

October 2020 Thesis Proposal to committee members:

Soil respiration and leaf litter production in the context of a long-term NxP fertilization experiment

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Project Goals and Supporting Objectives

The primary goal of this study is to test for an effect of soil nitrogen (N) and phosphorus (P) availability on litterfall and soil respiration, two of the largest fluxes in the carbon cycle of forests. These data will also allow for an estimation of belowground carbon allocation (BGCA) using the following approximation proposed by Raich and Nadelhoffer (1989): $BGCA = \text{soil respiration} - \text{litterfall}$. This approximation has been critiqued, however, for its assumption that soil carbon is at steady state, so a final objective of this study is to conduct analyses of uncertainty to better understand the consequences of that assumption. To do so, I will quantify the minimum detectable difference for a treatment effect on BGCA in a study of our size.

Project Justification and Relevance

Resource partitioning in plants

Over the last 150 years, the global carbon cycle has been disrupted by the anthropogenic enhancement of atmospheric carbon dioxide (CO₂) concentrations (Quéré et al., 2018). Because of the role of carbon as the foundational element of all life on Earth, its effects upon global climate patterns, and its influence upon a number of biogeochemical processes, understanding how carbon moves through natural systems and how those systems will be affected by the disruption of the carbon cycle is of critical importance. Vegetation constitutes a major pool in the global carbon cycle, storing as much as 650

gigatons of carbon, or the equivalent of 75% of the atmospheric carbon dioxide (CO₂) reservoir (Quéré et al., 2018). Through photosynthesis, plants transform CO₂ into chemically stable carbohydrates, an energy source that can be transported to support vital functions such as growth, defense, reproduction, and nutrient acquisition. All of a plant's chemical resources—and these energy-rich carbohydrates in particular—are distributed such that growth-limiting nutrients can be acquired at higher rates (Chapin et al., 1987); that is, when demand for a particular nutrient is high, available resources are invested heavily in tissues, cells, or proteins specialized in the acquisition of that nutrient. As such, nutrient supply and demand act as powerful drivers of resource partitioning, pushing all plants toward an optimized equilibrium state in which resources are allocated so that all nutrients are equally limiting to plant growth (Bloom et al., 1985; Litton et al., 2007; Rastetter & Shaver, 1992).

Litter production

Previous studies of litter production in fertilization experiments have shown that pre-treatment nutrient concentrations, intensity and duration of fertilization, and forest type determine the extent to which foliar production is affected by nutrient addition. For example, a fertilization experiment across two volcanic Hawaiian soils of differing developmental ages showed that N addition increased litter production in the younger soil but not its more developed counterpart (Vitousek et al., 1995). A NxP factorial study in an Indonesian rainforest showed that plots treated with a combination of N and P experienced increases in litterfall mass after two years of fertilization, suggesting that evidence of nutrient limitation takes time to appear, even in fast-cycling tropical settings (Mirmanto et al., 1999).

Studies conducted in temperate settings have produced varied results, with some reporting increases in litter mass in response to fertilization with N, P, and potassium (K) (Haynes & Gower, 1995) and others finding no such effect (Kim, 2008). To our knowledge, however, no full-factorial NxP experiments have studied nutrient limitation of leaf litter production in temperate settings, so the extent to

which these nutrients limit foliar production individually or interactively is unknown. Nitrogen has long been thought to limit productivity in northern hardwood settings, suggesting that litter production may be N-limited as well. However, since recent findings have begun to point toward P limitation (Goswami et al., 2018), it is possible that the traditional assumption of N-limitation may be unwarranted.

Soil respiration

Soil respiration describes the efflux of CO₂ from the soil to the atmosphere, all of which is ultimately derived from carbon that comes to the belowground system by way of plants' physiological processes. That carbon can be compartmentalized into three categories: (1) carbon derived from plant biomass, (2) carbon respired by fine roots, and (3) carbon exuded into the rhizosphere by fine roots to prime the decomposition of soil organic matter. Each of those categories would be expected to respond to changes in soil nutrient availability: when nutrients are readily available, plants' allocation of carbon to the belowground system decreases. It would stand to reason, then, that soil respiration should do the same.

