Katherine Dynarski
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Title: Decomposition dynamics and N fixation in temperate forests with N-rich bedrock

Summary: Of earth's nitrogen (N) that is already fixed (and thus reactive), 99% is found in the geosphere, yet geologic inputs are traditionally overlooked as a source of ecosystem-available N. Recent research has demonstrated that bedrock can provide a substantial amount of ecosystem-available N in moderate-to-high relief areas with N-rich sedimentary bedrock, and I have found that this input of N supports greater rates of biological N fixation in forest litter, further contributing to forest N fertility. I have established a reciprocal transplant litter decomposition experiment to differentiate the competing drivers of soil fertility and litter chemistry in regulating rates of N fixation in forests with differing inputs of N from bedrock.

Background/Rationale: Nitrogen (N) is considered to be one of the most frequently limiting elements to terrestrial ecosystem productivity worldwide. Atmospheric N exists as inert N_2, which must be transformed to NH_4^+ by N-fixing microbes via the energetically costly process of biological N fixation before it can be used by other organisms. Of earth's N that is already fixed (and thus reactive), 99% is found in the geosphere, yet geologic inputs are traditionally overlooked as a source of ecosystem-available N.

Recent research has demonstrated that bedrock can provide a substantial amount of ecosystem-available N in moderate-to-high relief areas with N-rich sedimentary bedrock (Morford *et al.*, 2015). In northern California, such areas have been demonstrated to support forests with approximately 40% more above-ground biomass C and 60% more soil C than otherwise similar forests underlain by N-poor bedrock (Morford *et al.*, 2011), demonstrating a clear link between long-term N cycling and modern terrestrial C cycling. The Houlton group has conducted extensive sampling of forests across various lithologies in the California coast ranges,

and characterized a set of sites that serve as a "rock N gradient" with rock N concentrations spanning from 50 mg N/kg bedrock to >1000 mg N/kg bedrock. We have found that N content in foliage, litter, and soil increases with increasing concentration of N in bedrock across these sites. Additionally, we have found evidence of accelerated N cycling in forests underlain by N-rich bedrock, with significantly higher rates of N mineralization in soils and N fixation in leaf litter in forests with high rock N content (> 500 mg N/kg bedrock) vs. forests with low rock N content (< 500 mg N/kg bedrock). These findings contrast those of most fertilization experiments, which generally show that increased N availability suppresses free-living N fixation (Reed *et al.*, 2011). Characterizing the feedbacks resulting in accelerated N cycling processes and increased plant-available N at sites with high bedrock N content has important implications for future forest productivity and C storage.

My dissertation research examines how differences in foliar, litter, and soil chemistry due to rock N inputs drive differences in soil microbial N cycling processes, particularly free-living N fixation in the organic horizon. The majority of free-living N fixing bacteria are heterotrophs and rely on litter as their C source, and must obtain other nutrients from litter and soil. The belowground input of N from rocks may therefore affect microbial activity by increasing N availability in soil as well as by influencing foliar chemistry, and thus the chemistry of litterfall. In my previous dissertation research, I have measured significantly higher rates of N fixation in the fresh litter (O_i) horizon vs. the decomposed litter (O_e and O_a) horizons, suggesting that C quality and availability plays a major role in regulating rates of N fixation. I have also found significantly greater molybdenum (Mo) content in soils at high rock N sites vs. low rock N sites. Because Mo is a commonly limiting nutrient to N fixation (Silvester, 1989; Barron *et al.*, 2009;

Wurzburger *et al.*, 2012), differences in soil Mo availability may also drive patterns of N fixation across sites.

In order to differentiate the competing drivers of soil fertility and litter chemistry in regulating rates of N fixation across our rock N gradient, I have established a reciprocal transplant litter decomposition experiment at three previously well-characterized sites. I collected freshly abscised leaves from three forests underlain by low N bedrock (<100 mg N/kg bedrock), high N bedrock (700 mg N/kg bedrock), and extremely high N bedrock (>1000 mg N/kg bedrock). I transferred leaves into mesh bags and allowed them to decompose for 100 days *in situ* (at the site that leaves were collected from) as well as at two "common garden" sites, one with the highest bedrock N content and one with the lowest bedrock N content, where leaf litter from all sites is decomposed. Decomposing leaves at their native site as well as at sites with differing bedrock N content allows for the differentiation of effects due to the site of decomposition and effects due to leaf chemistry.

