Linking global change and the N cycle: Interactions between temperature and nutrient availability in controlling rates of asymbiotic N_2-fixation

Introduction: Biological N_2-fixation, the process by which microbes convert unreactive atmospheric N_2 into ammonium (NH_4^+), is an important source of bioavailable nitrogen (N) in many terrestrial ecosystems[1]. N_2-fixing organisms can generally be divided into two categories: symbiotic (microbes living symbiotically in root nodules of leguminous plants) and asymbiotic (microbes such as cyanobacteria that can live freely within soil and leaf litter). Because the net primary productivity (NPP) of many ecosystems is limited by N availability[2], ecosystem N inputs play an important role in regulating ecosystem carbon (C) uptake and therefore climate change[3]. N_2-fixation is an important source of N, but global rates of the process are notoriously difficult to quantify[4]. One roadblock to assessing global N_2-fixation rates is an incomplete understanding of asymbiotic N_2-fixation, which is often considered to occur at negligible rates in comparison to symbiotic N_2-fixation. However, asymbiotic N_2-fixers are the most broadly distributed N_2-fixing organisms globally and may represent the dominant natural source of N for ecosystems in which symbiotic N_2-fixation rates are low[5]. Asymbiotic N_2-fixation may become especially important in the future as it is suggested to respond more quickly to changes in environmental factors, such as climate or nutrient availability, than does symbiotic N_2-fixation[6]. A better understanding of this process and the factors that control it is therefore critical in order to characterize global N cycling and C storage.

In general, asymbiotic N_2-fixation is thought to be limited by phosphorus (P) availability[7,8], as N_2-fixation is an ATP (and therefore P) intensive process, and suppressed by high N availability[9]. However, the magnitude of the effect of N and P availability is variable between studies. Another important control of asymbiotic N_2-fixation is temperature, which exerts a strong control over many N cycling processes[10]. Houlton *et al.* (2008) demonstrate a general relationship between temperature and N_2-fixation rates, in which rates increase in response to temperature up until an optimal temperature of 26° C, past which fixation rates decline[11]. Although these general trends associated with single factors have been established, there are key uncertainties regarding the relationships between factors. Recently, Reed *et al.* (2011) stressed the need for new studies to simultaneously address multiple factors controlling asymbiotic N_2-fixation, as they may interact in ways that cannot be predicted from single-factor studies[6]. For example, some studies suggest that molybdenum (Mo) availability may limit asymbiotic N_2-fixation[9], but moreso when P availability is low[7]. One key uncertainty is the relationship between P availability and N availability. N:P ratios may be better predictors of N_2-fixation rates than N or P availability alone[6], but this relationship is not yet clearly characterized. N:P ratios are likely to change in many ecosystems in the near future as human activities, such as fossil fuel combustion and agricultural fertilizer application, are increasing N availability on a global scale[12]. A second uncertainty is the role of temperature in combination with other factors. As global temperatures rise due to climate change, it is important to consider the relationship that temperature increase may have with changing N:P ratios in controlling rates of asymbiotic N_2-fixation.

I propose to examine interactions between P availability, N deposition, and temperature in controlling rates of asymbiotic N_2-fixation in the tropics via a full-factorial laboratory experiment. Globally, tropical forest ecosystems exhibit extremely high rates of both NPP and N_2-fixation, poising them as key systems in which to study controls of N_2-fixation. I will utilize tropical forest soil samples collected from the Bladen Nature Reserve in Belize, where UC Davis and members of the Houlton lab have established field sites. Bladen is located in the most

biologically diverse rainforest in Belize, yet its biogeochemistry has not been studied in detail. This study will make use of my previous experience in assessing rates of asymbiotic N_2-fixation in boreal peatlands using the acetylene reduction assay, a technique which has been successfully used in tropical forest soils.

