Temperature Response of the Respiratory Carbon Costs of Symbiotic Nitrogen Fixation

The problem: While most plants rely on soil nitrogen (N), plants capable of symbiotic N fixation (SNF) can acquire N directly from the atmospheric N_2. Because N_2 is inexhaustible, SNF is convenient. However, SNF has a high cost[2] due to the need to break the triple bond of N_2. Following previous literature[1,2], I define the C cost of SNF as the respiration (CO_2 flux) needed to drive SNF divided by SNF itself (N_2 flux). Biochemical calculations estimate the C cost of SNF to be slightly higher than using nitrate and much higher than using ammonium[1]. Measurements of these costs in nodules (the root structures that house symbiotic bacteria) have been close to the biochemical predictions[2]. However, these measurements have been carried out at constant temperatures. As explained below, the cost might vary widely across temperature.

The cost of SNF helps determine its effectiveness, both within a plant (using SNF vs. soil N) and across species (competition between N-fixing and non-fixing plants). A lower cost makes SNF viable even when soil N is abundant, whereas a higher cost makes SNF untenable even when soil N is scarce. Therefore, variation in the cost of SNF across temperature would have far-reaching implications. For example, it could help explain why N-fixing trees are successful in warm areas[3], and could also affect how SNF will change with climate. Despite its importance to fundamental biology, research on temperature responses of SNF has long been beset by technological constraints. Using a novel method that overcomes these constraints, I will ask one main question: **What is the temperature response of the C costs of SNF?** I will address this question using the tree *Robinia pseudoacacia*, which lives across a wide climatic range, accounts for 64% of tree-based SNF in the contiguous USA[5], and is common across Eurasia[3].

Hypotheses: My hypotheses are based on previous measurements of the components of the cost: respiration and SNF itself. Previous work[6] has observed that SNF plummets at low (near 0°C) and high (near 50°C) temperatures. There are few data on nodule respiration at different temperatures, but leaf respiration continues across 0-50°C[7], suggesting nodule respiration might too. Respiration rates well above 0 divided by SNF rates near 0 mean that ***(H1) the C cost of SNF will be well above the biochemical predictions at low and high temperatures*.**

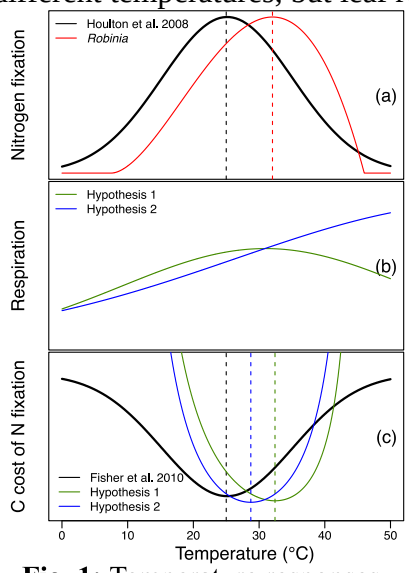

Fig. 1: Temperature responses of (a) N fixation, (b) nodule respiration (hypothesized), and (c) the C cost of N fixation (hypothesized). Dashed lines indicate temperature optima.

My hypotheses about temperature optima stem from measurements from my lab, which recently developed a system for non-destructive, extremely sensitive, and continuous measurements of nitrogenase activity[6]. Preliminary research using this system has shown that the optimal temperature for SNF is much higher (29-36°C) than previously assumed (25°C)[4], as shown in Fig. 1a. I do not know how nodule respiration will change with temperature, so I have competing hypotheses. ***(H2a) If respiration peaks near the same temperature as SNF (green curve, Fig. 1b), then the C cost will have a similar temperature optimum as SNF (green curve, Fig. 1c). (H2b) Alternatively, if respiration rises continually (blue curve, Fig. 1b), as leaf respiration does[6], then the temperature optimum of the C cost will be lower than the optimum of SNF (blue curve, Fig. 1c).***

Hypotheses H1, H2a, and H2b are represented in Fig. the equation for the C cost of SNF that is used in many

models[8](black curve). This equation assumes that the change in C cost of SNF with temperature is inversely proportional to the SNF rate and is scaled to the biochemical C cost of SNF (7.5-12.5 g C g N^{-1}). As explained above, I believe this model is flawed as it does not account for how nodule respiration changes with temperature.

