Investigating Soil Respiration Responses to N and P Additions

Chase Macpherson

Summer 2018

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Introduction

Soil is an enormous contributor to global carbon fluxes; it is the primary path for CO2 fixed by terrestrial vegetation to be released into the atmosphere (Schlesinger and Andrews, 2000), making it the third highest carbon sink by volume after oceans and land plants (Schlesinger 1997). Total soil respiration is comprised of root-associated respiration and microbial respiration (Hanson et al. 2000). As atmospheric carbon dioxide concentrations increase, understanding how soil respiration is changing in response to human environmental disturbances will greatly aid scientists and policymakers in accounting for its contribution to global climate change.

Soil respiration is heavily affected by nutrient availability and cycling of many elements. As anthropogenic disturbances continue in the cycling of nutrients like nitrogen and phosphorus, it is crucial that we understand how ecosystem functioning is and will be affected. Nutrient addition may modify the metabolic efficiency or activity of organisms, possibly altering soil and aboveground respiration.

Some research suggests that respiration decreases with addition of a system’s limiting nutrient, because trees allocate less biomass to fine root growth when nutrients are more readily available (Litton et al. 2007). There is debate over whether temperate deciduous forests are almost exclusively nitrogen-limited (LeBauer and Treseder 2008), or whether these systems are becoming nitrogen-saturated and therefore are now limited by other nutrients (Aber et al. 1989). Microbial respiration is less well-understood; added nitrogen suppresses microbial respiration (Janssens et al. 2010), but it is unclear whether this is because of changes in enzymatic activity, changes in allocation of resources to growth or enzyme production, or something else entirely (Högberg et al. 2006). The effects of phosphorus or a combination of nitrogen and phosphorus on microbial respiration are less well-understood for microbes as well, but ongoing work aims to explore this. The age of the soils is also an important factor in nutrient limitation; the young soils of the northeastern United States have undergone less weathering, and therefore generally have more available phosphorus. Applying multiple elements, such as both nitrogen and phosphorus, in limitation studies can give insight into system limitations (Rastetter and Shaver, 1992).

Researchers with the Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) project, in experimental forests in the White Mountains of NH, USA began measuring soil respiration in 2008. These studies include thirteen stands of three age classes (young, mid-age, and old), and four treatments have been applied (N addition, P addition, N+P addition, and control) since 2011. This project uses a full factorial design, and is the longest-running full factorial NxP experiment in a temperate forest in the world. This work has included the use of minirhizotrons for measuring fine root turnover, lab incubations of soil microbes to measure their respiration, and measuring soil N mineralization and litterfall N flux (Kang et al., 2016). Past data analyses from this project have found that soil respiration responses to nutrient addition depend on site fertility (Kang et al., 2016) and forest age (Li unpublished 2016), and that phosphorus and calcium can be important limiting nutrients when N is relatively abundant (Naples and Fisk, 2009). Variability in the respiration data, though, has been high, and has motivated desire for further study.

Objectives and Hypotheses

This study aimed to add to the existing data set of soil respiration for all four treatments of all three age classes, and to add to analyses done on the data set as a whole. Based on what is known about soil respiration and previous findings from this site, it was predicted that respiration responses would vary with forest age, although data on this relationship has been inconsistent (Li unpublished 2016, Kang et al. 2016, Fahey unpublished 2017). While Kang et al. (2016) found no significant interaction between age and treatment in respiration, Tim Fahey’s work in 2017 showed a greater treatment effect in fine root biomass in young stands than old ones, which could be caused by heightened competition and a stronger response to stimuli as a result. He found increases in fine root biomass with N addition, which would be expected to increase root respiration. The expected decrease in microbial respiration caused by N addition, though, could offset it. Therefore, it was hypothesized that the treatment effect on total respiration would be least significant in young stands and greatest in old stands, where fine root biomass responds the least to nutrient addition.

