Measuring plant nitrogen availability in forest soils with lab incubations and phytometer growth assays: a power analysis

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Abstract

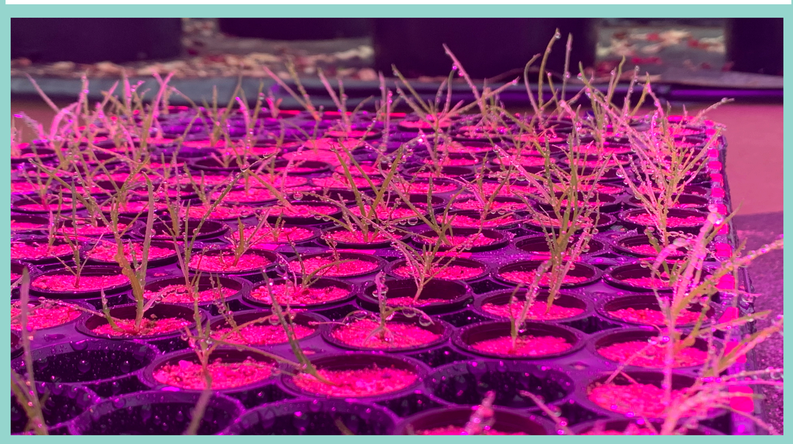
As the most commonly-limiting nutrient in terrestrial ecosystems, nitrogen plays a critical role in carbon sequestration and other ecosystem services. However, it is notoriously difficult to measure the availability of nitrogen in the forms that plants are able to take up. We conducted a combined lab and greenhouse experiment to determine the sampling sizes required to reliably measure plant nitrogen availability in forest soils collected from two plots at The Morton Arboretum, one angiosperm-dominated and the second gymnosperm-dominated. We used two methods to measure plant nitrogen availability in our forest soil samples: lab incubations and phytometer growth. Lab incubations measure mineral nitrogen concentration before and after a two-week incubation period to determine net nitrogen mineralization. Phytometer growth indexes nitrogen availability via height and biomass of seedlings grown in the soil. **Using 40 soil cores per plot, we will determine how many samples are required to have an 80% chance of detecting significant results between plots with a two-fold increase or decrease in nitrogen availability. By determining minimum sample sizes required, this pilot study will aid in the efficient design of an upcoming larger study comparing soil nitrogen availability across 18 plots at The Morton Arboretum**.

Introduction

- Because it is a critical limiting nutrient, plant nitrogen availability affects plant community composition and regulates ecosystem services, such as carbon sequestration. **Plant nitrogen availability is affected by numerous heterogeneous factors, including the history of a given soil and the influence of different plant species**.
- As part of a larger project, we will be estimating plant nitrogen availability in summer 2021 in 18 forest plots at The Morton Arboretum (Lisle, Illinois, USA). **What is the minimum number of replicates we will need per plot?** Using data from two plots dominated by different species, we conducted a power analysis to find out how many samples we will need to take to have an 80% chance of finding significant results between plots with half or double the nitrogen availability compared to a reference.
- There are numerous ways to estimate plant nitrogen availability within soils, and each has advantages and disadvantages. We considered **(1) common, but labor-intensive lab incubations to measure net nitrogen mineralization and net nitrification and (2) the less-common but comparably-easier growth of seedlings (i.e. "phytometers") in the soils in the SES greenhouse.** Lab incubations allow soil microbes to convert organic nitrogen to mineral nitrogen in a common lab environment. Measuring mineral nitrogen concentrations at the time of sample extraction as well as two weeks prior to sample extraction gives a baseline as to how much nitrogen the samples are able to mineralize for plant use in a given amount of time (**i.e. the net mineralization rate**) as well as conversion from ammonium to nitrate forms (**i.e. the net nitrification rate**). Phytometer experiments have long been used to indicate nutrient limitations on plant growth. In the case of our experiment, we measured phytometers in a greenhouse (rather than in a lab or field setting) to better control factors that would impact our measurements. A phytometer experiment allows for the **indexing of nitrogen availability** via its correlation with plant height and biomass (root and shoot).

Methods

- We collected soil samples in the late summer of 2020 at the Morton Arboretum. Of the 90 soil cores we collected, 45 soil cores are from an **angiosperm-dominated plot "QUIB-E" (*Quercus bicolor)*** and 45 soil cores are from a **gymnosperm-dominated plot "PIST-W" (*Pinus strobus*)**. We took these soil samples back to SES and refrigerated them to slow microbial processes until further analysis. All samples were sieved until no plant or animal matter remained in the samples.
- To compare the amount of nitrogen in our soil at the time of sample collection, as well as two weeks after collection, we used an **extraction and incubation method in the lab**. The extraction process is to prepare the samples for analysis of plant-available nitrogen in the soil. These "initial" extractions give data on the amount of nitrogen before microbes were given the opportunity to mineralize additional plant-available nitrogen. We placed 4.0 ± 0.1 g of each soil sample in a falcon tube and added water to rehydrate the soil. We extracted plant-available nitrogen using KCl and placed these "initial" extractions in a freezer. The incubated soil samples will be used to look at the nitrogen that the soil will make available within the span of two weeks- "final" extractions. We placed the soil samples in falcon tubes into a cool, dark tub for two weeks and replaced damp kimwipes on the falcon tubes every other day. After the two-week incubation, these samples underwent the same extraction process as the first round of "initial" extractions. We extracted plant-available nitrogen using KCl and placed the "final" extractions in the freezer as well. Researchers at the Morton Arboretum measured the nitrogen levels of the initial and final extractions.
- We identified the SES greenhouse as the best place to carry out our **phytometer experiment** and used *Poa pratensis* (Kentucky Bluegrass) because of its reliable growth and tolerance of greenhouse conditions. We germinated the seeds in petri dishes with sand prior to transplanting into narrow pots (i.e. "cone-tainers") with homogenized mixtures of 30 mL soil and 12 mL of sand in each cone-tainer. We transplanted similarly-sized emergent seeds into cone-tainers after four days of germination and measured the height of seedlings a week later (i.e. before soil fertility could exert much of an influence). We fertigated cone-tainers once a week with 2 mL Hoglands fertilizer solution containing no nitrogen and only watered lightly on those days. After 41 days, we harvested biomass, dried it to constant mass, and weighed it.



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Results and Conclusion

- We **calculated net nitrogen mineralization and net nitrification from the extraction data**. We used these findings in a power analysis to determine the number of samples needed to have an 80% chance of detecting statistically significant results assuming either half the mean rate or double the mean rate. The **phytometer results also underwent two separate power analyses– one accounting for initial height and total biomass, and one accounting simply for total biomass**. In the graphs, the red data points represent the angiosperm plot, and the blue data points represent the gymnosperm plot.
- **Applying these results to the future Morton Arboretum study, we have decided that taking 20 samples from each of the 18 plots gives a good chance of detecting results that are statistically significant between plots whose nitrogen availability differs by a factor of two.** The Summer 2021 Morton Arboretum study will focus on the residual phytometer data, as all 18 plots will be analyzed using phytometers to measure nitrogen availability in these forest soils. This will require less soil to be taken from each plot. **A random sample of eight of these plots will also undergo the incubation process, which will give us a manageable amount of data to correlate to plant growth measuring variability from the phytometer experiment**.