

Does nutrient enrichment interact with *Castilleja miniata* to alter plant communities in montane meadows?

Student: Corinne Calhoun

Mentors: Aimée Classen and Nathan Sanders

Advanced Independent Research, NSF funded REU

Summer 2012

ABSTRACT

Many studies have demonstrated the negative effects of hemiparasites on host performance, such as decreased host biomass and reproductive capabilities. Much less work, however, has examined the role of hemiparasites in shaping plant community structure, though it has been proposed that the effect of a hemiparasite can vary based on a number of factors, including nutrient availability. Using *Castilleja miniata* as a test species, I tested for an interactive effect of hemiparasite removal and nitrogen enrichment on plant community structure. I found no evidence of an interactive effect altering species richness, total plant cover, diversity, or evenness. Nitrogen availability and *C. miniata* presence or absence may have an additive effect on nitrogen-fixer productivity. Nitrogen-fixers grew more in plots under ambient soil conditions where *C. miniata* was removed. The importance of nitrogen-fixers in an ecosystem warrants further research into the dynamics between nutrient cycling, hemiparasites, and nitrogen-fixer performance.

INTRODUCTION

There are over 3,000 species of flowering parasitic plants (Bardgett et al. 2006) and they inhabit almost all major terrestrial ecosystems (Adler 2002). A hemiparasite is a kind of parasitic plant that not only gains carbon, nutrients, water, and defensive compounds from host plants, but also can photosynthesize and gain nutrients non-parasitically (Press and Phoenix 2005; Adler 2003 citing Arslanian *et al.* 1990).

Many studies have investigated the effects of hemiparasites on host performance (e.g. Seel and Press 1996; Matthies 1995; Mudrák and Leps 2012) and root hemiparasites, such as witchweeds (*Striga spp.*), have been shown to reduce the yield of major cereal crops (Malcom 1966; Press 1998 citing Parker and Riches 1993). In their two-year lab study, Seel and Press (1996) found that grasses infected with the hemiparasite *Rhinanthus minor* had less than half the mass of

non-infected grasses. Matthies (1995) also found negative effects of a hemiparasite on host growth rates.

However, despite the plethora of knowledge pertaining to the effects of a hemiparasite on a host, the mechanisms by which a host is affected and the community-level effects of a hemiparasite are not as well understood. By parasitizing a host plant, a hemiparasite can decrease its host's biomass (Mudrák and Leps 2010) and ability to compete with neighboring non-host species, thus changing community dynamics (Bardgett et al. 2006). If the hemiparasite negatively affects the more competitively dominant or common species in the community, this may allow non-host species to increase in abundance or biomass and positively affect the plant community diversity (Mudrák and Leps 2010).

There is evidence, however, that hemiparasite influence on plant community diversity may change with habitat type and resource availability (Gibson and Watkinson 1992; Press 1998; Westbury et al 2006). Gibson and Watkinson (1991) suggested that host selectivity in *R. minor* may depend on a number of factors, including the relative abundance of hosts, environmental conditions, and genetic structure of both host and parasite populations. Under laboratory conditions, they found decreased root connectivity between *R. minor* and its host when grown in nitrogen (N) rich soil as well as greater host biomass relative to hosts grown in N poor soil with *R. minor*. This variation in root connectivity suggests that hemiparasite colonization as well as the impact its colonization has on the surrounding plant community can vary based on nutrient availability. For example, at higher nutrient levels both host and hemiparasite invest less in their root system: the hemiparasite advantage of gaining nutrients from a host plant decreases (Mudrák and Leps 2010), possibly along with the negative effects on the host plant and cascading community level effects.

This study will further investigate how a hemiparasite structures plant communities under altered nutrient conditions by manipulating soil N availability and presence or absence of the long-lived, subalpine perennial root hemiparasite *Castilleja miniata* (Adler 2003). Nitrogen availability is cited as one of the most common nutrients that limit plant growth (Bowman *et al.* 1993). Manipulation studies in non-hemiparasite communities have shown an increase in total aboveground biomass with the addition of N and a decrease in total aboveground biomass with reduced N availability (Blue *et al.* 2011 citing Craine *et al.* 2003; Gruner *et al.* 2008; Cleland and Harpole 2010; Wedin and Tilman 1993; and Throop 2005). Chalcraft *et al.* (2008) demonstrated a scale-dependent effect of N enrichment on biodiversity: on a small scale, an increase in primary productivity due to N enrichment leads to a decrease in diversity.