The N+P interaction has not had a significant effect on respiration in the past, so this trend was predicted to continue. P addition has increased aboveground growth in previous years (Goswami et al. 2017), but has not significantly impacted respiration, so it is expected to continue not having a significant effect. Finally, it was hypothesized that treatment could affect flux responses to temperature changes. Nitrogen addition’s effect could change at higher temperatures as the balance between microbial activity and increased root biomass change, in favor of an overall increase in respiration.

Methods

This study took place at two sites in 2018; in the White Mountain National Forest, NH, USA. These are the Bartlett Experimental Forest (BEF; 44º2-4’N, 71º9-19’W; elevation 250-500 m) and Hubbard Brook Experimental Forest (HBEF; 43º’56’N, 71º44’W; elevation 500 m). Due to the difficulty of access, soil respiration measurements were not gathered from Jeffers Brook this year. Thus I collected data in 11 stands (9 at BEF and 2 at HBEF). This area is in a humid continental climate, with average temperatures of 19 ºC in July and -9 ºC in January (at 450 m elevation). Precipitation is evenly distributed throughout the year, with an average of approximately 140 cm (Bailey et al. 2003). The soils are largely Haplorthods derived from glacial till, originating from granitoid and high-grade metamorphic silicate rock (Bailey et al. 2003, Vadeboncoeur et al. 2014). Both sites’ soil textures are loamy sands under an organic horizon of variable thicknesses (Bailey et al. 2003, Vadeboncoeur et al. 2014).

Each stand contains 4 treated 50 x 50 m plots, with 30 x 30 m of measurement area and a 10 x 10 m area of buffer (treatment but no measurement). The exceptions to these areas are 2 smaller mid-age stands, one at Hubbard Brook and one at Jeffers Brook, which are 20 x 20 m with a 5 x 5 m buffer. Each plot is positioned on similarly rugged topography. Low levels of nitrogen, phosphorus, and a combination of the two were applied to the plots (at 30 kg/ha/yr of N and 10 kg/ha/yr of P), to alleviate any possible nutrient limitations (rather than saturate the system). The measurement areas for respiration are fertilized separately with 0.278 g NH4NO3 or 0.122 g NaH2PO4 (or a combination of the two) per area, to increase consistency and accuracy in fertilizer application.

Soil respiration was measured using a LI-8100 FCO2 system (Licor Biosciences, Lincoln, NE), which included measurements for CO2 flux, soil moisture, and temperature. Measurements were taken at every plot, each of which contained seven areas of measurement. PVC collars with a diameter of 20 cm were used to define the areas. The use of seven collars per plot helps to decrease the effect of the small, local differences in elevation and topography within each plot. Measurements were taken once for each stand, within the timeframe of approximately 9:30 am - 2:30 pm, and stands measured in the morning during one measurement period would be measured in the afternoon for the second measurement period (and vice versa).

For analysis, data from 2011 to 2018 (the years of treatment) was compiled and tidied. Data analysis was done in R (R Core Team, 2018), using a combination of parametric and non-parametric tests. The Shapiro-Wilk test was used to test for normality, and the Kruskal-Wallis test was used for explaining effects. Because the Kruskal-Wallis test cannot account for interaction, a factorial ANOVA blocked by stand was used for testing the interaction between N and P, because of the factorial design of the experiment. While this test does assume normality, and this is problematic for this data set, the interaction analysis was highly desirable. Challenges in R prevented the actual interaction analysis, but the results of the individual treatments are reported. Plot average data was used for all analyses, to avoid pseudo-replication. Power analyses using the package “stats” in R were done to examine the effects of past data variability on the power of these analyses, and finally, Tukey’s Honestly Significant Difference (HSD) test was used to judge inter-stand variation.

