Does conversion of shrublands to grasslands affect soil nitrogen trace gas emissions?

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Research aims and significance
In semi-arid ecosystems, trace gas emissions tend to be positively correlated with water availability; after rain events, nitrification and denitrification processing rates increase quickly as water-limited microbes become active in the soil (Austin et al., 2004; Butterbach-Bahl et al., 2013). Because semi-arid systems receive little water for a large portion of the year, NO\textsubscript{x} and N\textsubscript{2}O fluxes from these regions have long been assumed to be negligible and have therefore been largely ignored in calculating nitrogen budgets (Sullivan et al., 2015). However, recent work has refuted this idea with measurements of very high trace gas emissions in soils following water pulses in natural (Homyak & Sickman, 2014; Sullivan et al., 2015) and agricultural (Liang et al., 2015a; Oikawa et al., 2015) arid systems. The concept of pulse dynamics itself is relatively new and understudied (Austin et al., 2004), but pulses of water are particularly important for many biological processes to occur in arid environments. Further investigation is needed to identify how trace gases are generated in warm, semi-arid ecosystems and how biology influences nitrification/denitrification processes in these environments (Butterbach-Bahl et al., 2013).

California shrublands (e.g. coastal sage scrub and chaparral), including those on and near the Shipley-Skinner lands, are currently experiencing ecosystem type conversion to mainly-exotic, annual grasslands. Southern California in particular is also a hotspot for N deposition and is subject to warm and dry summers. However, to our knowledge, no investigation has been made into how the combination of climate and plant invasion influence trace gas fluxes in this region, but evidence from previous work implies that a combination of nitrogen sources could have a large impact on NO\textsubscript{x} and N\textsubscript{2}O fluxes, and therefore greenhouse gas emissions and air pollutants, into the atmosphere (Liang et al., 2015a).

Currently, little is known about how NO\textsubscript{x} and N\textsubscript{2}O respond to changes in plant and soil communities in semi-arid systems. I propose to expand the current knowledge of nitrogen cycling by exploring the question: How does conversion from shrubland to invasive grassland communities alter N\textsubscript{2}O and NO\textsubscript{x} fluxes in semi-arid climates? I propose two hypotheses to answer this question:

\textbf{H1: Invasive grass communities release higher concentrations of NO\textsubscript{x} and N\textsubscript{2}O than do intact shrubland communities due to disruptions of native plant and soil processes.}

\textbf{H2: Because litter of invasive grasses is rich in N compared to shrub litter, additional litter inputs from invasive annual grasses will increase NO\textsubscript{x} and N\textsubscript{2}O release from soils, particularly in intact shrub communities that have not been subjected to N-rich litter.}

To test these hypotheses, the proposed study will be separated into two complimentary activities:

1.) Analyze the differences between invasive grasslands and intact shrublands in their effluxes of NO\textsubscript{x} and N\textsubscript{2}O.
2.) Analyze the differences in trace gas fluxes in both ecosystems in response to N-rich litter additions.

Testing these hypotheses will help to explain the impacts of arid ecosystems on greenhouse gas emissions and air pollutants into the atmosphere and will give further insight into additional
effects that invasive plants have on fundamental processes in the soil. If invaded lands produce higher concentrations of nitrogenous greenhouse gases, an even stronger argument can be made for restoration of native ecosystems to restore less harmful processes and more healthy ecosystem function.

**Background and Rationale**

**Invasive plants alter native plant and soil communities with subsequent effects to biogeochemical processes**

With the implementation of the Haber Bosch process and of industrial agriculture, the amount of nitrogen (N) fixed from the atmosphere each year has approximately tripled over pre-industrial levels, and the intensity of N fertilization in agricultural lands has substantially increased cycling of terrestrial nitrogen (Schlesinger & Bernhardt 2013). Nitrification (i.e. conversion of ammonia (NH₄⁺) to nitrate (NO₃⁻)) and denitrification (i.e. conversion of NO₃⁻ to atmospheric nitrogen (N₂)) processes have increased in response to higher N availability in soils (Schlesinger & Bernhardt 2013; Homyak et al., 2014). However, both of these processes are not completely efficient and can generate byproducts -- nitrogen monoxide (NO) and nitric oxide (NO₂), often referred together "NOₓ," as well as nitrous oxide (N₂O). N₂O is a strong greenhouse gas and is highly responsible for ozone depletion in the atmosphere (Ravishankara et al., 2009). Similarly, NO has been linked to decreases in air quality and can redeposit on land after efflux from soils, contributing to higher N availability in non-agricultural systems such as arid shrublands, grasslands, and deserts (Holland & Lamarque, 1997). The addition of N in more N-limited systems such as these can disrupt native community structure and ecosystem processes and may leave ecosystems vulnerable to disturbance.

