

Potential Effect of Nutrient on Native/Invasive Plants in Disturbed Road-cuts

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Abstract

Invasive species is destructive to many habitats because it prevents several native species to grow. Controlling invasive species is challenging because invasive also prefer conditions what native likes (Gusewell 2004). The purpose of the experiment was to manipulate the nutrient available to *L. vulgarisulgaris*, *Cirsium arvense*, and native species to prevent invasive species' fast growth, and ultimately, domination. Among eight plots per a field experiment, I added sawdust and sugar to the experimental plot to determine whether percent cover and growth rate of invasive plants and native plants were affected. To test effects of carbon and nitrogen on the growth rate of juvenile *L. vulgaris*, I treated four different groups with fertilizer, sugar/sawdust, sugar/sawdust/fertilizer, and nothing. Carbon compositions of straw site plant community (*Bromus. sp*, $28.9\% \pm 0.58$) were significantly lower than control one (*Bromus. sp*, $38.3\% \pm 0.6$; *Hackelia.sp*, $7.44\% \pm 0.59$). In the pot study, plants with carbon (33%; viability) and carbon /nitrogen (58%) treatment died significantly more than plants with nitrogen (100%), and control (100%) treatment. Plants with carbon ($0.22\text{g} \pm 0.079$) and carbon/nitrogen ($0.28\text{g} \pm 0.082$) treatment had significantly higher biomass than plants with nitrogen ($0.45\text{g} \pm 0.076$) and control ($0.49\text{g} \pm 0.079$) treatments. Changes in the microbe community might inhibit the *L. vulgaris* root growth and explain the results in the pot study.

Introduction

Invasive species occupation destroys of ecosystem of various habitats (Marcia et al. 2004). Some invasive species are destructive because they occupy the area where the native species used to reside (Marcia at al. 2004). The lack of space for native plants eventually leads to a decrease of species diversity (Marcia at al. 2004). Diversity is necessary to the habitat because of direct and indirect reasons. For direct reasons, diversity has commercial, recreational, and

pharmaceutical benefit (Leopold 1952). For indirect reasons, diversity of species gives educational value (Leopold 1952). Because invasive species are decreasing this potential value, management of the invasive species population is absolutely necessary (Marcia et al. 2004).

Each specific organism is not destined to occupy a niche in a particular location. Whichever organism reaches to the place first gains the niche (Simberloff and Wilson 1970). Therefore, when competition between plants occurs in disturbed areas, those with better competitive ability and fast growth rate dominate successfully (Berendse 1998). This is the case for some invasive species, *Linaria vulgaris* and *Cirsium arvense*, for they dominate the disturbed area quickly using their stress tolerance and fast growth rate (Volenberg et al. 1999; Wilson 1979). These plants occupy the place where native species previously resided, hence reduce biodiversity and aesthetic quality of native plant community in Gothic, Colorado (Mitch 1993; Volenberg et al. 1999; Wilson 1979).

It is necessary to prevent invasive species domination by restoration (Marcia et al. 2004). Transplanting the native species is a good method of restoration, but this is time consuming and the application of large areas is limited (Oswald and Ransom 2002). Not only the town site with construction, but also other large outside areas need restoration (Eschen 2007). A second way to restore native plant communities could be to change the soil environment so that the native plants are better competitors, than invasive (Yarie and Cleve 1996). For example, nitrogen addition was presumed to restore native plant species (Brooks 2003, Marcia et al. 2004). However, addition of nitrogen by fertilization enhances growth of invasive species, thus it inhibits species diversity even more (Brooks 2003, Marcia et al. 2004). Invasive species occurred more frequently in soil with rich nitrogen (McDonald 2008).

