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Exploring the effects of C-amendment on the soil microbial community in a Puget lowland prairie

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ABSTRACT

Scotch broom is a non-native plant that has invaded plant communities worldwide, including that of the Pacific Northwest in the United States. It associates with soil bacteria to fix nitrogen (N) from the atmosphere, thereby elevating N-levels in the soil and crowding out native plants by promoting the invasion of other non-natives like itself. Researchers have used carbon (C)-amendment to lower soil N and restore native plant growth. However, little is known about C-amendment's effects on the soil microbial community that correspond to changes in soil N and plant community composition. Our study aims to investigate how C-amendment affects the functionality of the soil microbial community in a Puget lowland prairie invaded by Scotch broom. By understanding the effects of C-amendment on both the plant and microbial communities, we can better assess its effectiveness at native plant restoration in the future.

INTRODUCTION

Add C
↓
Increase microbes
↓
Decrease soil N
↓
Decrease non-native plant coverage
↓
Increase native plant coverage

Researchers propose that C-amendment affects different bacteria at different times following treatment. To begin addressing the question of how C-amendment affects the structure of the soil microbial community, we will first determine whether or not there are functional differences in the metabolism of microbes in untreated (control) versus treated soil.

MATERIALS & METHODS

Figure 1. Glacial Heritage Preserve, Olympia, WA

Study Site & Experimental Setup

We set up twenty 0.50 m^2 plots (ten each of control and sugar-treated) with moderate broom coverage at Glacial Heritage Preserve in Olympia, WA (Figure 1). We added 1000 g of C total (≈ 3 cups of sugar) to sugar-treated plots in increments of one cup every two weeks. We collected soil samples every two weeks (with one exception).

Timeline

Week 0: collected soil samples before treatment added first cup of sugar
↓
Week 2: collected soil samples before treatment added second cup of sugar
↓
Week 4: added third cup of sugar
↓
Week 6: collected soil samples
↓
Week 8: collected soil samples

MATERIALS & METHODS (continued)

BIOLOG Ecoplates

To determine functional differences between the microbial communities of control and sugar-treated soils, we used 96-well plates called BIOLOG Ecoplates (Figure 2). Each well contains one of 31 carbon sources and a dye, tetrazolium violet, that changes color when NADH is produced during cellular respiration. If the microbes are metabolizing the carbon source in the well, then the well turns purple. Soil samples were placed in water and centrifuged to extract the microbial organisms. We pipetted the supernatant into each well and then incubated the plates at 20° C. Using a 96-well plate reader, we measured the absorbance of each well 24 hours after incubation and every 12 hours after that up to 96 hours.

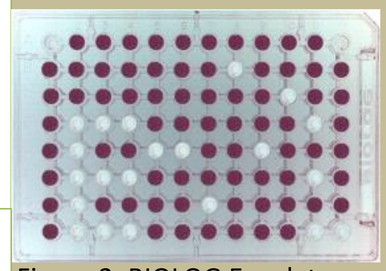


Figure 2. BIOLOG Ecoplate

Soil Nitrogen

Soil samples were placed in a drying oven, then ground and sieved. We weighed out a portion of the soil into a Falcon tube and centrifuged. The supernatant was used to measure NO_3^- levels with a Cole Parmer NO_3^- selective probe.

RESULTS

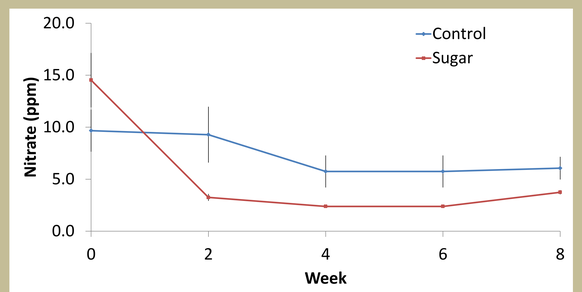


Figure 3. Mean soil nitrate of control and sugar-treated plots per week of treatment. Error bars represent standard error. NO_3^- of control and C-treated plots differed in Week 2 (t-test, df=18, F=19.144, p<0.001), Week 4 (t-test, df=18, F=15.238, p=0.001), Week 6 (t-test, df=18, F=15.238, p=0.001), and Week 8 (t-test, df=18, F=14.415, p=0.001). NO_3^- of control and C-treated plots did not differ in Week 0, before sugar-amendment (t-test, df=18, F=0.011, p=0.917).