Invasive plants have become increasingly prevalent in almost every ecosystem type on earth because of their abilities to quickly exploit favorable growing conditions, including higher N availability (Yahdjian et al., 2014). During the invasion process, diverse native communities are effectively homogenized by competitively superior invaders. The shift in plant species, and in some cases, plant functional type – for example, the conversion of native shrublands to invasive grasslands taking place in California -- often alters biogeochemical cycles through invaders’ own physiologies or through their disruption of other organisms in the community (Wolkovich et al., 2010; but see McCarron et al., 2003). Since successful invasive plants tend to be fast-growing annuals by nature, their litter tends to be higher in N content compared to native vegetation (Ehrenfeld, 2003; Wolkovich et al., 2010), and ecosystems that have been invaded tend to have correspondingly higher N content in soils (Liao et al., 2008) and emissions of trace gases (NOₓ and N₂O) than do intact systems. However, relatively little effort has been made to assess the combined effects of water pulse dynamics apparent in arid lands with grass encroachment on trace gas fluxes. By analyzing differences in NOₓ and N₂O outputs between intact CSS and invasive grasslands in Riverside County, CA, I will address this issue.

**Research plan**

**Site selection**

I will test my hypotheses by conducting a field experiment at Motte Rimrock Reserve in Perris, CA. The Motte Reserve is dominated by sage scrub (CSS) communities and transitional grasslands and is surrounded by urban lands, providing both intact and degraded semi-arid plant communities for study. This reserve is experiencing similar shifts in plant communities and a similar climate to the Shipley-Skinner lands and other areas of inland Southern California; thus,
conclusions of this study could be applied to larger spatial scales. It also receives little nitrogen deposition compared to other shrublands in Riverside County (CCB Biocomplexity Map 2002), so effects of invasive plants on trace gas emissions will likely be more pronounced than if this study were done in an area of higher deposition with correspondingly higher N availability. I will conduct the proposed experiment in Summer 2016 to explore the combined effects of invasion and water pulses on NO\textsubscript{x} and N\textsubscript{2}O emissions.

**Activity 1: Analyze the differences between invasive grasslands and intact shrublands in their effluxes of NO\textsubscript{x} and N\textsubscript{2}O**

*H1: Invasive grasses disrupt soil communities and increase available N in soil and these changes will cause increase N trace gas emissions from invaded CSS than intact CSS.*

Within the Motte Reserve, I will select 6 1-m\textsuperscript{2} plots located at least 20 meters apart from each other and on flat topography, with relatively homogeneous in plant cover for each ecosystem type (e.g. shrub-dominated and grass-dominated, n = 12 plots total). Within each plot, three 30 cm diameter PVC soil collars measuring will be installed to 10 cm depth with addition 5 cm above the soil (n = 36 collars total). Prior to and concurrent with trace gas measurements, temperature and moisture will be measured. Additionally, 3 soil cores will be taken from the top 10 cm of soil within each plot (but outside collars, n = 36 cores total). Each core will be sieved (2 mm mesh) and homogenized in the lab and analyzed for total C and N (FlashEA 1112 NC Analyzer, Thermo Fisher Scientific, Waltham, MA), as well as extractable N, to verify that replicates within each ecosystem type have similar soil N availability. Collar treatments will be randomized within replicates and will include a non-watered collar for measurement of gas fluxes (referred to here as "Collar 1"), a watered collar for measurement of gas fluxes in response to water pulses ("Collar 2"), and a watered collar containing grass litter for measurement of gas fluxes in response to invasive litter inputs ("Collar 3") (see Figure 1).

![Figure 1](image1.png)

*Figure 1.* Proposed experimental design for measuring trace gas flux differences between invasive grass (left) and intact CSS (right) communities. Soil collar numbers signify experimental treatment: 1 = ambient soil conditions; 2 = simulated 3-cm rain event; 3 = 50 gram litter addition. The fourth subplot ("Other meas.," dotted circle) will not have a soil collar but will be used for obtaining C\&N cores and taking other plot-level climate measurements.
Trace gas fluxes tend to be correlated with soil moisture; therefore, semi-arid lands tend to release higher concentrations of NO$_x$ and N$_2$O after a wetting event (Austin et al., 2004). I will measure emissions in dry conditions that are present during the majority of Riverside County's summer as well as emissions that may occur immediately following a rain event, simulated by water addition. Therefore, Collar 1 in each plot will be unaltered, while Collar 2 will receive enough water to simulate a 3-cm rain event. Immediately before adding water to Collar 2, NO$_x$ and N$_2$O will be measured from each collar to serve as pre-treatment controls. Following wetting, measurements will be taken at 15 minutes and 1, 6, 12, 24, and 48 hours.