One nutrient that contributes to the fast plant growth is nitrogen (Gusewell 2004). According to recent studies, adding carbon by adding sawdust and sugar increases microbe that consume nitrogen, and this decreases nitrogen composition in the treated soil (Yarie and Cleve 1996; Eschen et al. 2007). Gothic has nutrient rich soil, and this may enable fast domination of invasive species (Campbell et al. 2000). For the invasive species that grow fast in order to occupy the habitat first, reducing nitrogen could negatively impact their diversity, cover, and biomass (Steers 2011). Study has shown that addition of nitrogen, although with the addition of carbon, significantly increase biomass of plant (Hines 2006). If decreasing nitrogen facilitates biodiversity, this method can have wide and reasonable applications for large disturbed area (Gusewell 2004; Yarie and Cleve 1996; Eschen et al. 2007).

Rapid exotic species coverage at construction road cut is an opportunity to restoring native plant communities. **My research goal was to look at the effect of nutrient on invasive species plant growth and percent cover of native species.** By changing nitrogen availability, the competitive dynamics between plants may change. In the field, using *Linaria vulgaris* and *Cirsium arvense*, I removed nitrogen at experimental group and compare growth of plants with those at control group. Specifically, I added sawdust and sugar, a source of carbon to decrease nitrogen levels in soil. In a separate study, I looked at effects of increasing nitrogen and decreasing nitrogen on *L. vulgaris* growth, in the absence of competitors.

Method

Study Species/ Site

Linaria vulgaris (common name: Yellow Toadflax, Butter and Eggs), and *Cirsium arvense* (common name: Canada thistle) are common invasive plants (Mitch 1993). *L. vulgaris* is Figwort family and it came from Europe (Mitch 1993). This species competes with native grass

(volenberg at al. 1999). It reproduces is via seed and root, and this makes the management hard (volenberg at al. 1999). A *L. vulgaris* produces 30,000 seed in a year (volenberg at al. 1999) *C. arvense* also occupies disturbed land, like abandoned ground and burned prairie (Wilson 1979). Most often it lives in cool, air-bound, nutrient rich, deep and moist soil (Wilson 1979). This dioecious, shade tolerant plant produces up to 103 seeds per head (Wilson 1979).

In order to test the effect of carbon/nitrogen on invasive species, I set up the following experiments. I conducted two experiment, each using eight two square meter plots located at the road-cut in front of the Swallow's Nest dormitory at Rocky Mountain Biological Station, Gothic, Colorado (*Latitude* 38°45'N, *longitude* 106°59'W, *altitude* 2895.6m).

Experiment 1

The first experiment tested whether carbon addition affected the nutrient treatment on *L. vulgaris*, *C. arvense*, and other natives. Straw was put down to prevent erosion, so I assumed that some amount of carbon has been added. Among these eight plots, I gave sawdust-sugar treatment at four plots. I placed 2kg of sawdust, and 2kg sugar per two square meter experimental plot (Eschen at al. 2007). Last October 2013, 20g of *Rumex* species, 3g of *Elymus* species, 30g of *Bromus sp.*, 0.40g of *Caryx* species, 0.70g of *Polemonium foliossissimum*, 8g of *Heckelia* were sowed to each of eight plots. In 2014 after treatment were applied, once in every two weeks, I measured the height of *L. vulgaris* to determine the growth rates. During every other week, I measured the composition of invasive and native species. We were testing weather carbon addition affects percent cover of native and invasive plants. After calculating the average value per plot number, I decided to use it in the height analysis. I used a one way ANOVA to determine whether adding carbon affects growth rate of invasive plants. After obtaining the change of percent cover from July third to July 28th, I used PERMANOVA to test the effect of

carbon addition on the plant community composition. I used Simper analysis to test the contribution of the major plant species that showed the effect on carbon addition. I tried to measure the bloomed flower, but *L. vulgaris* did not mature enough to have flower. I was interested in measuring *C. arvense*, but the plot did not have any *C. arvense*.