Results

The Shapiro-Wilk Normality Test on the flux and log of flux data revealed that the data are not in fact normally distributed. A Kruskal-Wallis test on flux explained by treatment provided a p-value of 0.77 for 2018, and 0.57 for the compiled 2011-2018 data (see Figures 1 and 2). Contradictory to the initial hypothesis, there was a reduction in total respiration in young stands with P addition (p=0.02). Additionally, there was a marginally significant overall interaction between age and treatment (p=0.09), but no significant interaction specifically between age and N treatment.

Plotting flux and temperature displayed the expected positive relationship between the two variables (see Figure 3). The appearance of a slightly different slope for the N-treated plots suggests that there could be a difference in temperature response in flux for N-treated soil, but this was not verified.

Lastly, a power analysis using the standard deviation of all of the combined data and a power of 0.8 revealed that the necessary number of collars would be approximately 381. Tukey’s HSD test comparing stands revealed that the most dissimilar stand from the others in its age class were C1 for the young, C4 for the mid-aged, and JBO for the old. Among the old stands, C7 and C8 were very similar, as were C9 and HBO, so this age class had the most variability.

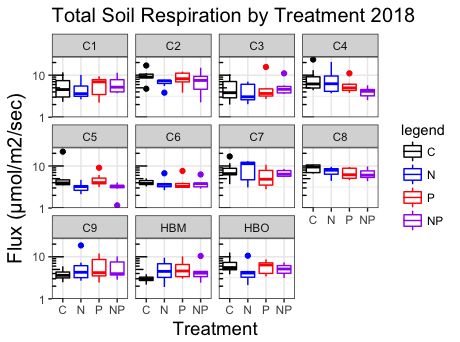


Figure 1. Total flux data from 2018 only (one round of collection) separated by stand and on a log scale.

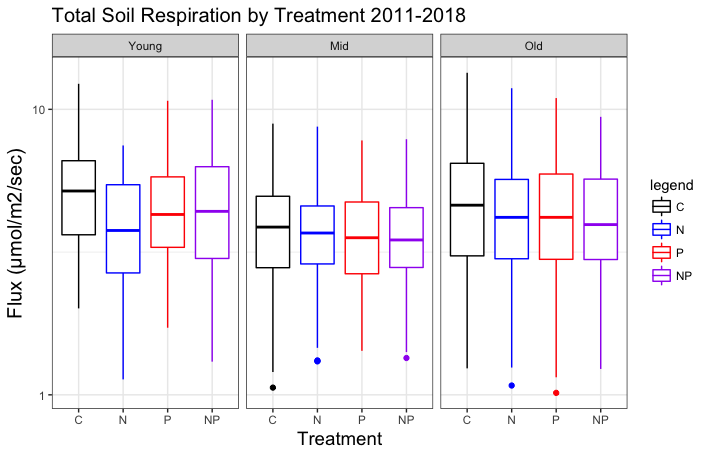


Figure 2. Total flux data from all years of treatment separated by stand age and on a log scale.

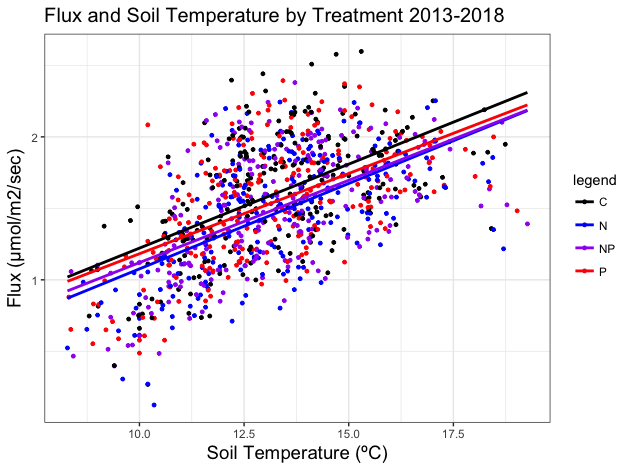


Figure 3. Flux data from 2013-2018 (excluding years 2011 and 2012, due to inaccurate soil temperature data collection). Trend lines separated by treatment.