Table 1. Number of Metabolized Carbon Substrates in Category Type Extracted by Stepwise Linear Regression per Treatment Based on PCA1

Category	Control	Sugar-treated
Carbohydrate	2	5
Carboxylic Acid	5	5
Polymer	3	1
Amine/Amide	0	2
Amino Acid	5	3
Misc	1	2
Total	16	18

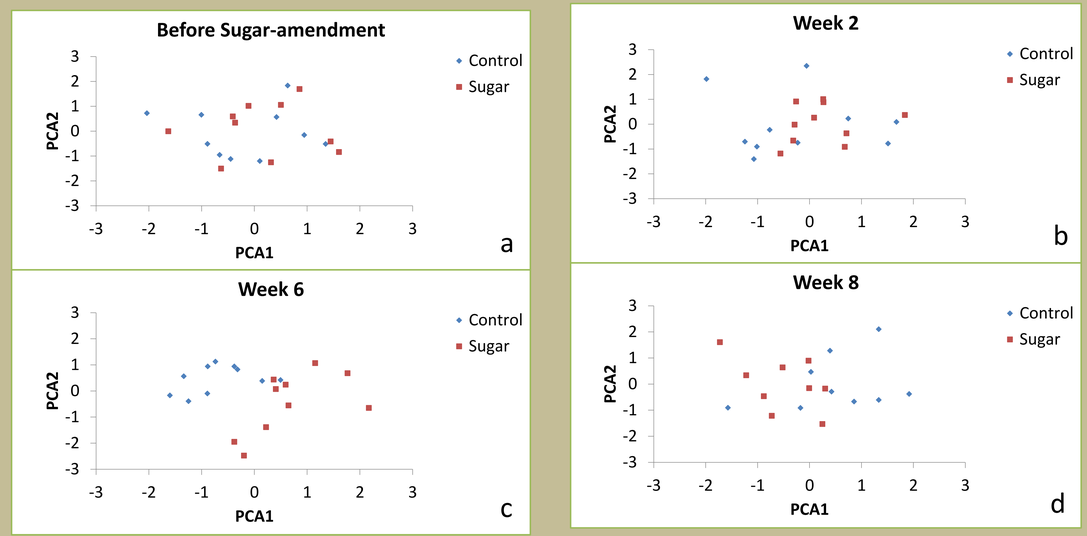
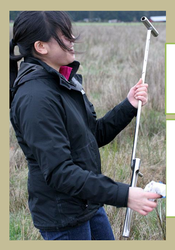


Figure 4. Principal Components Analyses of Absorbance Measurements. Data are taken from absorbance readings of BIOLOG ecoplates at 72 hours of incubation. Control and sugar-treated plots did not differ in PCA1 or PCA2 values Before Sugar-amendment (Panel a), Week 2 (Panel b), or Week 8 (Panel d) (t-test, df=18, F=5.788, p > 0.137). At Week 6, control and sugar-treated plots differed in PCA1 (t-test, df=18, F=0.188, p=0.001) and PCA2 values (t-test, df=18, F=6.210, p=0.039). The effect of treatment on PCA1 value did not depend on soil nitrate (ANCOVA test, Treatment*Nitrate, df=2, F=0.540, p=0.594) or soil moisture (ANCOVA test, Treatment*Soil moisture, df=2, F=0.313, p=0.737).

DISCUSSION & FUTURE RESEARCH

Our results suggest that sugar-amendment does produce a shift in the functionality of the soil microbial community. Two weeks after administering the full amount of the sugar treatment (Week 6), we observed a clear separation in PCA values between control and sugar-treated plots (Figure 4). Microbial communities in sugar-treated plots tended to use a wider range of carbohydrates and amines/amides compared to those in the control plots (Table 1). In addition to the two carbohydrates metabolized in control soils, β-methyl-D-Glucoside and i-Erythritol, the sugar-treated soil microbes metabolized D-Mannitol, N-acetyl-D-glucosamine, and α-D-Lactose, which are all derivatives of glucose. The two amines/amide carbon substrates metabolized by sugar-treated soil microbes were phenylethylamine and putrescine. The increase in carbohydrate use is to be expected in the sugar-treated plots because sugar is a carbohydrate. It is surprising, however, that we did not observe this functional shift until Week 6, since microbial populations are thought to undergo changes relatively quickly. As expected, nitrate levels dramatically decreased with the addition of sugar to levels below those of the control plots (Figure 3). In the future, I will investigate 1) why the control and sugar-treated microbial populations metabolized certain substrates rather than others, 2) whether sugar-amendment does in fact increase microbial populations, and 3) determine if sugar-amendment influences populations of microbes that are responsible for specific biological processes, such as nitrogen fixation.



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