Experiment 2

Similarly, I was interested in the effect of nitrogen reduction on *L. vulgaris* and *C. arvense* in second experiment. The straw has not been covered in this area, nor were native seeds broadcasted. I used eight plots (4m²) that no additional seed had been distributed. Among the four plots, I added 2kg of sawdust and 2kg sugar per a plot (Eschen et al. 2007). I monitored the height of *L. vulgaris* and height/leaf number of *C. arvense* every two week. I measured plant percent cover every other week. I wanted to measure bloomed flower, but *C. arvense* did not bloomed by the time of research. After making an average per plots for the leaves, height, and flower bud analysis I used one way ANOVA for the number of leaves and number of flower pod. After subtracting first percent cover from final percent cover data, I used PERMANOVA to test whether carbon addition affected the change in plant percent cover over the summer. I used Simper analysis to test contribution of major species that showed whether carbon addition was effective.

Pot experiment

To understand how increasing and decreasing nitrogen affects *Linaria vulgaris* growth, I set up a third experiment. I conducted a 2x2 experiment crossing carbon addition and nitrogen addition. I prepared forty pots (0.01m²) and forty *L. vulgaris* juveniles from Gothic town site, and let the juveniles grow for five weeks (Eschen et al. 2007). I had ¼ of the pots with nitrogen rich fertilizer (0.21g per pot), ¼ with sawdust and sugar (0.005kg sawdust, 0.005kg sugar,), ¼

with both the fertilizer (0.21g) and sawdust/sugar(0.005kg sawdust, 0.005kg sugar,), and ¼ with none of the treatment (Eschen at al. 2007). Every two weeks, I revisited the pot to measure plant height for growth rate. I used MANOVA test to determine effect of nutrient on the invasive species growth over the summer.

We found a lot of dead *L. vulgaris* during the experiment. To investigate further whether this is treatment effect or frequent watering, I sampled two scoops of each 48 sample pot soil. The purpose of this was to measure wet soil mass and dry soil mass for moisture content. I waited two days when the sample is in the drying oven at 105°C. Additionally, I measured plant dry biomass. I separated plant from the soil, placed it in labeled box, and put it in forced air oven at 60°C. After waiting two days, I measured/recorded the plants dry masses. I used one way ANOVA to test whether addition of carbon/nitrogen influenced the soil moisture content. I used two ways ANOVA to test the effect of carbon/nitrogen addition on plant dry masses.



Figure 1. The research site of plot and pot study.

Results

There was an effect of carbon addition on plant communities. There was a trend towards there being different plant communities in two treatments: carbon and control (PERMANOVA; $F_{1,7}=3.65$, $p=0.087$; Figure 2). Simper analysis revealed that *Bromus sp.* (53.15%), *Hackelia sp.* (31.85%), and *Thlaspi sp.* (6.75%) accounted for most of the plant community differences between treatments (Figure 3). There was no effect of carbon addition on *L. vulgaris* height. (ANOVA; $F_1=0.14$, $p=0.73$). Similarly, we did not find significant relationship between carbon addition and plant growth in non-straw sites. There was no effect of carbon addition on plant

community composition. (PERMANOVA; $F_{1,7}=0.12$, $p=1$; Figure 4). Simper analysis did not revealed significant difference in the non-straw plot five main species. The species that contributed the most to any potential differences in plant community were *Bromus sp.* (35.40%), *C. arvense* (34.84%), *Hackelia sp.* (13.03%), *Polemonium reptans* (4.65%), and *Polygonum* (2.86%) (Figure 5). There was no effect of carbon addition on *C. arvense* leaf numbers (ANOVA; $F_1=0.013$, $p=0.92$), heights (ANOVA; $F_1=0.22$, $p=0.67$), and flower pods number ($F_1=4.49$, $p=0.17$). Likewise, no effect appeared of carbon addition on *L. vulgaris* height ($F_1=0.096$, $p=0.79$).

