The Effects of Soil Warming and Freezing on Microbial Activity:
Substrate-induced Respiration as a Measurement of Microbial Biomass

Emily Whalen[1,2]; Carol Adair[1,2]; Stephanie Juice[2]

[1]Vermont EPSCoR, Research on Adaptation to Climate Change (RACC), [2]University of Vermont

Introduction

The Northern Forest Mesocosm Climate Change Experiment (NForM) aims to understand how climate change – which is projected to induce climate warming, reduce cold wintertime temperatures, and increase soil freezing – will impact C, nutrient and water balances in the Northern Forest[4]. The study integrates two soil types ("Kullman" and "Milton"), with three treatments: heat, snow removal (to induce soil freezing), and a control.

To better understand the factors driving C and nutrient cycling in the NForM Experiment, and more broadly, in the Northern Forest, this study aims to determine how soil microbial populations will be affected by climate change. Soil microbes influence nutrient mineralization, creating a labile source of major plant nutrients[2,5]. Soil microbial biomass C measurements are also used as an indicator of soil health[2,3].

Methods and Materials

Substrate-induced respiration (SIR) is a rapid, laboratory-based technique commonly used to estimate carbon (C) concentrations in soil microbial biomass[1]. We performed soil incubations utilizing glucose as a substrate to stimulate microbial respiration (SIR) and without glucose as an added substrate (basal respiration, BR). CO_2 evolution from each incubation was measured using gas chromatography. In SIR, CO_2 evolution is related to microbial biomass C through the equation: mg biomass C 100 g^{-1} soil = 40.04*(mL CO_2 h^{-1}100g^{-1} soil) + 0.37.

Sample Preparation.
- 11 ¾" 0-10cm soil cores taken from each treatment replicate. Samples homogenized and sieved to 2mm to remove rocks and roots.
- Two 50g dry weight equivalent subsamples weighed into 2 specimen cups, one for SIR and one for BR.
- Two preliminary incubations performed to gauge glucose saturation and microbial growth curves.
- Appropriate volume and concentration of glucose solution added to each SIR sample, and inserted into quart-size Mason jar. Equivalent volumes DDI water added to corresponding BR jars.
- 15mL gas samples collected every hr for 5 hr from each jar.
- Gas samples inserted into 10mL vials and analyzed for [CO_2] using a Shimadzu GC-17A Gas Chromatograph.

Data analysis. Two separate 2 (Soil Type) x 3 (Treatment [Control, Heated, Snow exclusion]) ANOVAs were conducted to analyze microbial biomass C and BR measurements. All comparisons were tested at an *a priori* significance level of p=0.05 using JMP and R statistical software.

Hypotheses: We expected that:
1) Soil warming and freezing would reduce microbial biomass in both soils, and
2) microbial biomass would be lower in Kullman vs. Milton soils, due to water stress induced by the low water-holding capacity of Kullman.

Figure 2. Incubation jar set-up

Results

•**SIR**. The main effect for Soil type (Kullman, Milton) approached statistical significance (p=0.08), but failed to meet the *a priori* significance level. There was no main effect for Treatment or interaction (p > 0.05). However, a *post hoc* t-test showed that microbial biomass C was lower in Milton compared to Kullman soil after snow exclusion (p=0.053).

•**BR**. No significant main effects and no significant interaction were observed (all p's > 0.05).