Indeed, sites with greater N mineralization have been found to have lower rates of soil respiration than otherwise comparable sites with lower N mineralization (Bae et al., 2015). In the same sites, analysis of soil respiration data collected after three years of repeated fertilization showed that nutrient treatments significantly reduced soil respiration but only in those plots with low pre-treatment N fertility (Kang et al., 2016). Fertilization aside, mature stands were also found to have significantly higher rates of CO₂ efflux than their early successional counterparts. In another study in a northern hardwood forest, repeated N fertilization resulted in a reduction of soil respiration beginning in the second year of fertilization and continuing thereafter. Those reductions in soil respiration were mirrored by reductions in microbial respiration, and declines in the productivity of trees in N-treated sites suggested that root respiration and exudation likely fell as well (Bowden et al., 2004). However, Maier et al. (2004) cast

some doubt upon the suggested negative effect of fertilization upon root respiration. They found that while the proportion of total plant respiration represented by soil respiration did indeed fall in fertilized plantation plots, coarse and fine root respiration increased. This trend was not statistically significant, but it illustrates how the many component processes of soil respiration render it an unpredictable phenomenon warranting further study. Investigating how P may affect soil respiration directly or interact with N could offer further insight into the complicated nature of fertilization's effects on soil respiration.

BGCA and nutrient availability

The sum of the three carbon fluxes to the belowground system--(1) incorporation into root biomass by way of belowground net primary productivity (BNPP), (2) respiration in processes of cell and tissue maintenance, or (3) exudation of carbohydrates from roots into the soil matrix for nutrient acquisition--is referred to as belowground carbon flux (TBCF) or belowground carbon allocation (BGCA) (Litton et al., 2007; Raich & Nadelhoffer, 1989). BGCA allows roots to be created and maintained so nutrients and water may be taken up by plants; it sustains microbial communities so nutrients may be mobilized from soil organic matter (SOM); and as plant material decomposes and is incorporated into the soil matrix, this flux ultimately contributes to the enormous pool of carbon stored in soils across the globe (Quéré et al., 2018).

Roots allow plants to absorb critical nutrients such as N, P, and K from soils to then be partitioned throughout the organism. As described above, plant allocation of resources to root systems generally has an inverse relationship with soil nutrient concentrations: as nutrient availability decreases, BGCA increases (Bloom et al., 1985; Hermans et al., 2006). A meta-analysis found that BGCA decreased in response to fertilization in all five studies analyzed (Litton et al., 2007), and BGCA was found to be inversely correlated with N availability in a study conducted in a northern hardwood forest in the White Mountains of New Hampshire (Bae et al., 2015). It is possible, however, that these

relationships are mediated by the magnitude of the nutrient addition. For example, a recent study by Zeng et al. (2020) indicated that BGCA in a temperate forest increased with a low-N addition but decreased in response to a high-N addition. The latter finding would be consistent with soil respiration outcomes in Kang et al. (2016) described above. Of course, this is not a conclusive finding for treatment effects on BGCA, but since soil respiration is an important component of BGCA, it does indicate in which direction a treatment effect may go.

Components of BGCA

By studying subcomponents of BGCA (root production, root maintenance, and root exudation), researchers have been able to shed light on the mechanisms underlying the relationships between nutrient availability and BGCA, specifically its variability across forest types and ages. For example, after N and P additions began in the 2015 N-availability study by Bae et al., fertilization was found to have no effect upon fine-root turnover (Kang et al., 2016). However, in a fertilization study of a Brazilian eucalypt plantation, fine root biomass was found to decrease in response to N addition (Jourdan et al., 2008). Similarly, fine roots in N-treated tropical settings were found to turnover more quickly than their unfertilized counterparts, meaning fewer resources were invested in their upkeep. These findings would suggest that pre-treatment nutrient conditions are an important factor in determining how BGCA in forest ecosystems responds to nutrient additions.