The number of dead *L. vulgaris* in carbon treatment and carbon/nitrogen treatment were significantly higher than Control and nitrogen addition ($\chi^2=19.32$, $p=0.0002$; Figure 6). Difference between the plant dry biomass of carbon and non-carbon treatments was significant, with the non-carbon treatment having higher mass (2-way ANOVA; carbon: $F_{1,44}=7.67$, $p=0.0082$; Nitrogen: $F_{1,44}=0.022$, NS; Carbon*Nitrogen: $F_{1,44}=0.44$, NS; Figure 7). The soil moisture content in carbon added treatments was not significantly different with non-carbon treatments (one way ANOVA; $F_{1,3}=1.42$, $p=0.25$; Table I) Nutrient addition did not affect the plants growth rate. (MANOVA; $F_{1,3}=1.84$, $p=0.076$; Figure 8)

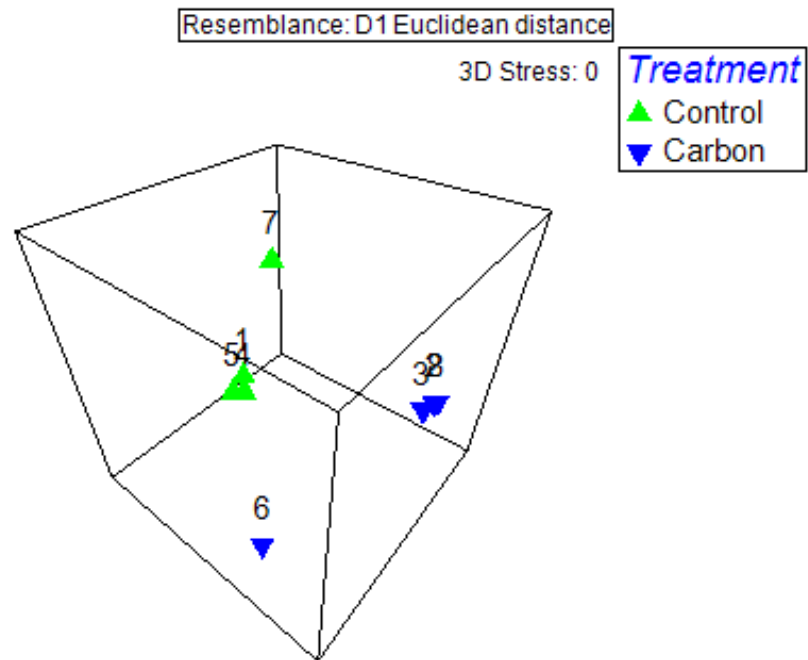


Figure 2. Multi-Dimensional Scaling (MDS) analysis for the straw site, the dependent variable is percent cover change over four weeks, distinguished by carbon addition treatment. Plot values that are close together indicate data are similar.