Hypotheses: I expect my results to support previous research suggesting that individually, increased P availability supports greater N_2-fixation, increased N deposition suppresses N_2-fixation, and temperature increases past ~25°C inhibit N_2 fixation[11] **(H1)**. I further hypothesize that N deposition will suppress asymbiotic N_2-fixation more strongly in substrates with lower P availability **(H2)**. Several studies suggest that N_2-fixation is highest in ecosystems with low N:P ratios[3,13]. Therefore, substrates with greater P availability may still be able to sustain relatively high rates of N_2-fixation even in the presence of increased N deposition. Finally, I hypothesize that temperature will exert the most powerful control over N_2-fixation; that is, regardless of P and N availability, asymbiotic N_2-fixation will decrease at temperatures deviating from ~25°C **(H3)**. Temperature provides a direct control over N_2-fixation through the inhibition and even degradation of the enzyme nitrogenase, which catalyzes fixation.

Methods: Leaf litter and soil samples from the organic horizon will be collected from two locations along the P gradient within the study site: an area on P-rich parent material (limestone substrate) and an area atop relatively P-poor parent material (volcanic substrate). To examine the relationship between P availability and N deposition, soil and leaf litter samples from each location will be divided into two groups: N addition (NH_4NO_3 solution) and control (distilled water). To determine the role of temperature, samples from each treatment group will be further divided into temperature groups: 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C. Samples will be equilibrated at the appropriate temperature for one week prior to further analysis.

Soil and leaf litter samples will be assessed for N_2-fixation using the acetylene reduction assay (ARA) described by Hardy *et al.*[14] and calibrated with $^{15}N_2$. Mixed headspace gas samples will be collected in 10 mL syringes after 24 hours and analyzed for ethylene content on a gas chromatograph equipped with a flame ionization detector.

Significance and Broader Impacts: Asymbiotic N_2-fixation plays an important, but critically understudied, role in providing available N to ecosystems worldwide. The results of this research will contribute to our understanding of controls of this process. This will be useful in understanding and modeling how rates of N_2-fixation in tropical forests are likely to change in the near future due to both climate change and anthropogenic N inputs. Because the N cycle and C cycle are tightly coupled[3], this data can be used to improve current models of global N cycling, global C cycling, and climate change. To that end, I will make my data publicly available via Dryad, an online database for the long-term storage of ecological data. I will work with undergraduates through the Department of Land, Air, and Water Resources mentorship program and engage them in my lab work, teaching them important skills for research in biogeochemistry. I will also serve as a mentor for high school students from underprivileged backgrounds through SEEDS/EnvironMentors throughout the duration of this project and will help them develop their own projects in N_2-fixation. In this way, I will help prepare a new generation of scientists to tackle important environmental questions.

References: [1]Galloway, J. N. *et al. Biogeochemistry*, (2004). [2]LeBauer, D. S., & Treseder, K. K. *Ecology*, (2008). [3]Wang, Y.-P., & Houlton, B. Z. *Geophys. Res. Letters*, (2009). [4]Hedin, L.O., *et al. Annu. Rev. Ecol. Evol. Syst.* (2009). [5]Vitousek, P. M., *et al. Philos. Trans. R. Soc. Lond., B, Biol. Sci.*, (2013). [6]Reed, S.C., *et al. Annu. Rev. Ecol. Evol. Syst*., (2011). [7]Reed, S. C., *et al. Biogeochemistry*, (2013). [8]Augusto, L., *et al. Global Biogeochemical Cycles*, (2013). [9]Barron, A.R., *et al*. *Nature Geoscience* (2009). [10]Amundson, R., *et al. Global Biogeochemical Cycles*, (2003). [11]Houlton, B.Z., *et al*. *Nature*, (2008). [12]Galloway, J. N., *et al*. *Science*, 892 (2008). [13]Vitousek, P. M., *et al. Biogeochemistry*, (2002). [14]Hardy, R. W. F., *et al. Plant Phys.*, (1968).