To monitor changes throughout decomposition, replicate litterbags were collected after 1, 2, 7, and 30 days of decomposition. One set of litterbags remains in the field to be collected after 100 days of decomposition. I am measuring free-living N fixation activity, mass loss, total C/N/P, and C fractions (soluble, hemicellulose + cellulose, lignin) in all litterbags in order to investigate the effect of nutrient and C dynamics throughout decomposition on free-living N fixation. Measuring differences in litter C fractions between sites and throughout decomposition is of particular interest, as C availability appears to play a central role in regulating organic horizon heterotrophic N fixation. Litter samples with more soluble C and less lignin C are considered to be higher quality substrates for heterotrophic metabolism. I am seeking Jastro

support to fund my final litterbag collection field excursion and to fund an undergraduate intern to perform the litter C fractionation procedure on all leaf litter samples for this project.

Objectives:

1. Measure mass loss, chemical composition (total C, total N, total P, $\delta^{15}N$, C quality fractions), and free-living N fixation in leaf litter throughout 100 days of decomposition.
2. Determine the relative roles of soil fertility and litter chemistry in influencing rates of free-living N fixation in leaf litter throughout decomposition.

Hypotheses:

1. If litter chemistry is the dominant control over decomposition dynamics and free-living N fixation, patterns of mass loss, chemical composition changes, and N fixation will be consistent for litter samples from the same source site, regardless of the site of decomposition.
2. If soil nutrient availability is the dominant control over decomposition dynamics and free-living N fixation, patterns of mass loss, chemical composition changes, and N fixation will be consistent within each site, regardless of the source of litter.

Results to date: My dissertation research, as well as the work of other Houlton lab members, at these sites has found that soil N concentration, foliar N concentration, and litter N concentration all increase as rock N concentration increases. Additionally, I have measured significantly higher rates of free-living N fixation in leaf litter at high rock N sites vs. low rock N sites. $\delta^{15}N$ of soil, foliage, fresh litter, and decomposed litter is elevated at high rock N vs. low rock N sites, further indicating accelerated N cycling in forests receiving significant inputs of N from rocks.

Budget:

	Justification	Unit cost	Quantity	Total cost
Field work				
SUV rental from fleet services – daily fee	Site access requires 4X4 SUV. I estimate I will need to do one 3-day field trip to visit my sites and collect remaining litterbags.	$57/day	3 days	$171
SUV rental from fleet services – per mile fee	Sites are located in northern California. Furthest site is 200 miles from Davis, driving between field sites is required.	$0.35/mile	500 miles	$175
Lab analyses				
Hiring student intern to perform litter carbon fractionation procedure		$10.50/hr (wages + fees)	~8 hrs per batch of 12 samples x 350 samples = ~250 intern hours	$2,625
Total				$2,971

Research progress/timeline:

Already completed – preliminary characterization of rock, soil, foliar, and litter chemistry at study sites, preliminary measurements of free-living N fixation, construction and deployment of litterbags, collection of litterbags at days 1, 2, 7, and 30 days of decomposition, measurement of mass loss of collected litterbags, total C/N/P analysis of collected litterbags, measurements of free-living N fixation in collected litterbags

Timeline for proposed research:

October 2016: Final litterbag collection

October 2016-February 2017: Litter C fractionation procedure on all litterbag samples (to be performed by undergraduate student intern)

February 2017-May 2017: Data analysis

Past Jastro Support: I have received Jastro support during the 2014-2015 and 2015-2016 academic years. I used Jastro funds in 2014-2015 to analyze microbial communities associated with N cycling in the Mojave Desert using qPCR and purchase equipment necessary for my fieldwork. I used Jastro funds in 2015-2016 to support my dissertation research examining N fixation, soil chemistry, and litter chemistry across a rock N gradient in northern California.