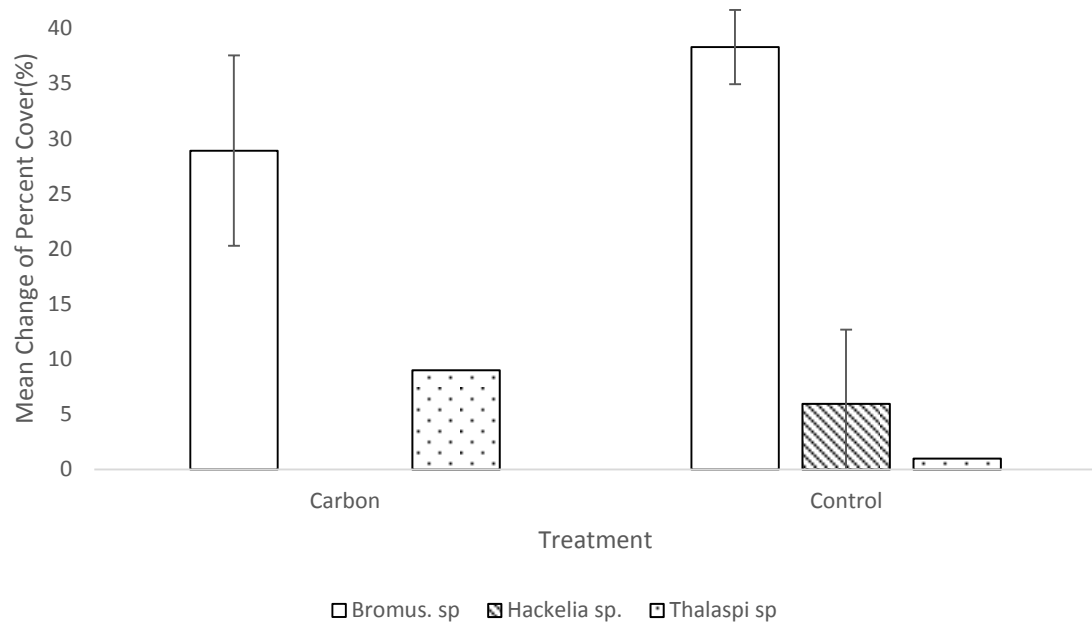


Figure3. Straw site mean percent change in composition ($\% \pm \text{SD}$) of *Bromus sp.* *Heckelia sp.* and *Thlaspi sp.* among two different treatments: carbon and control. The change in percent cover was over five weeks.

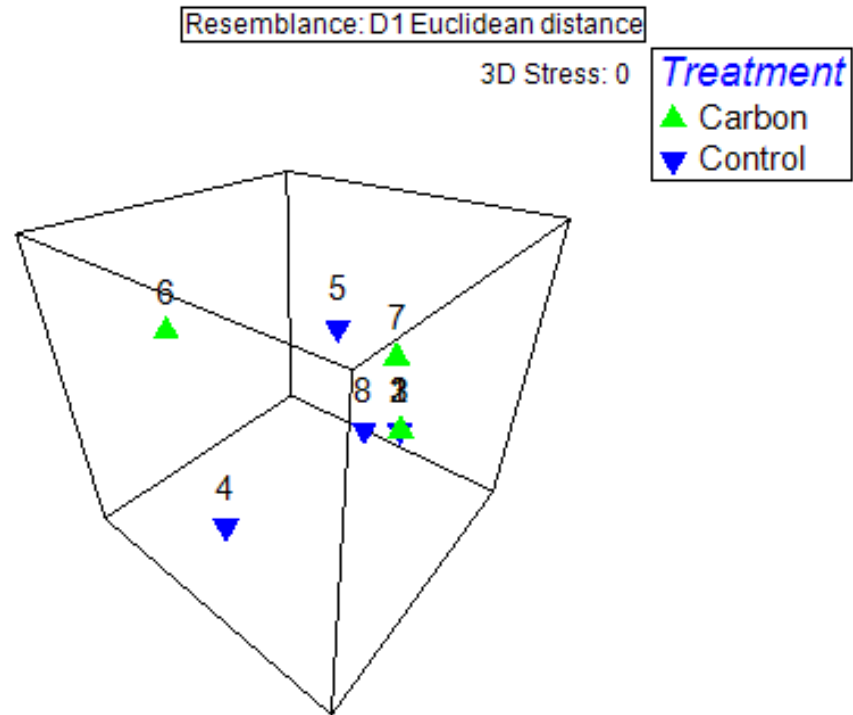


Figure4. MDS for the non-straw site, dependent variable is percent cover change over four weeks, independent variable is carbon addition treatment.

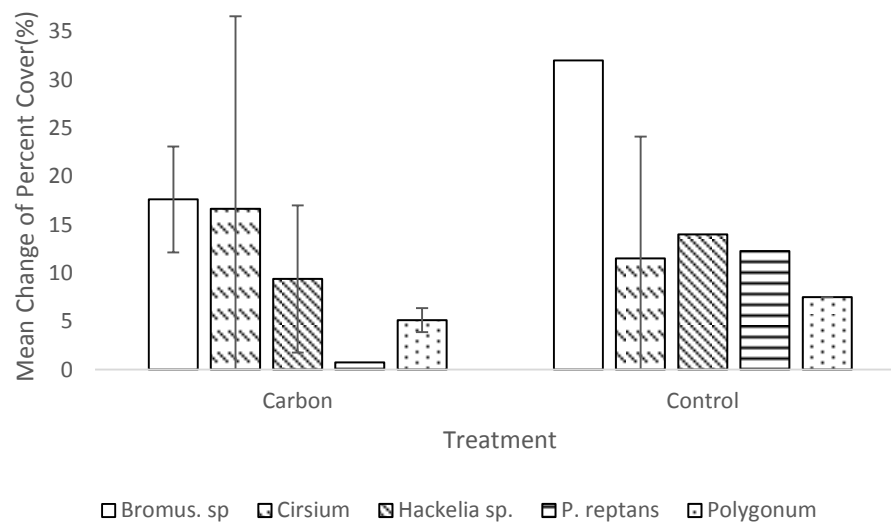


Figure5. No straw sites mean change in percent composition ($\% \pm \text{SD}$) of major plants among two different treatments: carbon and control. The data are based on the change from start to end research period.