Methods: Using growth chambers at Columbia University, I will grow 30 *Robinia pseudoacacia* seedlings (from seed) under a temperature regime of 26°C during day and 20°C during night using a 14-hour light and 10-hour dark photoperiod with relative humidity and CO_2 concentrations of 70% and 400 ppm to emulate controlled climate conditions[6]. The seedlings will be inoculated with slurries of crushed nodules as well as bacteria cultured from these nodules to ensure the plants can establish symbiotic partnerships, and will be fertilized with limited levels of N (1.5 g N m^{-2} yr^{-1}) but ample amounts of all other nutrients to promote SNF.

The nodules will be measured for SNF and respiration continuously across 1-50°C over the course of 3 hours. The excised nodules will be placed in a sealed chamber with 2% acetylene (the concentration at which the system measures nitrogenase activity most precisely and accurately[6]). After accounting for leakage and other factors[6], the rate at which acetylene is reduced to ethylene (measured with a Picarro G2106 laser) gives a measurement of nitrogenase activity[9]. Preliminary work in our lab has shown that *Robinia* nodules have stable nitrogenase activity at least 6 hours after excision, and that the ratio of $^{15}N_2$ to acetylene reduction is stable across the temperature range of our study (TA Bytnerowicz, pers. comm.).

CO_2 flux in the chamber will be synchronously measured by a Licor LI-6262[6] to determine the temperature response of nodule respiration. I will process and analyze the data by modifying R scripts previously developed in the Menge lab (ref. 4 for processing, TA Bytnerowicz, pers. comm. for temperature responses of SNF). The analysis will yield temperature response curves for SNF, respiration, and the ratio of the two (the C cost of SNF).

Intellectual Merit: This research will answer questions fundamental to the biology of the symbiotic relationship between legumes and N-fixing bacteria. At the level of plant ecophysiology, at what temperatures is it energetically favorable for *Robinia pseudoacacia* to fix N? At the level of community ecology, how does *Robinia pseudoacacia* compete against non-fixing plants if it relies on SNF?

Broader Impacts: The paucity of knowledge on how SNF and its C cost respond to temperature has been a major constraint on global biogeochemistry and climate modeling. As described above, temperature response functions for SNF and for the C cost of SNF are already in use in terrestrial biosphere models, despite few data for the temperature response of SNF itself and zero data for the temperature response of the C cost of SNF. My work will lead to direct improvements in the representation of SNF in these models, and thus will directly influence our ability to predict global biogeochemistry and climate change.

In addition to publishing in academic journals, I will present my work at academic conferences, such as SACNAS' National Diversity in STEM Conference, and outreach programs, such as the Ecological Society of America's SEEDS program and Women In Science at Columbia (WISC). As a former McNair Scholar, I am well aware of the disparity of resources within underrepresented populations. Because of this, I willalso contribute my mentorship to the Environmental Justice and Urban Ecology Summer Research Program, a funded program for high school students at the Washington Heights Expeditionary Learning School.

[1]Gutschick 1981, The American Naturalist. [2]Tjepkema & Winship 1980, Science. [3]Steidinger et al. 2019, Nature. [4]Houlton et al. 2008, Nature. [5]Staccone et al. 2020, Global Biogeochemical Cycles. [6]Bytnerowicz et al. 2019, Methods in Ecology & Evolution. [7]Heskel et al. 2016, PNAS. [8]Fisher et al. 2010, Global Biogeochemical Cycles. [9]Hardy et al. 1968, Plant Physiology.