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Title: Interactions between bedrock nitrogen reservoirs and biological nitrogen fixation

Summary: Of earth's N that is already fixed (and thus reactive), 99% is found in the geosphere, yet geologic inputs are traditionally overlooked as a source of ecosystem-available N. Recent research has demonstrated that bedrock can provide a substantial amount of ecosystem-available N in moderate-to-high relief areas with N-rich sedimentary bedrock, and I have found that this input of N supports greater rates of biological N fixation in forest litter, further contributing to forest N fertility. I am working to use chemical and microbial analyses to understand the mechanism of this interaction, with implications for forest carbon storage.

Background/Rationale: Nitrogen (N) is considered to be one of the most frequently limiting elements to terrestrial ecosystem productivity worldwide. Atmospheric N exists as inert N_2, which must be transformed to NH_4^+ by N-fixing microbes via the energetically costly process of biological N fixation (BNF) before it can be used by other organisms. Of earth's N that is already fixed (and thus reactive), 99% is found in the geosphere, yet geologic inputs are traditionally overlooked as a source of ecosystem-available N.

Recent research has demonstrated that bedrock can provide a substantial amount of ecosystem-available N in moderate-to-high relief areas with N-rich sedimentary bedrock (Morford *et al.*, 2015). In northern California, such areas have been demonstrated to support forests with approximately 40% more above-ground biomass C and 60% more soil C than otherwise similar forests underlain by N-poor bedrock (Morford *et al.*, 2011), demonstrating a clear link between long-term N cycling and modern terrestrial C cycling. Rock N inputs appear especially significant to ecosystem function when bedrock contains >500 ppm N (Houlton &

Morford, 2015). However, the potential link between this largely unaccounted for N input and other important terrestrial N cycling processes such as BNF has not been studied in detail.

Bedrock N inputs can plausibly affect BNF through both direct and indirect mechanisms. Directly, rates of BNF are regulated by N availability; generally, BNF decreases as N availability increases, reflecting its extreme energetic cost (Cusack *et al.*, 2009; Zackrisson *et al.*, 2009; Matson *et al.*, 2014). Indirectly, the amount of N available to plants via bedrock may alter plant tissue chemistry and therefore plant litter quality. For example plants with greater access to N due to bedrock inputs may produce leaves with fewer recalcitrant C compounds such as lignin, improving litter quality (Hobbie, 2000). Many N fixing microbes in plant litter are heterotrophs and rely on litter as their C source, so increased litter quality could support greater rates of BNF (Vitousek & Hobbie, 2000). The overall effect of geologic N inputs on litter BNF (i.e. suppression vs. stimulation) is therefore dependent on if the direct or indirect effects of rock N inputs are dominant in regulating BNF.

My preliminary research across a rock N gradient in northern California (bedrock N concentrations ranging from 30-800 ppm N) found that rates of litter BNF are positively correlated with bedrock N concentration. Further, lignin:N ratios in foliage (a common proxy for litter quality; lower lignin:N indicates higher quality litter for heterotrophic consumption) decrease across the rock N gradient, suggesting that bedrock N inputs may drive increased litter quality, supporting increased rates of asymbiotic BNF in litter. This represents a previously unknown mechanism by which bedrock N reservoirs may enhance forest N fertility and support C storage.

I am seeking Jastro support to continue researching the interaction between geologic N and litter BNF. Specifically, I propose to expand my sampling sites to examine a wider range of rock N concentrations as well as include “replicate” sites of similar rock N concentration.

Because rates of BNF vary wildly both within and between sites, replication is critical to be able to tease out the effects of a particular factor (such as bedrock N inputs) on BNF. I also seek to connect patterns of BNF to microbial community composition by measuring the relative abundance of N fixing microbes using quantitative PCR.

Objectives:

1) Measure rates of BNF using the acetylene reduction assay (Hardy *et al.*, 1968) in forest litter across 8 additional forest sites in northern California where we have already measured rock N content (concentrations range from 30 ppm to >1000 ppm N in rock.
2) Elucidate the mechanism for the interaction between rock N inputs and litter BNF by measuring litter quality (concentrations of C, N, and lignin), $\delta^{15}N$ of relevant ecosystem compartments (foliage, litter, soil, and rock), and proportion of N-fixing microbes in the soil and litter microbial community.