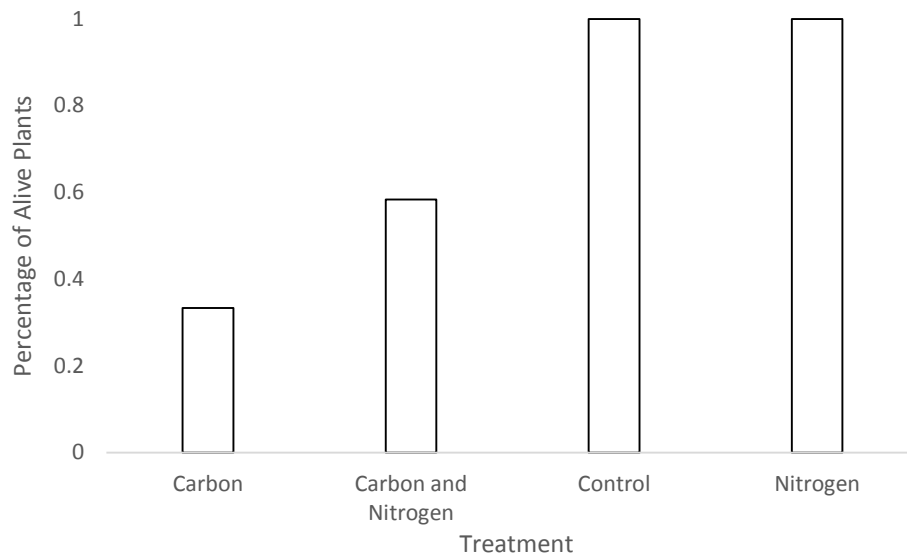


Figure 6. Percentage of live *Linaria vulgaris* in the carbon, carbon and nitrogen, control, and nitrogen treatment

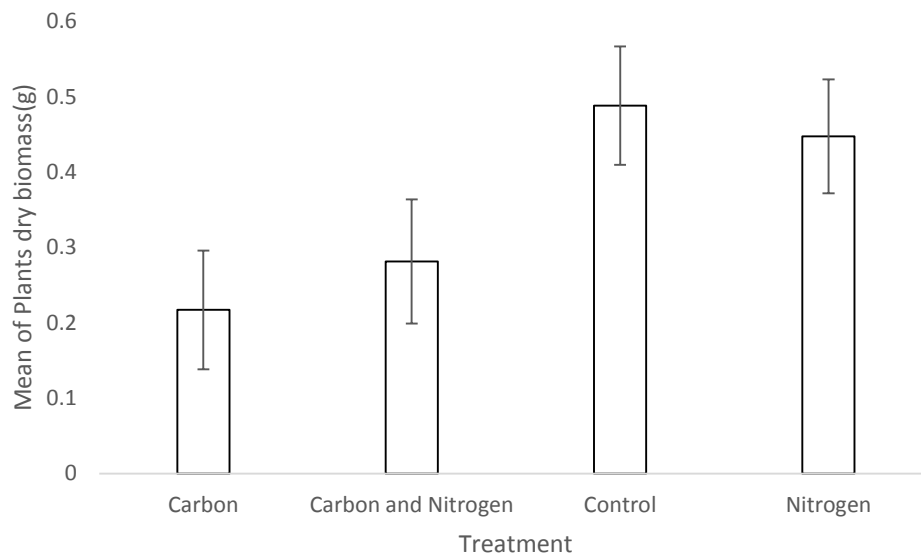


Figure7. Plant dry biomass (g) from four different treatment: Carbon, Carbon and Nitrogen, Control, and Nitrogen. Data shown as Mean $\pm$ SE