Soil nutrient concentrations control the rate at which root exudates are released as well. Fertilization studies have shown that when the support system of soil organisms is no longer needed for N mineralization, trees invest much less of their carbohydrate resource to sustaining the belowground communities they had previously depended upon for a supply of nutrients. For example, isotopically labeled CO₂ was used to demonstrate that N additions decreased root exudation by 60% in a Swedish

boreal forest (Högberg et al., 2010). Together, these findings indicate that BGCA and its subcomponents are affected by nutrient availability.

Objectives

The goal of this study is to discover how soil respiration and litterfall--two of the largest carbon fluxes in forest ecosystems--are controlled by nutrient availability in northern hardwood forests. Pre- and post-treatment datasets from the MELNHE study stretching from 2008 to the present will be used to analyze both litterfall and soil respiration. Next, annual BGCA will be approximated and tested for responses to N and P addition. This approximation will be made using the method proposed by Raich and Nadelhoffer: $BGCA \approx R_s - P_a$, where R_s represents soil respiration and P_a represents aboveground detrital inputs (Raich & Nadelhoffer, 1989). This approach has been criticized for its assumption that changes in soil carbon in closed-canopy settings are so small as to be insignificant relative to soil respiration and litterfall. For that reason, this study will be accompanied by an uncertainty analysis to determine the implications of that assumption. In sum, the following questions will be explored:

- 1) Is soil respiration affected by N and P addition?
- 2) Is leaf litter production affected by N and P addition?
- 3) To what extent do treatment effects on soil respiration and leaf litter depend upon pre-treatment nutrient conditions?
- 4) How are patterns of BGCA affected by nutrient additions? If potential effects are undetectable, could that be the result of uncertainties inherent in measuring soil respiration and litter production?

Research Approach and Methodology

Site Description

This study will be conducted in thirteen stands in the White Mountains of New Hampshire, USA. Stands are of three successional stages: young (30-35 years old), mid-successional (42-50 years old), and mature (91-137 years old). Stands are spread across three sites in the White Mountain National Forest: nine at Bartlett Experimental Forest (BEF), two at Hubbard Brook Experimental Forest (HBEF), and two at Jeffers Brook (JB). Tree species composition varies across stands, but mature stands are representative of the typical northern hardwood forest: sugar maple (*Acer saccharum*), yellow birch (*B. alleghaniensis*), and American beech (*Fagus grandifolia*). Young and mid-successional consist of red maple (*A. rubrum*), striped maple (*A. pensylvanicum*), pin cherry (*Prunus pensylvanica*), paper birch (*Betula papyrifera*), yellow birch, and American beech. Soils in all stands were formed in glacial till and are predominantly Spodosols with a wide range of drainage characteristics (Bailey, 2020; Vadeboncoeur et al., 2012). Precipitation is evenly distributed throughout the year and amounts to about 127 cm annually. The average annual temperature range in this humid, continental climate is -5 °C to 32 °C (Adams et. al, 2003).

The study stands are part of the Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) study, established in 2011 as the first long-term full-factorial N×P fertilization experiment in a temperate forest. Within each of our 13 stands are four treatment plots, each of which receives one of four nutrient treatments annually: N addition (30 kg N ha⁻¹ yr⁻¹ in the form of NH₄NO₃), P addition (10 kg P ha⁻¹ yr⁻¹ in the form of NaH₂PO₄), N plus P (at the same rates), or neither. In 10 stands, each plot measures 30m x 30m, with a 10m buffer on each side to better ensure that trees with roots that extend beyond the 30x30 area receive the full fertilization. In two stands, each plot measures 20m x 20m with a 5m buffer. In one stand, each plot measures 30m x 30m with a 7.5m buffer. All soil respiration and litterfall measurements will be made in the innermost 30m x 30m or 20m x 20m area.