Hypotheses:

1) Higher concentrations of rock N are associated with greater rates of BNF in forest litter.
2) Higher concentrations of rock N and greater rates of BNF are correlated with increased litter quality, indicated by decreased lignin:N ratios in foliage and litter.
3) Higher concentrations of rock N are associated with greater $\delta^{15}N$ in litter, reflecting the source of litter ($\delta^{15}N$ of foliage increases across the rock N gradient) as well as accelerated rates of fractionating N cycling processes, indicating a more active N cycle (including BNF) at N-rich sites.
4) Greater rates of BNF will be measured in samples with a greater relative abundance of N fixing microbes, as measured by the proportion of gene copies of *nifH* (a gene associated with BNF) to gene copies of 16S (a gene found in all bacteria).

Results to date: My preliminary research across a rock N gradient in northern California (bedrock N concentrations ranging from 30-800 ppm N) found a correlation between rates of BNF and rock N content (R^2=0.11, p=0.0035). Rates of BNF at high rock N sites (>500 ppm N in rock) are nearly double rates of BNF at low rock N sites (<500 ppm N in rock). Further, foliar lignin:N ratios decline with increasing rock N content (R^2=0.11, p=0.019).

Budget:

	Justification	Unit cost	Quantity	Total Cost
Field work				
SUV rental from fleet – daily fee	Site access requires 4X4 SUV. I estimate I will need to do two 3-day field trips to visit my sites.	$57/day	6	$342
SUV rental from fleet services – per mile fee	Sites are located in northern California/southern Oregon (where N-rich sedimentary rock is prevalent). Furthest site is ~400 miles from Davis.	$0.35/mile	900	$315
Food	Breakfast, lunch, and dinner while camping in field	$100/trip	2	$200
Lab analyses				
PowerSoil DNA extraction kit	Extract DNA from soil samples	$500/kit of 100 preps	1	$500
Reagents for qPCR	Analyze abundance of N fixers in microbial communities	$100/per plate	8	$800
Reagents for lignin analyses	Lignin analysis requires acetyl bromide and hydroxylamine hydrochloride	$200	1	$200
^{15}N of soil analysis at SIF	I plan on collecting 10 litter samples per site x 8 sites = 80	$8/sample	80	$640
Total				$2,997

Past Jastro Support: I have received Jastro support during the 2014-2015 academic year. I used Jastro funds to analyze microbial communities associated with N cycling in the Mojave Desert using qPCR and purchase equipment necessary for my fieldwork.

References:

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Title: A Multi-Scale Exploration of Coupled N Cycling Processes in the Mojave Desert

Summary (100 words): Though asymbiotic N fixation and denitrification are globally significant pathways of N input and loss, their rates in natural ecosystems are difficult to quantify and scale up. I propose to examine a potential coupling between these related gaseous N processes via acetylene gas assays, measurement of $\delta^{15}N$ of bulk soils, and microbial community characterization. I plan to perform this research at the Sweeney Granite Mountains reserve in the Mojave Desert, which serves as a natural laboratory for examining the effects of vegetation and climate on N cycling due to its distinct vegetated and barren areas and its highly seasonal precipitation.

Background/Rationale: Biological N fixation, the process by which microbes convert unreactive atmospheric N_2 into ammonia (NH_3), is an important source of bioavailable nitrogen (N) in many terrestrial ecosystems (Galloway *et al.*, 2004). Inorganic N added to the soil via N fixation can serve as the substrate for denitrification, the pathway though which inorganic N is converted back to gaseous forms and subsequently lost from the soil, suggesting an intrinsic link between these two processes. However, almost no studies have been conducted to explore a potential coupling between N fixation and denitrification in terrestrial ecosystems. Quantifying inputs and outputs of N within ecosystems is becoming increasingly important, as the net primary productivity (NPP), and therefore carbon (C) uptake capacity of many ecosystems is constrained by N availability (LeBauer & Treseder, 2008).