Discussion

The reduced respiration in young stands with phosphorus addition suggests P limitation. This is in accordance with the 2017 findings, so perhaps the increased nutrient availability increased fine root biomass or microbial growth. The fact that young stands seem to be phosphorus limited suggests that weathering could be a limiting process, keeping phosphorus locked in inorganic material.

It is possible the initial hypothesis was correct about the balancing effect of increased fine root biomass and decreased microbial respiration. However, there is not a stronger N effect in older stands, so overall the expected relationship between nitrogen addition and respiration is not supported. It is interesting that the effect of N on respiration seems stronger than that of P in the graphic representation of the data, but that the statistical interpretation was opposite.

In the future, many more analyses could be done on this data set. Rather than using somewhat robust parametric tests with non-normal data, finding the actual distribution and accounting for that in models would improve the accuracy of the results. It is likely that the log of the flux data are in a Poisson distribution, but using the fitdistrplus package in R could confirm this. Once the distribution is confirmed, mixed effect models could be made, and by comparing their AIC values, the most parsimonious one could be determined. Furthermore, looking more into the temperature response data analysis could be beneficial. Using an exponential fit would better suit this relationship, and then calculating a temperature coefficient, such as Q10, for each treatment would reveal whether there really is a significant difference between changes in flux with changes in temperature.

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Appendix 1:

Chase Macpherson Collaborators:

June 11, 2018 Ruth Yanai

MELNHE Melany Fisk

Tim Fahey

Alexander Young

Alexandrea Rice

Investigating Soil Respiration Responses to N and P Additions

Introduction

Soil is an enormous contributor to global carbon fluxes; it is the primary path for CO2 fixed by terrestrial vegetation tobe released into the atmosphere (Schlesinger and Andrews, 2000), making it the third highest carbon sink and source by volume after oceans and land plants (Schlesinger 1997). Total soil respiration, or flux, (FCO2) is mainly comprised of root-associated and microbial respiration (Hanson et al. 2000). As atmospheric carbon dioxide concentrations increase, understanding how soil respiration is changing in response to human environmental disturbances will greatly aid scientists and policymakers in accounting for its contribution to global climate change.

Soil respiration is heavily affected by nutrient availability and cycling dynamics of many elements besides carbon. As anthropogenic disturbances continue in the cycling of nutrients like nitrogen and phosphorus, it is crucial that we understand how ecosystem functioning is and will be affected. Nutrient addition may modify the metabolic efficiency of soil organisms, altering the resultant respiration and ecosystem level carbon flux.

Some research suggests that respiration decreases with added nitrogen, because trees allocate less biomass to fine root growth when nutrients are more readily available (Litton et al. 2007). This trend should be the most significant for the most limiting nutrient in the system. There is debate over whether temperate deciduous forests are almost exclusively nitrogen-limited (LeBauer and Treseder 2008), or whether these systems are becoming nitrogen-saturated and therefore are now limited by other nutrients (Aber et al. 1989), so using multiple elements can give more insight into this process (Rastetter and Shaver, 1992). Microbial respiration is less well-understood; research has shown that added nitrogen suppresses microbial respiration (Bowden et al. 2004), but it is unclear whether this is because of changes in enzymatic activity, changes in allocation of resources to growth or enzyme production, or something else entirely. The effects of phosphorus or a combination of nitrogen and phosphorus on microbial respiration are under-studied, but ongoing work at Bartlett Experimental Forest in part aims to explore this.