Field Methods

Litter collection

Leaf litter has been collected annually in the MELNHE stands since 2008. Collections are made from five litter traps with a collecting area of 0.146 m² systematically arranged in each 30m x 30m or 20m x 20m treatment area. In the larger plots, four baskets are about 21m from the corners of the treatment area, and one is in the center. In the smaller plots, four baskets are about 11m from the corners of the treatment area, and one is in the center. Twigs are removed from collected samples, and the samples are oven-dried at 60°C before their oven-dried mass is recorded. For this study, the litterfall record spanning 2008 to 2019 will be analyzed.

Soil respiration

Soil respiration has been measured in these stands since 2008 using LiCOR devices. The number of measurements recorded in a given year and the stands in which those measurements were made has varied from one year to the next (Table 1). Respiration collars are systematically distributed throughout each inner treatment area, and 90-second measurements of soil CO₂ efflux are made at each collar. The number of collars in each plot varies from one year to the next but in most cases ranges from 5 to 7 collars. Soil temperature has been measured at each collar simultaneously with soil respiration, and soil moisture values were recorded from 2013-2018.

The 2020-2021 sampling year will be the most complete sampling year in the dataset, with measurements taking place at every stand beginning from spring 2020 and lasting until the first snowfall of autumn 2020. Three-season measurements were also made during 2010 as a pre-treatment benchmark.

Table 1. Timing of soil respiration measurements in each year

Year	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
2008					X	X			
2009				X	X	X	X	X	X
2010	X	X	X	X	X	X	X	X	X
2011			X	X	X	X	X	X	X
2012			X	X	X	X	X	X	
2013			X	X	X	X	X	X	
2014				X	X	X	X		
2015				X	X				
2016				X	X	X	X	X	
2017				X	X				
2018				X		X			
2019				X	X				
2020		X	X	X	X	X	X	X	X

Belowground carbon allocation

Finally, to achieve an annual estimate of soil respiration, daily soil temperature measurements will be modeled based on relationships between soil temperatures measured alongside our respiration measurements and air temperatures measured at HBEF and at the National Ecological Observatory Network (NEON) at BEF. Those modeled daily temperatures will then be used to model soil respiration based off of relationships between soil temperature and soil respiration observed in our stands. The following function will be used to model soil respiration based off of temperature data collected alongside each measurement of soil respiration: $R_s = ae^{bT}$, where a and b are constants, R_s is soil respiration, and T

is temperature (Van't Hoff, 1884). Soil moisture data could also be used to improve estimates of daily soil respiration.

Data analysis (November 2020 - January 2020)

The hypotheses to be tested in this study include:

H₁: Soil respiration will be reduced by N addition but not by P addition

H₂: Litter biomass will be increased in P-treated plots

H₃: BGCA will be reduced in N- and P-treated plots

H₄: Treatment effects on fine-root biomass will mirror treatment effects on BGCA

H₅: Treatment effects on enzyme activity and microbial biomass growth will mirror treatment effects on soil respiration

Treatment effect on soil respiration, litter biomass, and BGCA

Both litterfall mass and soil respiration will be analyzed using a repeated-measures ANOVA in R with the lmer function in the lme4 package. Individual sampling locations--baskets in the case of litterfall and collars for soil respiration--will be used as observations in analysis. Plot-level BGCA values ($\text{g C m}^{-2} \text{ yr}^{-1}$) will be calculated by subtracting average plot-level litterfall ($\text{g C m}^{-2} \text{ yr}^{-1}$) from average plot-level soil respiration ($\text{g C m}^{-2} \text{ yr}^{-1}$). Measures of uncertainty for both litterfall and soil respiration will be propagated using the sum of squares.

Then, each response variable--litterfall mass, soil respiration, and average BGCA--will be compared across treatments and stands using analysis of variance (ANOVA) in a randomized block design. Two factors will be included: stand age (3 levels) and nutrient treatment (4 levels). Fixed effects will include stand age and treatment, and pre-treatment nutrient conditions will be included as covariates for analysis of soil respiration and BGCA. An example ANOVA model statement would be as follows:

soil.resp ~ stand.age + pre.treat.Nmin + pre.treat.resinP + N*P + (1|year/round/stand/stand.treatment).