While *Castilleja* spp. can parasitize a wide range of hosts (Spasojevic and Suding 2011), factors contributing to host selectivity and the community-level effects of the hemiparasite are still not well understood. It has been demonstrated that *Castilleja miniata* can increase community evenness: previous work in this ecosystem found that removal of *Castilleja* spp. decreased evenness of the surrounding plant community but had no significant effect on species richness (Reed *et al.*, unpublished data). If increasing available nutrients shifts the relationship between *Castilleja* and its hosts, as it does in laboratory studies with *R. minor*, I predict an observable change in *Castilleja*'s effect on the surrounding plant community, specifically community evenness.

In this study, I manipulated the direct and interactive effects of nutrient availability and *Castilleja* presence on total plant cover, plant community diversity, and evenness. Suding *et al.* (2006) demonstrated the importance of species function in removal manipulations and the community's response to species loss, so I also broke down treatment effects based on functional groups (non N-fixer forbs, graminoids, and N-fixers) that, due to their functional differences, may respond to

N enrichment and *Castilleja* removal differently. I hypothesized that (i) *Castilleja* removal and N addition will have an interactive effect and increase total plant percent cover and decrease evenness of the plant community ii) N addition will have a main effect of increasing overall percent plant cover, decrease percent cover of N-fixers, and decrease the evenness of the plant community. iii) *Castilleja* removal will increase the percent cover of one or a few species, likely within the same functional group, and decrease evenness of the plant community. Unlike many previous studies, this manipulation illuminates some of the factors determining the effect of a hemiparasite on a plant community in a non-laboratory setting.

METHODS

Study site: We established this study in an aspen meadow near the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA. This site is roughly 3000 meters above sea level and is located along the Judd Falls trail, approximately 200 meters from the trailhead. Soils are rocky and well drained. I established experimental plots in three distinct sites located within 25 meters of one another. Sites were chosen based on the location of abundant patches of *Castilleja miniata* and all sites have roughly SW facing slopes. Across all sites, the most abundant species were *Castilleja miniata*, *Carex spp.*, *Erigeron speciosus*, and *Potentilla pulcherrima*.

Experimental design: Each of the 24 experimental plots was 1.5 × 1.5 m with a minimum 1 m buffer area between plots. I randomly assigned plots to one of four treatments: (1) *Castilleja* removal, (2) N addition, (3) *Castilleja* removal × N addition, and (4) control plots. Prior to treatment establishment, we measured percent cover of each species in a 1 × 1 m quadrat within each 1.5 × 1.5 m plot, in addition to the cover of *Castilleja* within each plot. Ten days and twenty-three days after I applied the treatments, we measured percent cover of every species within each plot.

Castilleja removal: I removed all aboveground *Castilleja* biomass using hand-held pruning shears from each 1.5 m² plot assigned the removal treatment. If *Castilleja sulphurea* was present, it was also removed. I regularly clipped *Castilleja* removal plots throughout the summer.

Nitrogen addition: On the same day as the original *Castilleja* removal, I added N to the assigned 1.5m² N-addition plots using 30-0-3 (30% N, 0% phosphorus, 3% potassium) lawn fertilizer. Each N-addition plot was sprinkled with fertilizer once during the summer at a density of 20 g urea N per m² as per the methods in Blue et al. (2011).

Soil measurements: In addition to percent cover, I also measured soil moisture (0-12 cm) as percent volumetric water content (%VWC) and temperature (0-10 cm, °C) to ensure plots did not vary in this regard. On any given day, neither temperature nor %VWC varied significantly by treatment (Table 1). I measured soil moisture using a hand-held probe and temperature in °C with an analog thermometer. I averaged two soil moisture measurements taken at random locations in each plot and measured temperature from the center of each plot, both at a depth of 7 cm due to rockiness of soil. I took these measurements jointly three times throughout the study.

Calculations: From the percent cover measurements, I calculated 1) species richness 2) the Shannon-Wiener diversity index ($H' = \sum p_i \ln p_i$), and 3) evenness using the equation: $\text{Evenness} = (1/\sum p_i^2) (1/S)$ where p_i is the proportion of each species and S is the total number of species. All calculations were done in the statistical program R. I classified each species found in the plots as a non-N-fixer forb, graminoid (families Poaceae and Cyperaceae), or N-fixer (family Fabaceae) then calculated the Mean Cover Ratio within each treatment type of 1) total percent cover and 2) that of each functional group using the equation: $\text{Mean Cover Ratio} = (\sum (p_{2t}/p_{1t}))/P$ where p_{1t} is the total percent cover of all concerned species in plot X pre-treatment application, p_{2t} is the percent cover of all

concerned species in plot X twenty-three days after treatment application, and P is the number of plots within the treatment type.

Statistical analyses: I ran four separate ANOVAs with the statistical program JMP 10 to analyze the effect of treatment type on species richness, diversity, evenness and total plant percent cover. I also analyzed the effect of treatment on Mean Cover Ratio of functional groups and total plant cover with four ANOVAs.