Researchers with the Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) project, based at the Bartlett Experimental Forest in NH, USA began measuring soil respiration in 2008 (before annual fertilization, which began in 2011). Thirteen stands of three age classes (young, mid-age, and old) have been given four treatments; N addition, P addition, N+P addition, and control. This project uses a full factorial design, and is the longest-running full factorial NxP experiment in a temperate forest in the world. This work has included the use of minirhizotrons for measuring fine root turnover, lab incubations of soil microbes to measure their respiration, and measuring soil N mineralization and litterfall N flux (Kang et al., 2016). Past data analyses from this project have found that soil respiration responses to nutrient addition depend on site fertility (Kang et al., 2016) and forest age (Li unpublished 2016), and that phosphorus and calcium can be important limiting nutrients when N is relatively abundant (Naples and Fisk, 2009). Variability in the data, though, has been high, and has motivated desire for further study.

Objectives and Hypotheses

This study aims to add to the existing data set of FCO2 for all four treatments of all three age classes. Based on what is known about soil respiration and previous findings from this site, I hypothesize that the addition of N will decrease respiration due to decreased belowground C allocation and decreased microbial activity. I expect it to decrease most significantly in N-poor sites (with low net N mineralization, as measured in 2015), which include BEF C1, C2, and C8 (see Table 1). These sites will likely have the highest initial proportion of carbon in root biomass, so the decreases will be proportionally large. I also predict that respiration responses will vary with forest age, although data on this relationship has been inconsistent (Li unpublished 2016, Kang et al. 2016, Fahey unpublished 2017). In one case, a 50% difference in fine root biomass between stands of different ages did not produce significant differences in respiration. This is inconsistent with previous observations on the relationship between root biomass and respiration, which would suggest that respiration would respond to nutrient addition, so this has been another motivator for further data collection.

Although we see some things changing with treatment, the N+P treatment has not had a significant effect in the past, so I predict that this trend will continue and we will observe neither significant increases nor decreases in respiration.There is also variability in this data, though, and some age classes have shown slight decreases under this treatment, so it is possible we will see some variation again. Finally, P addition will likely not have a significant effect, as it has not significantly impacted respiration in previous trials.

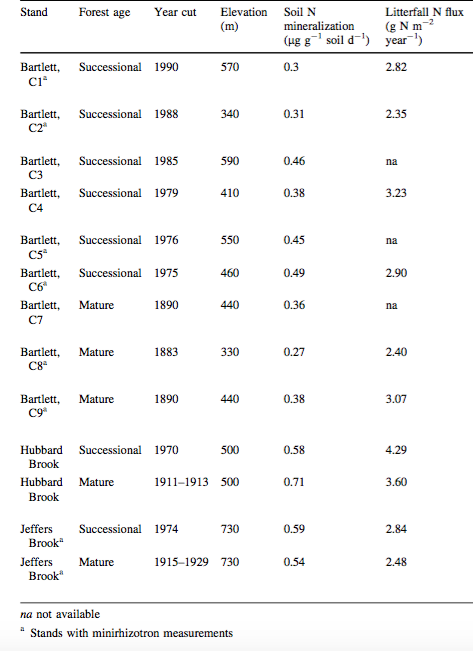


Table 1. Site details and information on nitrogen cycling for Bartlett, Hubbard Brook, and Jeffers Brook Experimental Forests from Kang et al. (2016). Soil N mineralization is used as an indicator for site fertility, and is assumed to not have changed significantly since measurement in 2015.

Methods

This study would take place at two sites in the White Mountain National Forest, NH, USA. These are the Bartlett Experimental Forest (BEF; 44º2-4’N, 71º9-19’W; elevation 250-500 m) and Hubbard Brook Experimental Forest (HBEF; 43º’56’N, 71º44’W; elevation 500 m), and they are comprised of a total of 11 stands (9 at BEF and 2 at HBEF). This land is in a humid continental climate, with average temperatures of 19 ºC in July and -9 ºC in January (at 450 m elevation). Precipitation is evenly distributed throughout the year, with an average of approximately 140 cm (Goswami et al. 2017). The soils are largely Haplorthods derived from glacial till, originating from granitoid and high-grade metamorphic silicate rock at Bartlett Experimental Forest and Hubbard Brook (Bailey et al. 2003, Vadeboncoeur et al. 2014). Both sites’ soils are loamy sands under an organic horizon of variable thicknesses (Bailey et al. 2003, Vadeboncoeur et al. 2014).