This gap in research may be attributed to the difficulty of quantifying asymbiotic N fixation (N fixation carried about by microbes living freely within soil and plant litter) and denitrification at both an ecosystem and a global scale (Hedin *et al.*, 2009; Philippot *et al.*, 2009). It is difficult to scale field measurements of N fixation and denitrification up to an ecosystem or global scale because of the heterogeneity associated with both processes; measured rates can vary wildly between seemingly identical soil samples taken just a few inches apart. This variability can be partially attributed to the

heterogeneity of the soil microbial communities responsible for these chemical transformations, even across small spatial scales (Reed *et al.*, 2010). Studies of N fixing and denitrifying microbial communities have pointed to both the abundance and diversity of these microorganisms as drivers of these two N cycling processes, making the characterization of microbial communities useful for explaining and understanding variation in measured rates (Hsu & Buckley, 2009; Philippot *et al.*, 2013).

I propose to examine coupling between N fixation and denitrification using a combination of techniques across a range of spatial scales: *in situ* gas assays, characterization of soil microbial communities, and measurement of bulk soil $\delta^{15}N$ (elevated soil $\delta^{15}N$ suggests a "leaky" ecosystem N cycle with a greater proportion of gaseous N losses). I am especially interested in how microbial abundance/diversity, vegetation, and climate may affect this coupling. By working at scales ranging from the microscopic (microbial community characterization) to the broad ($\delta^{15}N$ of bulk soil) across environmental factors, I hope to connect the microscopic-scale, difficult to measure processes of N fixation and denitrification to more easily measured soil and ecosystem properties.

The Sweeney Granite Mountains Desert Research Center (SGM) in the Mojave Desert is an excellent place to examine the effects of variations in vegetation and climate due to its natural heterogeneity. The landscape of SGM is characterized by stands of the dominant *Larrea tridentata* shrub (commonly known as creosote) with barren interspaces between the shrub stands. Previous researchers have found that carbon (C), N, and microbial populations tend to be concentrated beneath the creosote stands, and PLFA analysis indicates that the microbial community underneath creosote stands is distinct from that found in the interspace (Ewing *et al.*, 2007). The climate is also bimodal, with rainfall occurring almost exclusively during short periods in the late summer and the late winter, creating distinct dry and moist seasons. I will explore how differences between soil microbial communities, driven by these differences in vegetation and moisture, affect rates of N fixation and

denitrification and if these two processes are coupled to each other under some conditions.

Objectives:

1. Measure rates of N fixation and denitrification in Mojave desert surface soils in the interspace and underneath creosote stands in both the dry and wet seasons using the actylene reduction (N fixation, Hardy *et al.*, 1968)/acetylene block (denitrification, Yoshinari *et al.*, 1976) assays.

2. Measure δ^{15}N of bulk soil from these same samples.

3. Extract DNA from soil samples using MoBio PowerSoil DNA extraction kit

    3a. Quantify gene copies of *nifH* (N fixation), *nirS* (denitrification), *nirK* (denitrification), and 16S (all bacteria) in these soil samples using quantitative PCR.

    3b. Estimate composition and diversity of N fixing microbial community in these soil samples by amplifying and sequencing the *nifH* gene (MiSeq sequencer at UC Davis Genome Center).

Hypotheses:

1. "Hotspots" of high N fixation and denitrification rates occur in the same spaces. That is, greater N availability due to N fixation drives subsequent N loss via denitrification.

2. Microbial communities (abundance and diversity) are directly linked to measured rates of N fixation and denitrification.

    a. Higher rates of N fixation are correlated with more *nifH* gene copies, and higher rates of denitrification are correlated with more *nirS/nirK* gene copies.

    b. Higher rates of N fixation are also correlated with greater N fixer diversity.

3. Higher rates of N fixation and denitrification are associated with greater δ^{15}N in bulk soil.

Results to Date/Discussion:

My preliminary results from the Sweeney Granite Mountains Reserve in the Mojave Desert, California suggest that greater N fixation may occurs in interspace soils; the only detectable rates of N fixation (estimated by ethylene production, as per the acetylene reduction assay) were found in interspace soils. This was surprising, as previous studies have found greater microbial biomass underneath shrub stands,

which I would expect to be associated with higher rates of N fixation. Rates of denitrification were uniformly low (Figure 1) and were not significantly different between locations (t-test, p=0.57). I found no correlation between N fixation and denitrification (Figure 2, p=0.827, R^2=0.029).