RESULTS

What effect do Castilleja removal and N-addition have on total plant percent cover, species richness, diversity, and evenness of the entire plant community?

Ten days after treatment application, I found no significant difference in species richness, total plant percent cover, diversity, or evenness of the plant community based on *Castilleja* and N treatment (Table 2). Similarly, *Castilleja* and N treatment had no interactive or main effects on species richness, diversity, or evenness twenty-three days after treatment application (Table 2, Fig. 1a-1d). In other words, plots did not vary in total plant percent cover, diversity, or evenness based on treatment type at any time after treatment application. All following results are based on percent cover measurements taken twenty-three days after treatment application.

How does Castilleja removal and N-addition affect total growth and growth of different functional groups in the community?

Neither *Castilleja* removal nor N addition caused a different response in Mean Cover Ratio for total percent cover, percent cover of non N-fixer forbs, or percent cover of graminoids based on treatment (Fig. 2a-2c). In the case of graminoids and N-fixers, figures suggest that N addition had little or a negative effect on percent cover (Fig. 2b and 2c). There was, however, a difference in the Mean Cover Ratio of N-fixers ($F=4.0022$ _{3,23}; $p=0.0220$): Growth of N-fixers was

significantly less in N treated plots with *Castilleja* present versus in plots under ambient soil conditions where *Castilleja* was removed (Fig. 2d). In all cases, percent cover seemed to increase from pre-treatment to post-treatment measurements most in plots where *Castilleja* was removed and N was not added (Fig. 2a-2d).

DISCUSSION

Hemiparasites have been shown to increase the evenness of the surrounding plant community (Reed et al. 2011, unpublished data). However, the factors contributing to such community level effects are largely unstudied. Gibson and Watkinson (1992) suggested the effect a hemiparasite has on the plant community may be partially determined by nutrient availability. I therefore predicted that nutrient enrichment would alter the way in which the hemiparasite *Castilleja miniata* affects its plant community using percent cover, species richness, diversity, and evenness as response variables.

What effect does Castilleja removal and N-addition have on total plant percent cover, species richness, diversity, and evenness of the entire plant community?

I did not see an effect of treatment type on species richness, which was as expected based on the previous study done in this system (Reed et al. 2011, unpublished data). In contrast to Reed et al. (2011, unpublished), however, I did not see a decrease in evenness with the removal of *Castilleja*. This may be due to the time constraints of the present study. Seel and Press (1996) suggest the effects of a hemiparasite on its host endure even after hemiparasite death, so long-term results may yield stronger trends.

How does Castilleja removal and N-addition affect total growth and growth of different functional groups in the community?

Functional group analysis did not indicate the presence of an interaction effect between *Castilleja* treatment and N treatment as I had previously hypothesized. It did reveal, however, an additive effect of both the treatments on the mean N-fixer cover ratio. N-fixers grew less well in N enriched plots and increased more readily in plots where *Castilleja* was removed. The decreased growth found in N enriched plots is to be expected, as N-fixers only hold a competitive advantage in N poor ecosystems (Skogen et al. 2011). However, the main effects of N enrichment on graminoids, forbs and total plant cover, suggesting a small or negative growth response, were not consistent with the hypothesis that in an N-poor ecosystem, N enrichment would increase productivity and decrease diversity. It may be the case, then, that N was not the most limiting resource to plant growth in this system during the summer of 2012. At the time of this study, there was a particularly short winter preceding a dry summer. Instead of N, water may have been limiting plant growth this year.

Results also indicate a trend towards all functional groups growing more in plots under ambient soil conditions where *Castilleja* was removed. On average, *Castilleja* negatively affected all functional groups such that in its absence, these groups increased in cover. If *Castilleja* presence only decreases its hosts' biomass, this may be an indication that in this system *Castilleja* is demonstrating generalist host preference, instead of targeting a single group or species.

This study has demonstrated that when *Castilleja miniata* is present in this system, N-fixer performance decreases. N-fixers are keystone species that, unlike other functional groups, can bring in new N to the system. This has profound implications for hemiparasite systems if parasitization also decreases N-fixers' ability to perform this ecosystem function. Hemiparasites, however, have been shown to contain higher nutrient concentrations in their leaf litter and may serve the ecosystem by redistributing these nutrients: either by transferring them from comparatively N-rich hosts to other members of the community or unlocking slowly released nutrients from slow growing, long-lived perennial hosts

(Press et al 1998). The effects of a hemiparasite on N-fixers and the overall plant community may depend heavily on host preference; therefore further research should investigate mechanisms of host preference and trace the transfer of nutrients between host, hemiparasite, and the community.