Table I Soil moisture content (g) of four different treatment. Data shown as Mean $\pm$ SE

Treatment	Carbon	Carbon and Nitrogen	Control	Nitrogen
Mean $\pm$ SR	5.66 $\pm$ 0.33	4.96 $\pm$ 0.35	4.79 $\pm$ 0.33	4.90 $\pm$ 0.32

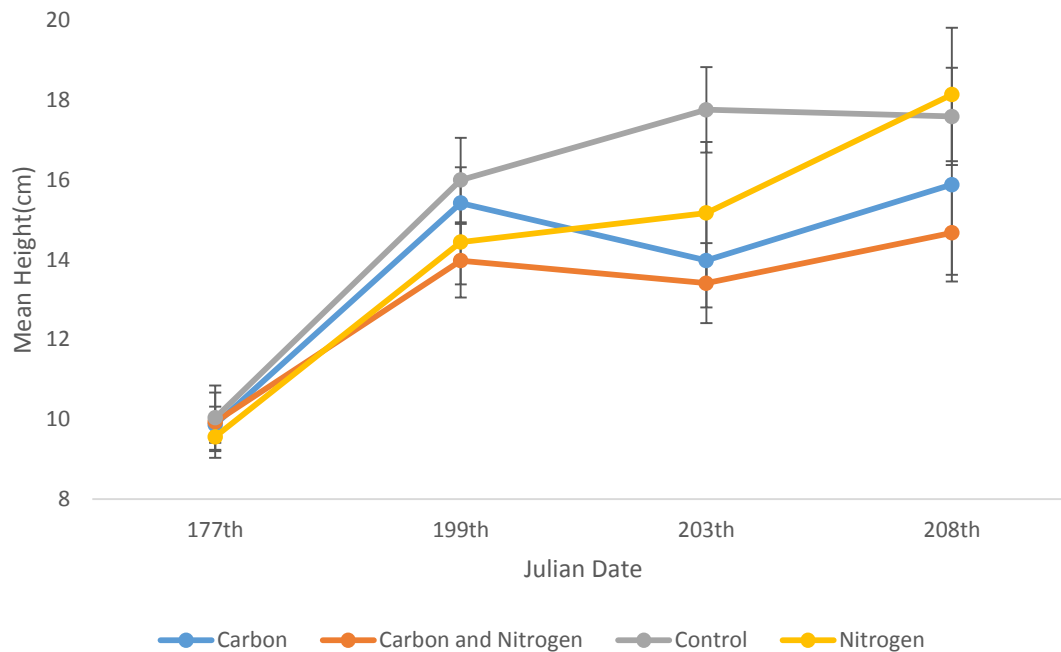


Figure 8. Relationships between four different treatments and their height over six weeks, data shown as mean $\pm$ SD

Discussion

Adding carbon affected plant communities at the straw site where native seed had been sown. Increasing carbon increases microbes, which consume nitrogen that is necessary for the plant growth (Yarie and Cleve 1996; Eschen et al. 2007). Due to the native seed sowing practice from previous year, I was able to see many *Bromus sp.* species in the straw site. *Bromus sp.* and *Hackelia sp.* were more prevalent in control than carbon site because these plants need nitrogen while they are growing (Figure 3). On the other hand, *Thlaspi arvense* lived better with decreasing nitrogen, indicating that this invasive species can live well in the environment where nitrogen is low (Figure 3). This might be because *mustard sp.* like *Thlaspi arvense* lived does not

have mycorrhizae that might decrease by carbon addition. *Thlaspi arvense* grows in disturbed and low nutrient area; this might contribute to the plant's capacity to colonize carbon added plots. In the straw site, not many *L. vulgaris* were there, and the existing samples were short. There was no effect of carbon addition on *L. vulgaris* height. It will be interesting to see these plants in 2015. Based on the result of pot study, the control plot would have more *Linaria vulgaris* next year.