Rates of both N fixation and denitrification were extremely low during the dry season sampling period, likely due to extremely low soil moisture limiting rates of both processes. The slightly higher rates of N fixation in the interspace soils may be due to the increased moisture in those soils, probably because there is no plant uptake of water in the interspace. Overall, the low rates measured during the dry season indicate that most N cycling probably occurs in the moist season. Sampling during the moist season and/or conducting a wet-up experiment will hopefully make trends more clear.

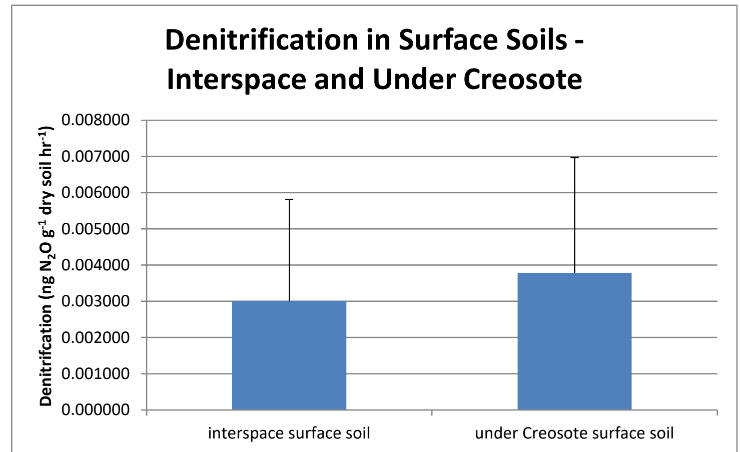

Figure 1 (above): Denitrification rates (ng N_2O produced g^{-1} dry soil hr^{-1}, measured using acetylene block) plotted vs. N fixation (ng ethylene produced g^{-1} dry soil hr^{-1}, measured using acetylene reduction. No significant correlation was found (linear regression, p=0.827, R^2=0.029)

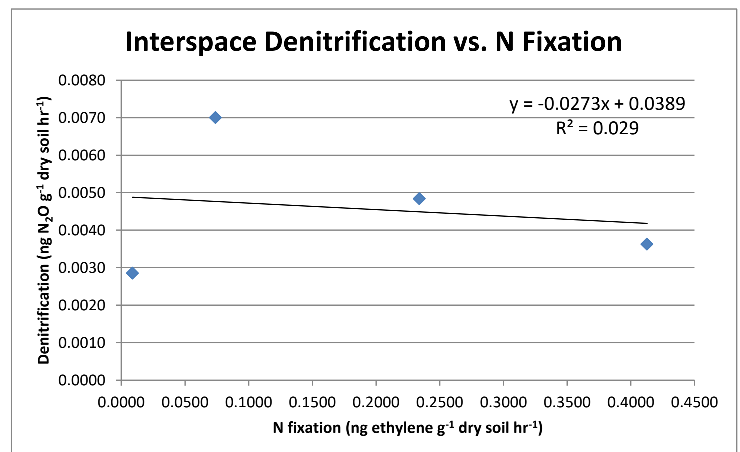

Figure 2 (above): Denitrification rates (ng N_2O produced g^{-1} dry soil hr^{-1}, measured using acetylene block) of interspace surface soils and surface soils under creosote shrub stands. No significant difference was found (t-test, p=0.57). Error bars represent standard deviation.

Interspace soils were significantly enriched in $\delta^{15}N$ relative to the soils underneath creosote stands (Figure 3, t-test, p=0.038). This suggests that interspace soils experience a greater proportion of gaseous losses of N than do soils under creosote. These losses most likely occur during the moist season, when anaerobic conditions are more likely.