N$_2$O and NO$_x$ will be measured simultaneously at each timepoint by fitting over the soil collar a chamber top that is connected to two separate instruments which allow for analysis in situ. N$_2$O efflux will be measured using cavity-enhanced laser absorption spectroscopy with a tunable diode laser (Model 908-0014, Los Gatos Research, Inc., Mountain View, CA). NO$_x$ efflux will be measured using the quantitative reaction of NO with ozone followed by UV detection of ozone depletion in combination with a molybdenum convertor (Model 410 and Model 401, 2B Technologies, Boulder, CO). This method has been used in previous lab and field work conducted in the Jenerette lab (Liang et al., 2015b; Oikawa et al., 2015; Eberwein et al. 2015 AGU oral presentation) and has been shown to be effective for analyzing trace gases in arid shrub-dominated ecosystems. By comparing ambient and wetting treatment emissions between community types, I can develop conclusions about how annual grass invasion into shrublands impacts trace gas emissions in a semi-arid climate. I can also begin to address the influence that ecosystem type has on how pulsed rain events impact nitrogen fluxes.

**Activity 2: Analyze the differences in trace gas fluxes in both ecosystems in response to N-rich litter additions**

*H2: Trace gas emissions will be higher in litter-enriched soils than unenriched soils, particularly in intact shrublands, as invasive litter increases available soil N.*

Concurrent with Activity 1, I will measure trace gas emissions of soils that have been enriched with invasive grass litter to simulate additional litter inputs into the soil that would occur as a result of invasion by annual grasses. 4 days prior to the beginning of the study, I will collect a mix of live invasive grass species from the Motte Reserve and dry them at 70 degrees Celsius for 72 hours. After the study begins, immediately before collecting trace gas measurements, I will spread 50 grams grass mixture within Collar 3 of all plots. Concurrent with Component 1, I will take an initial measurement of Nox and N2O emissions and then wet soil to simulate a 3-cm rain event. I will measure at 15 minutes and 1, 6, 12, 24, and 48 hours after wetting. By comparing fluxes of litter-enriched and ambient soils between both CSS and grass ecosystems, I can make predictions about how additional litter inputs from annual grasses affect trace gas fluxes as semi-arid shrublands continue to be invaded. I can also assess how litter influences the impacts of water pulses in semi-arid lands.

**Statistical analysis and future plans**

For each component of this study, I will conduct ANOVA and multiple regression analyses to compare Nox and N2O emissions separately to treatments. If invasive grasslands have increased trace gas fluxes compared to intact shrublands, I will find support for Hypothesis 1. If invasive litter increases the rate of Nox and N2O efflux compared above "bare" soil in both ecosystem types, I will find support for Hypothesis 2. While the proposed experiment can serve as a standalone study, I will continue to explore the effects of grass invasion and rain events on trace gas fluxes across seasons. There is evidence that trace gas emissions vary seasonally in arid ecosystems (Homyak & Sickman,
2014), but seasonal variation in fluxes may be different between intact shrub and invasive grass communities. Nox and N2O emissions show sensitivity to temperature in addition to moisture availability (Liang et al., 2015b); while Southern California lands are mainly moisture-limited, temperature may play a role in seasonal variation in fluxes as well. I plan to maintain soil collars at Motte Reserve for at least a year following experimental setup and replicate my experimental design in Fall 2016, Winter 2016/2017, and Spring 2017 to monitor differences in fluxes between shrublands and grasslands across a suite of temperature and precipitation regimes that Southern California receives throughout a calendar year.

Additionally, I plan to explore changes in the soil microbial communities that result from grass invasion into shrublands that may influence differences in trace gas emissions. The proposed work will determine if and how changes in dominant vegetation affect Nox and N2O fluxes; however, pinpointing a mechanism behind differences in nitrogen cycling will require investigation into the functioning of soil microbial communities between ecosystems. The vast majority of nitrification and denitrification processes is done by a limited suite of soil microbes, and attributing differences in Nox and N2O emissions to differences in plant species is only part of the story. By analyzing soil communities we can begin to explain a mechanism by which intact CSS cycles nitrogen and how invasive plants alter those cycling processes.

**Proposed budget**

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<td><strong>TOTAL</strong></td>
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**Budget justification**

We request one summer quarter of GSR funding ($6442) plus fees and benefits ($273) for Holly Andrews to conduct the proposed study. Because our equipment to measure trace gases is large and must be transported by a model of pick-up truck that is not available through UCR Fleet Services, we will rent a truck through Enterprise. We request PVC piping which will be cut for soil collars. We also request funds to conduct soil C and N, as well as extractable N, analysis at UCR. In total, we request $7954.09 for the proposed project.

**References**