Analysis will be conducted in R.

Uncertainty analysis

Using measures of variance in BGCA data, we will be able to quantify the minimum detectable difference of a treatment effect in a study of this size. This will be done using the method outlined in Zar (1984). That minimum detectable difference will then be compared to the size of treatment effects on BGCA found in this study.

Budgets

Timeline:

- April 17, 2020 - April 19, 2020: Thomas orients Brendan to the stands
- April 20 - June 1: Brendan collects soil respiration data
- June 1 - August 7: Thomas collects soil respiration data
- August 7 ~ November 15: Brendan collects soil respiration data
- November 15 - January 31: Data analysis
- February 1 - March 31: Writing

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Appendix

1. Future applications

The three-season soil respiration data that we will collect for this study will be vital to the development of a more comprehensive carbon budget in the future. That project will address one of the primary objectives of the MELNHE project: to determine how patterns of resource allocation in plants respond to nutrient availability.

2. Comments on research methods

Measuring BGCA

The method used to calculate BGCA in this study was developed by Raich and Nadelhoffer in 1989, and it represents a creative and relatively simple way of understanding how vegetation allocates carbon to the belowground system. They began from the premise that the carbon trees allocate belowground is either respired by roots or incorporated into structures or substances that are ultimately respired by decomposers in the soil system. Raich and Nadelhoffer refer to this collection of carbon-containing substances as belowground detritus. This premise can be represented by the following equation:

$$(1) \text{BGCA} \approx P_b + R_r$$

wherein P_b represents belowground detritus and R_r represents root respiration. Next, Raich and Nadelhoffer posit that because the carbon that fluxes out of soil systems due to heterotrophic respiration (R_h) is derived from either aboveground detritus (P_a), such as leaves and woody debris, or belowground detritus, it can be represented using the following equation:

$$(2) R_h = P_a + P_b$$

Subsequently, because the total carbon that fluxes out of soil systems (R_s) is either respired by roots or heterotrophs, the following statement can be made:

$$(3) R_s = R_r + R_h$$

Thus, equations 2 and 3 can be combined algebraically to provide

$$(4) R_s = R_r + P_a + P_b$$

and it follows that using equation 1, equation 4 can be simplified to

$$(5) R_s \approx P_a + \text{BGCA}$$

Finally, algebraic rearrangement of equation 5 provides

$$(6) \text{BGCA} \approx R_s - P_a$$

or, stated verbally, TBCF is the difference of total soil respiration and aboveground detrital inputs (Raich & Nadelhoffer, 1989).

The elegance of Raich and Nadelhoffer's approximation of TBCF is that it allows for a belowground process that is difficult—if not impossible—to observe to be estimated using two easily measurable processes. Both soil respiration and aboveground detrital inputs are commonly measured ecological variables, and combining them in this way allows for an additional ecological and biogeochemical process to be understood with little additional input. Its simplicity, however, belies a crucial and problematic assumption. The method assumes that soil carbon storage in a closed-canopy forest is constant, or, at the very least, its change is negligible in comparison to those other fluxes described in the equations outlined above. This has been challenged by a number of researchers since the method was first published, and methods have been developed in order to account for that important consideration (Gower *et al.*, 1996; Litton *et al.*, 2007). In spite of these concerns, the Raich and Nadelhoffer method was used for the present study because of its relative simplicity and the fact that it could be utilized with available data or data that could be easily gathered.

3. Undergraduate technician quality assurance

Undergraduate technicians will only be used for processing 2019 litter. All of the undergraduates responsible for this data collection have been trained by members of the MELNHE lab group, and I will regularly check in with them to guarantee that the appropriate procedures are being followed.

4. Background information for thesis (chapter one)

It is very likely that a lot that I have included in the project justification and relevance section could be moved here.