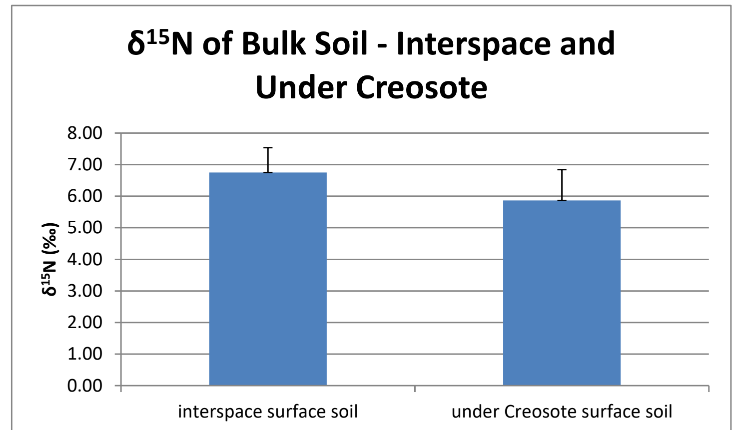

Figure 3 (above): δ15N of interspace surface soils and surface soils under creosote shrub stands. A significant difference was found (t-test, p=0.038). Error bars represent standard deviation.

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Budget/Justification: I am seeking Jastro funding to cover the cost of qPCR reagents, primers, and standards, as well as the cost of isolating and sequencing the *nifH* gene from my soil samples. For sequencing, all of my *nifH* samples can be pooled into a single lane on the MiSeq, so only one lane is needed. For qPCR, I need to run one plate per gene per round of sampling. I am examining four genes with two different rounds of sampling (dry season and moist season), so eight plates are needed. I already have a MoBio PowerSoil DNA extraction kit with sufficient preps for the work proposed here. My lab will fund all $\delta^{15}N$ of bulk soil analyses. The total cost for sequencing and all PCR/qPCR reagents and supplies is $2,307 (see below budget).

Item	Justification	Cost/Unit	Units	Units Needed	Total Cost
MiSeq P250 run	Sequence *nifH* genes in soil sample	$1,179.00	per lane	1	$1,179.00
PCR reagents + tubes	Amplify *nifH* for sequencing	$328.00	per 250 rxns	1	$328.00
qPCR reagents, primers, standards	Quantify *nifH*, *nirS*, *nirK*, and 16S gene copies	$100.00	per plate	8	$800.00

total	$2,307.00

Research Progress/Goals/Timeline:

Progress to Date: So far, I have measured rates of N fixation and denitrification using acetylene reduction/block assays during the Mojave dry season in surface soils from both the interspace and from underneath *Larrea tridentata* stands. I have also measured $\delta^{15}N$ of bulk soil in these soil samples. I set aside a subsample of all soil samples at -80°C for future DNA extraction and microbial community characterization.

Goals for this year: This year, I plan on returning to the Sweeney Granite Mountains reserve during the winter moist season. I will measure rates of N fixation and denitrification in surface soils from the interspace and from underneath *Larrea tridentata* stands. I will also measure the $\delta^{15}N$ of these soil samples. For all soil samples, dry and moist seasons, I will extract DNA and perform qPCR for the *nirS* (denitrification), *nirK* (denitrification), *nifH* (N fixation), and 16S (all bacteria) genes. I will also perform PCR on the DNA extracts to amplify the *nifH* gene. The resulting *nifH* amplicons will be pooled into a single lane for sequencing using the MiSeq sequencer at the UC Davis Genome Center. I will analyze the resulting *nifH* sequences and compare them to sequences deposited in the robust *nifH* database organized by the Buckley lab at Cornell. I will use this database to identify the main N fixers present in my soil samples, as well as approximate the N fixer community diversity within my samples. Bioinformatic analyses will be performed in collaboration with graduate student members of the Eisen lab (UC Davis, Department of Microbiology & Immunology); collaboration with bioinformatics experts will enable me to make use of the wealth of information obtained from sequencing.

Timeline:

September-October 2014: qPCR on dry season desert soils, *February 2015:* SGM moist season soil sampling, measure rates of N fixation and denitrification, $\delta^{15}N$ of bulk soil analysis (UCD Stable Isotope Facility), *March 2015:* qPCR on wet season desert soils, PCR on all desert soils to amplify *nifH* gene, *nifH* sequencing at the UCD Genome Center, *April-June 2015:* data analysis

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