At the non-straw site, there was no effect of carbon addition on plant communities (Figure 4, Figure 5). This may have been due to there being large variability. In the percent cover of plants among plots; some plots were bare ground while other had many plants. Additionally, this site was not controlled for sowing seed, so we did not know how many plants might appear. Furthermore, some plot were covered with plastic over winter and spring while other plots were not, resulting in a non-congruent emergence of various plant species. However, it is possible that changes in microbial community due to carbon addition did not impact growth rate and composition of *C. arvense*. *L. vulgaris* did not contribute much to the non-straw site percent cover (figure 5). Moreover the *L. vulgaris* was not common and didn't grow much. The amount carbon added for both site was half of the amount from what Eschen et al. (2007). Adding more carbon may have resulted in an effect at the no straw site.

L. vulgaris in the pot study were likely to die when carbon was added (figure 6). The dry biomass of four different treatments was marginally significant (figure 7). This is because less nitrogen in the carbon addition treatment prevented the plants from obtaining available resources. This result might have been due to the frequent watering and sawdust's potential to hold water. However analysis on soil moisture content showed that soil moisture was the same across treatments. Water is not the defining factor.

Adding carbon likely changed microbial community in a way that inhibited *Linaria vulgaris* growth. While I am doing biomass measure, I found out these two pot treatments did not have sufficient root development. Moreover, most of the two treatment with carbon, pots did not have new sprouts from seeds. I speculate that increase of microbes inhibited the plant's root growth, which are essential for nutrient and water uptake. Medve (1984) said vesicular arbuscular mycorrhizae fungi is present in *L. vulgaris*, so perhaps microbe might inhibit the growth of fungi, though this is a speculation. It would be interesting to explore the invasive species and fungi interaction in relation to nitrogen deprived soil. The heights of *L. vulgaris* were not different between treatments because I was not able to measure the height of dead plants, which had the most difference in height. Therefore, I had a reduced sample size later in the summer (figure 8).

Nitrogen did not affect *Linaria vulgaris* growth. This is surprising because Hines (2006) said that increase of nitrogen significantly increase plant biomass. Our study showed that *L. vulgaris* biomass did not significantly increase with increasing nitrogen when comparing control treatment. In fact, increasing carbon thus decreasing nitrogen decreased the plant's biomass (figure 7). Maybe *Linaria vulgaris* is an early colonizer, but does not need much nitrogen then what the soil already has. Steers (2011) said that the species that tend to occupy first do not proliferate with the absence of nitrogen, this may be true for to the native species like *Bromus. sp* and *Hackelia sp.*(figure 3).

Microbe interaction in the soil is a key factor for plant survival. Yarie and Cleve (1996) said the addition of sugar and sawdust would increase the microbe that would decrease nitrogen, and our pot study found support that there is soil difference amongst treatment (figure 6, figure 7). In future studies, it would be interesting to measure soil nitrogen amounts or type of

microbe. McDonald (2008) said invasive species are more frequent in nitrogen rich soil. That is not so in our study. It depends on invasive species type, for example, *Thlaspi*.sp were more prevalent in nitrogen poor soil. Unlike Yarie and Cleve (1996) suggestion that change in environment might reduce invasive species, changing soil environment might also change favorable conditions for native species. Beremdse (1998) said that invasive species have fast growth, this was the case of our non-straw site *C. arvense* (figure 5), and some of the *L. vulgaris* (figure7).

When comparing plant communities at the straw and seed addition site to the bare soil site I noticed more native plant species. Oswald and Ransom (2002) said that transplanting is good method of native restoration, though not tested here sowing is also good option to restore native plants.

Invasive species are destroying the native habitat by creating monoculture and outcompeting natives. This study suggest that adding carbon to soil increase *Linaria vulgaris* mortality, but our plot study shows little support for carbon addition effect on the growth of native versus invasive species over a single season. If we could replicate conditions that led to pot study results, carbon addition would be good method for inhibition of invasive plant species and restoration of native plant species.

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