soils will be incubated with 15N labeled NO3 or NH4. After extracting DNA, ultracentrifugation and fractionation will be performed to separate DNA by density. A density curve will be measured using qPCR and a refractometer to identify fractions of DNA labeled with 15N. This qSIP experiment analyze how soil microbiomes respond to wet deposition of N with extensive controls to quantify isotope enrichment, control for impact of H2O alone, and detect the difference between undisturbed and rewet soils. Incubations will include treatments combining H218O and 15N to assure that responses to N treatment are captured if 15N incorporation alone does not increase DNA density sufficiently for successful separation.

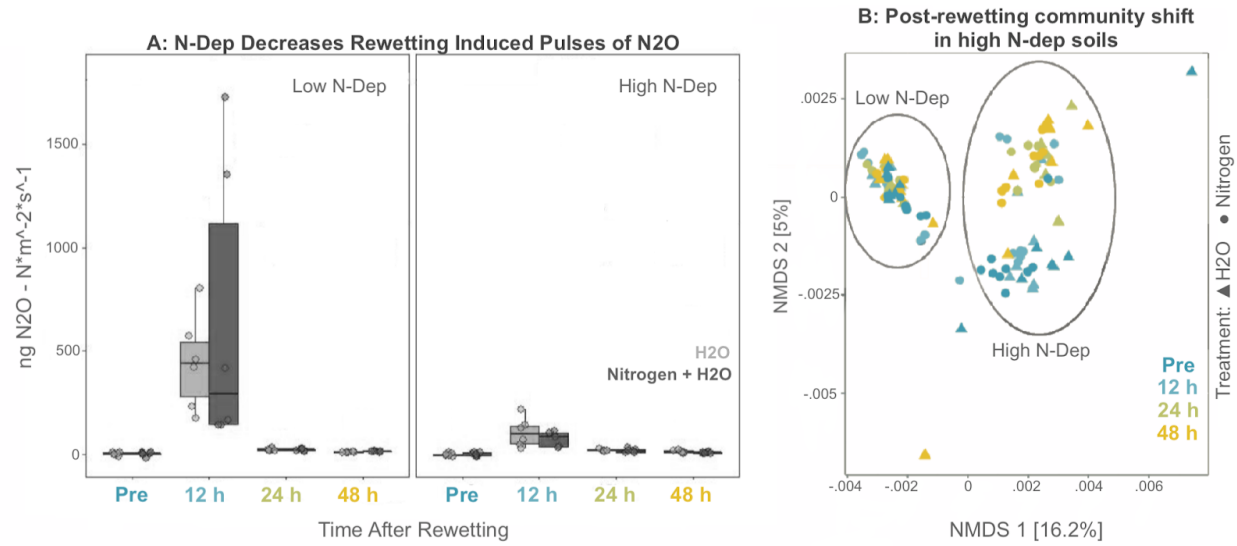
Marker Gene Sequencing: DNA from SIP fractions will then be sequenced to identify taxonomic patterns of N uptake for NO3 or NH4. I will sequence 16S rRNA genes in all SIP fractions on the Illumina MiSeq Platform. 16S data will be processed using QIIME and the qSIP informatics pipeline to identify taxa that actively consume different N species and quantify taxon-specific N assimilation rates. 16S data will then be used to pick samples for metagenomic sequencing that capture spatial heterogeneity and drivers of diversity

Metagenomics: Shotgun metagenomic sequencing will then be completed for SIP fractions of interest using the Illumina NovaSeq platform. Metagenomic reads will be analyzed in MG-RAST to identify putative gene sequences, and match predicted gene clusters to proteins. Quantitative analysis of annotated proteins translated from predicted coding regions is enhance by the qSIP method, as degree of isotope enrichment and read depth are a measure of a gene's response to treatment.

Data Analysis: I will first determine the environmental drivers of nitrogen cycling processes with a canonical correspondence analysis. Blomberg's k metric will be used to determined phylogenetic patterns of N assimilation. Regression analysis will be performed on flux data to analyze how variation in peak and cumulative N2O fluxes vary in response to relative expression of functional genes. I will then use a structural equation model to test how the effect of N-dep on N cycling taxa and genes impact N emissions and measures of soil chemistry as well as synergistic relationships between N source and other microbial processes activated during rewetting identified in the metagenome, e.g. stress responses and organic matter mineralization.

Expected Results and Conclusions: There is a growing interest in the microbial functions that immobilize mineral nitrogen as microbial biomass N in soils. By probing the soil microbiome with isotopically tagged mineral nitrogen, I will stimulate N uptake into microbial biomass. The metagenome from the microbial bodies produced using this nitrogen will allow me to characterize the metabolic capacity of N assimilating microbes. In desert systems where nutrients are very limited, atmospheric deposition of nitrogen may provide a novel N source for microbial metabolism. Therefore, by comparing metagenomes enriched with two different sources of mineral N (NH4 and NO3) I can analyze atmospheric nitrogen deposition as a selective pressure on N source and metabolism. By coupling these molecular methods to gas flux measurements in the field, I hope to identify functions which may be contributing to rewetting pulses of C and N.

Figure 1: Correlation between N_2O emissions and microbial community resilience during rewetting of desert soils in summer (Eberwein et al., In Review). A) Rewetting induced emissions of N_2O tracked for a period of 48 hours following treatment to stimulate 2cm of rain with H_2O alone or an aqueous solution of 30 kg NH_4NO_3 ha^{-1}. Error bars represent standard deviation and color represents treatment. **B)** Nonmetric multidimensional scaling analysis of 16S amplicon sequencing data from soils collected at key timepoints.



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DATA MANAGEMENT PLAN

- **Flux data**: Raw output from gas analyzers and data analyzed in R will be stored on computers and backed up on external hard drives in Dr. Aronson's lab. Data will also be stored in the same way in the lab of our collaborators, Dr. Pete Homyack and Dr. Darrel Jenerette, who are in possession of the gas analysis equipment.
- **Soil chemical and physical analyses:** These data will be recorded by hand in lab notebooks as the methods described above are performed in the lab. Data will then be digitally transcribed and stored on computers and backed up on external hard drives in Dr. Aronson's lab.
- **Marker gene and metagenomic sequences:** Sequence data will be stored in Dr. Aronson's directory on UCR's high performance computing cluster. Raw sequence data will also be backed up onto external hard drives in Dr. Aronson's lab. After publication of the results of this experiment, results of the sequence data analysis will be publically accessible through the Integrated Microbial Genomes (IMG) portal, the NCBI RefSeq database for prokaryotic genomes, and the metagenomics RAST server.
- In addition to the specific data management plans above, all data will be stored in a Google Drive folder for backup and so any current or future collaborators may access it.

BUDGET NARRATIVE

Item	**Budget Requested**
Graduate Tuition and Fees for 1 quarter	$7,237
GSR for 1 quarter	$5,391
Kits to extract DNA from soils	$1000
Reagents to prepare gene libraries for sequencing, including DNA polymerase and primers.	$1000
Cost of sequencing on the Illumina Platform at UCR's genomics core	$3000