Each stand contains 4 treated 50 x 50 m plots, with 30 x 30 m of measurement area and a 10 x 10 m area of buffer (treatment but no measurement). Each plot is positioned on similarly rugged topography, in attempt to control for forest composition and soil quality. Low levels of nitrogen, phosphorus, and a combination of the two were applied to the plots (at 30 kg/ha/yr of pelletized NH4NO3and 10 kg/ha/yr of granular NaH2PO4), to alleviate any possible nutrient limitations (rather than saturate the system). Total soil respiration (FCO2) would be measured using a LI-8100 FCO2 system (Licor Biosciences, Lincoln, NE), which includes measurements for CO2 flux (µmol/m2/sec), soil moisture (volts), and temperature (ºC). Measurements would be taken at every plot in every stand (C1-C9 at BEF, HBO [old], and HBM [mid-age]), and would be measured using the seven PVC collars set up in each plot that define the area of measurement, which each have a diameter of 20 cm. The presence of seven collars per plot helps to decrease the effect of the small, local differences in elevation and topography within each plot. These collars are fertilized separately from the rest of the plots with 0.278 g NH4NO3 or 0.122 g NaH2PO4 (or a combination of the two) per collar, to increase consistency and accuracy in fertilizer application. Measurements would be taken twice for each stand, within the timeframe of approximately 9:30 am - 2:30 pm, and stands measured in the morning during one measurement period would be measured in the afternoon for the second measurement period (and vice versa).

Expected Results

I expect to find that young stands have higher respiration in control plots, and that nitrogen fertilization has a greater suppressing effect on young stands’ soil respiration (see Figure 1), and also that sites that had lower measured net N mineralization will show greater decreases in respiration after N addition (see Figure 2).

Total Soil Carbon Flux (µmol/m2/sec)

Young

Mid-age

Old

Treatments

= Control

= N addition

Figure 1. A graph of projected results for soil respiration response to nitrogen addition for young, mid-age, and old stands at Bartlett Experimental Forest. I expect to see higher respiration in control plots and greater decreases in respiration in N addition plots for younger stands than older ones.

Initially N-Poor

Initially N-Rich

Total Soil Carbon Flux (µmol/m2/sec)

Treatment

= Control

= N addition

Figure 2. Projected results for respiration response to added N based on inherent site fertility. I expect to see greater decreases in respiration from N addition in less fertile (more N-poor) sites.

Budget and Timeline

Researchers at the Hubbard Brook Experimental Forest own a Li-8100 that we have access to, so purchasing one would not be necessary. The measurements would be collected over two approximately 6-day periods, requiring the work of about 2 people each day. If time and weather permitted, a third collection period would be added.

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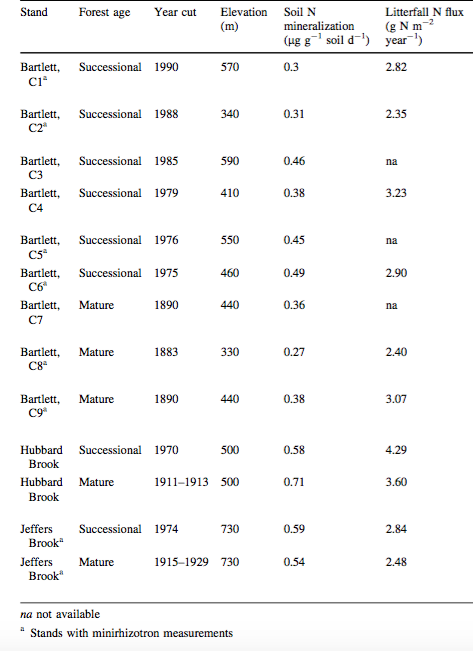


Table 1. Site details and information on nitrogen cycling for Bartlett, Hubbard Brook, and Jeffers Brook Experimental Forests from Kang et al. (2016). Soil N mineralization is used as an indicator for site fertility, and is assumed to not have changed significantly since measurement in 2015.