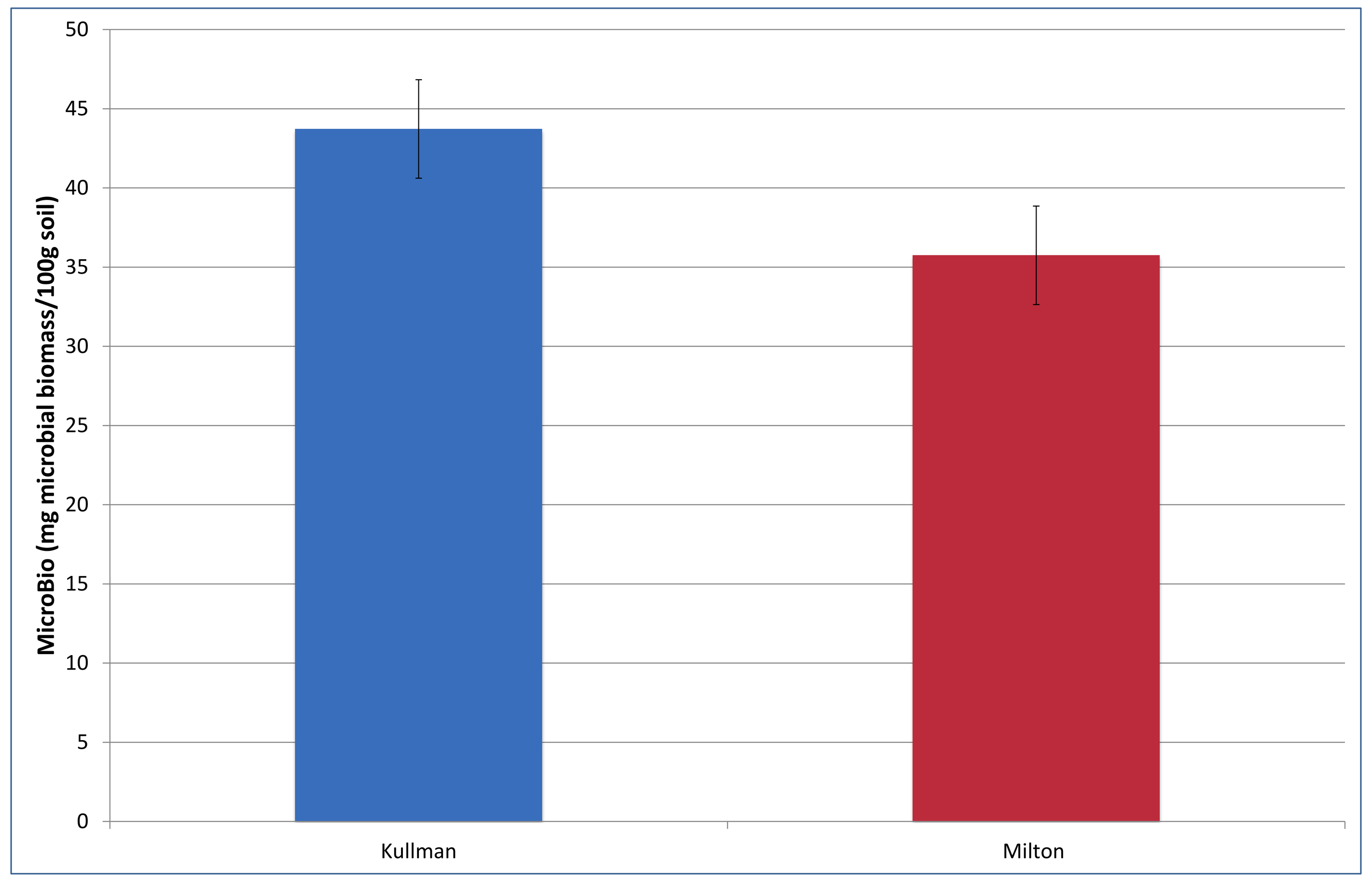

Chart 1. Least squares mean comparison of Kullman and Milton Soils (SIR); p=0.08

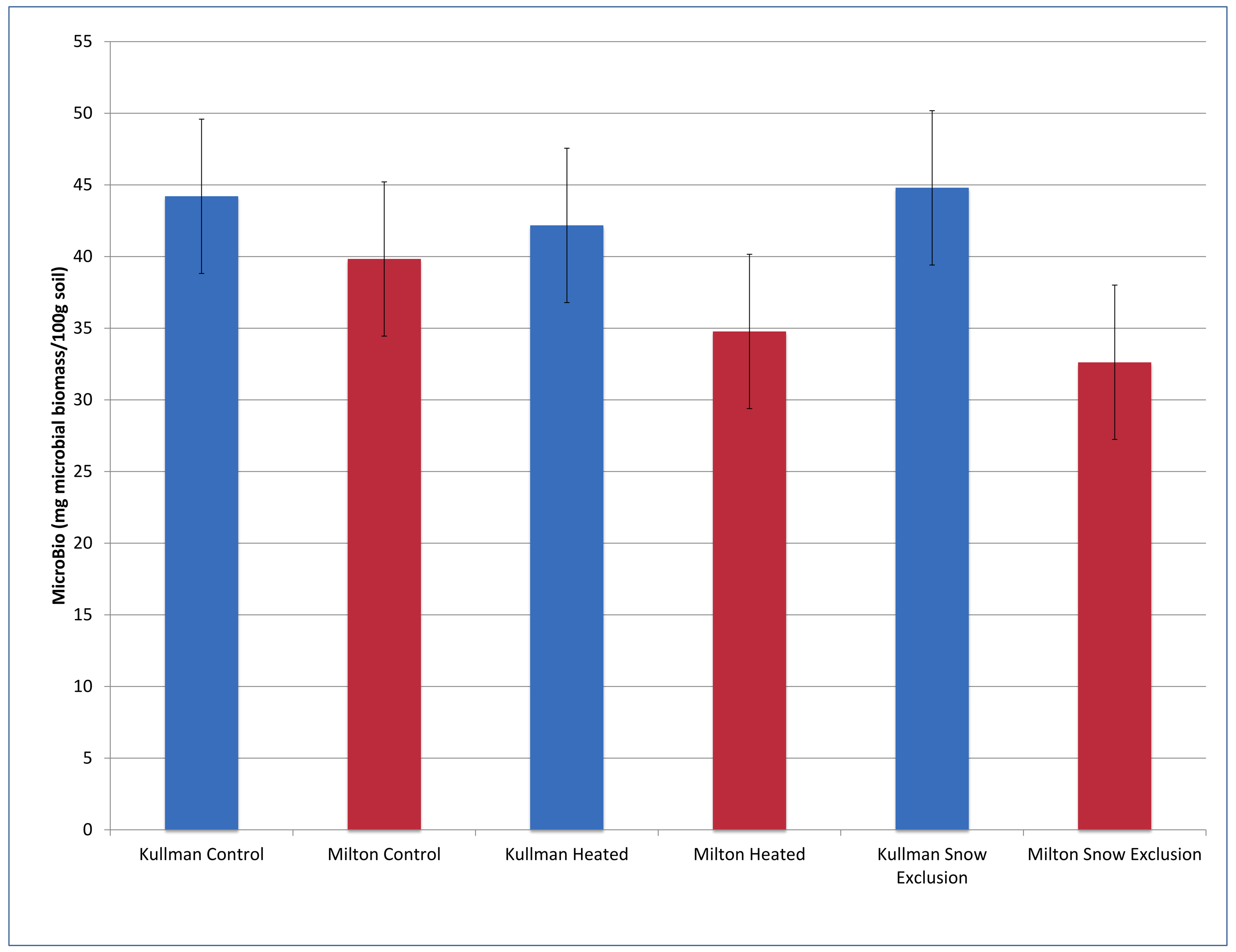

Chart 2. Least squares mean comparison of microbial biomass C ('MicroBio') by soil and treatment (SIR); p=0.77

Figure 3. View of study area and mesocosms

Discussion

We sought to assess the effect of temperature manipulations (i.e., heat vs. snow removal) on microbial biomass C ('MicroBio') levels in two types of soil (i.e., Milton vs. Kullman). In the modest study executed here we did not observe any large effects of these treatments.

However, we observed a trend toward greater levels of MicroBio in Kullman soil regardless of treatment condition, and a *post hoc* trend for MicroBio levels to be lower in Milton soil after snow exclusion.

The *post hoc* finding that snow exclusion might have decreased MicroBio levels specifically in Milton soil should be followed up. It is possible that a soil texture difference between Milton and Kullman (Milton=smaller particle size) increases soil freezing in Milton soils, due to increased retention of soil pore water. Given the lack of interaction in the omnibus ANOVA, the *post hoc* test performed here was meant to be purely descriptive. These possibilities will remain speculative until future studies show this effect to be reliable.

As the larger NForM Experiment was initiated in spring 2013, it is likely that treatments had not yet significantly impacted soil microbial populations. In addition, the snow removal treatment may not have been evident due to seasonal effects. That is, since soil samples were collected in August 2014, the effects of snow removal may have been minimized by the time samples were collected in the summer. Future studies could compare the present results with samples collected in autumn (early soil freezing) or spring (late soil freezing/early thaw), to elucidate any such temporal effects on microbial activity.

Conclusions

•Results are preliminary. The *post hoc* nature of the finding that snow exclusion might have decreased MicroBio in Milton should be followed up to show results to be reliable.

•It is recommended that future studies increase sample size to potentially decrease variability of SIR and BR measurements.

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Contact

Emily Whalen
University of Vermont, '14
Email: emilywhalen92@gmail.com
Phone: 603-715-7101

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