Methods

This study would take place at two sites in the White Mountain National Forest, NH, USA. These are the Bartlett Experimental Forest (BEF; 44º2-4’N, 71º9-19’W; elevation 250-500 m) and Hubbard Brook Experimental Forest (HBEF; 43º’56’N, 71º44’W; elevation 500 m). Due to the difficulty of access, Jeffers Brook is not proposed for soil respiration measurements this year. Thus I will collected data in 11 stands (9 at BEF and 2 at HBEF). This land is in a humid continental climate, with average temperatures of 19 ºC in July and -9 ºC in January (at 450 m elevation). Precipitation is evenly distributed throughout the year, with an average of approximately 140 cm (Goswami et al. 2017). The soils are largely Haplorthods derived from glacial till, originating from granitoid and high-grade metamorphic silicate rock (Bailey et al. 2003, Vadeboncoeur et al. 2014). Both sites’ soils are loamy sands under an organic horizon of variable thicknesses (Bailey et al. 2003, Vadeboncoeur et al. 2014).

Each stand contains 4 treated 50 x 50 m plots, with 30 x 30 m of measurement area and a 10 x 10 m area of buffer (treatment but no measurement). Each plot is positioned on similarly rugged topography, in attempt to control for forest composition and soil quality. Low levels of nitrogen, phosphorus, and a combination of the two were applied to the plots (at 30 kg/ha/yr of pelletized NH4NO3and 10 kg/ha/yr of granular NaH2PO4), to alleviate any possible nutrient limitations (rather than saturate the system).

soil respiration (FCO2) would be measured using a LI-8100 FCO2 system (Licor Biosciences, Lincoln, NE), which includes measurements for CO2 flux (µmol/m2/sec), soil moisture (volts), and temperature (ºC). Measurements would be taken at every plot in every stand (C1-C9 at BEF, HBO [old], and HBM [mid-age]), and would be measured using the seven PVC collars set up in each plot that define the area of measurement, which each have a diameter of 20 cm. The presence of seven collars per plot helps to decrease the effect of the small, local differences in elevation and topography within each plot. These collars are fertilized separately from the rest of the plots with 0.278 g NH4NO3 or 0.122 g NaH2PO4 (or a combination of the two) per collar, to increase consistency and accuracy in fertilizer application. Measurements would be taken twice for each stand, within the timeframe of approximately 9:30 am - 2:30 pm, and stands measured in the morning during one measurement period would be measured in the afternoon for the second measurement period (and vice versa).

Expected Results

I expect to find that young stands have higher respiration in control plots, and that nitrogen fertilization has a greater suppressing effect on soil respiration in young stands (Figure 1), and also that sites that had lower measured net N mineralization will show greater decreases in respiration after N addition (Figure 2).

Total Soil Carbon Flux (µmol/m2/sec)

Young

Mid-age

Old

Treatments

= Control

= N addition

Figure 1. A graph of projected results for soil respiration response to nitrogen addition for young, mid-age, and old stands at Bartlett Experimental Forest. I expect to see higher respiration in control plots and greater decreases in respiration in N addition plots for younger stands than older ones.

Initially N-Poor

Initially N-Rich

Total Soil Carbon Flux (µmol/m2/sec)

Treatment

= Control

= N addition

Figure 2. Projected results for respiration response to added N based on inherent site fertility. I expect to see greater decreases in respiration from N addition in less fertile (more N-poor) sites.

Budget and Timeline

Researchers at the Hubbard Brook Experimental Forest own a Li-8100 that we have access to, so purchasing one would not be necessary. The measurements would be collected over two approximately 6-day periods, requiring the work of about 2 people each day. If time and weather permit, a third collection period will be added.

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