LITERATURE CITED

- Arslanian, R. L., G. H. Harris, and F. R. Stermitz. New quinolizidine alkaloids from *Lupinus argenteus* and its hosted root parasite *Castilleja sulphurea*: stereochemistry and conformation of some naturally occurring cyclic carbinolamides. *Journal of Organic Chemistry*. 55. (1990): 1204-1210
- Adler, L. S.. Host effects on herbivory and pollination in a hemiparasitic plant. *Ecology*. 83.10 (2002): 2700-2710
- Adler, L. S.. Host species affects herbivory, pollination, and reproduction in experiments with parasitic *Castilleja*. *Ecology*. 84. (2003): 2083-2091
- Bardgett, R. D., R. S. Smith, R. S. Shiel, S. Peacock, J. M. Simkin, H. Quirk and P. J. Hobbs. Parasitic plants indirectly regulate below-ground properties in grassland ecosystems. *Nature*. 439. (2006): 969-972
- Bowman, W. D., T. A. Theodose, J. C. Schardt, R. T. Conant. Constraints of nutrient availability on primary production in two alpine tundra communities. *Ecology* 74.7 (1993): 2085-2097
- Chalcraft, D. R., S. B. Cox, C. Clark, E. E. Cleland, K. N. Suding, E. Weiher, D. Pennington. Scale-dependent responses of plant biodiversity to nitrogen enrichment. *Ecology* 89.8 (2008): 2165-2171
- Cleland, E. E., W. S. Harpole. Nitrogen enrichment and plant communities. *Ann NY Academy of Sciences* (2010): 46-61
- Craine, J. M., P. B. Reich, D. Tilman, D. Ellesworth, J. Fargione, J. Knops, S. Naeem. The role of plant species in biomass production and response to elevated CO₂ and N. *Ecology Letters* 6. (2003): 623-630
- Gibson, C. C. and A. R. Watkinson. Host selectivity and the mediation of competition by the root hemiparasite *Rhinanthus minor*. *Oecologia*. 86. (1991): 81-87
- Gibson, C. C. and A. R. Watkinson. The role of the hemiparasitic annual *Rhinanthus Minor* in determining grassland community structure. *Oecologia*. 89.1 (1992): 62-68
- Gruner, D. S., J. E. Smith, E. W. Seabloom, S. A. Sandin, J. T. Ngai, H. Hillebrand, W. S. Harpole, J. J. Elser, E. E. Cleland, M. E. S. Bracken, E. T. Borer, B. M. Bolker. A cross-system synthesis of consumer and nutrient resource control on producer biomass. *Ecology Letters* 11. (2008): 740-755

- Malcom, W.M. Root parasitism of *Castilleja coccinea*. Ecology 47.2 (1966): 180-186
- Matthies, D.. Parasitic and competitive interactions between the hemiparasites *Rhinanthus serotinus* and *Odontites rubra* and their host *Medicago sativa*. Ecology 83. (1995): 245-251
- Mudrák, O., J. Leps. Interactions of the hemiparasitic species *Rhinanthus minor* with its host plant community at two nutrient levels. Folia Geobot 45. (2010): 407-424
- Press, M. C.. Dracula or Robin Hood? A functional role for root hemiparasites in nutrient poor ecosystems. Oikos. 82.3 (1998): 609-611
- Press, M. C. and G. K. Phoenix. Impacts of parasitic plants on natural communities. New Phytologist. 166. (2005): 737-751
- Seel, W. E. and M. C. Press. Effects of repeated parasitism by *Rhinanthus minor* on the growth and photosynthesis of a perennial grass, *Poa alpina*. New Phytologist. 134. (1996): 495-502.
- Skogen, K. A., K. E. Holsinger, Z. G. Cardon. Nitrogen deposition, competition and the decline of a regionally threatened legume, *Desmodium cuspidatum*. Oecologia. 165. (2011): 261-269
- Spasojevic, M. J., and K. N. Suding. Contrasting effects of hemiparasites on ecosystem processes: can positive litter effects offset the negative effects of parasitism?. Oecologia. 165. (2011): 193-200
- Suding, K. N, A. E. Miller, H. Bechtold, W. D. Bowman. The consequence of species loss on ecosystem nitrogen cycling depends on community compensation. Oecologia 149.1 (2006): 141-149
- Throop, H. L.. Nitrogen deposition and herbivory affect biomass production and allocation in an annual plant. Oikos 111. (2005): 91-100
- Wedin, D., D. Tilman. Competition among grasses along nitrogen gradient: initial conditions and mechanisms of competition. Ecological Monographs 63.2 (1993): 199-229
- Westbury, D. B., A. Davies, B. A. Woodcock, N. P. Dunnett. Seeds of change: the value of using *Rhinanthus minor* in grassland restoration. Journal of Vegetation Science 17.4 (2006): 435-446

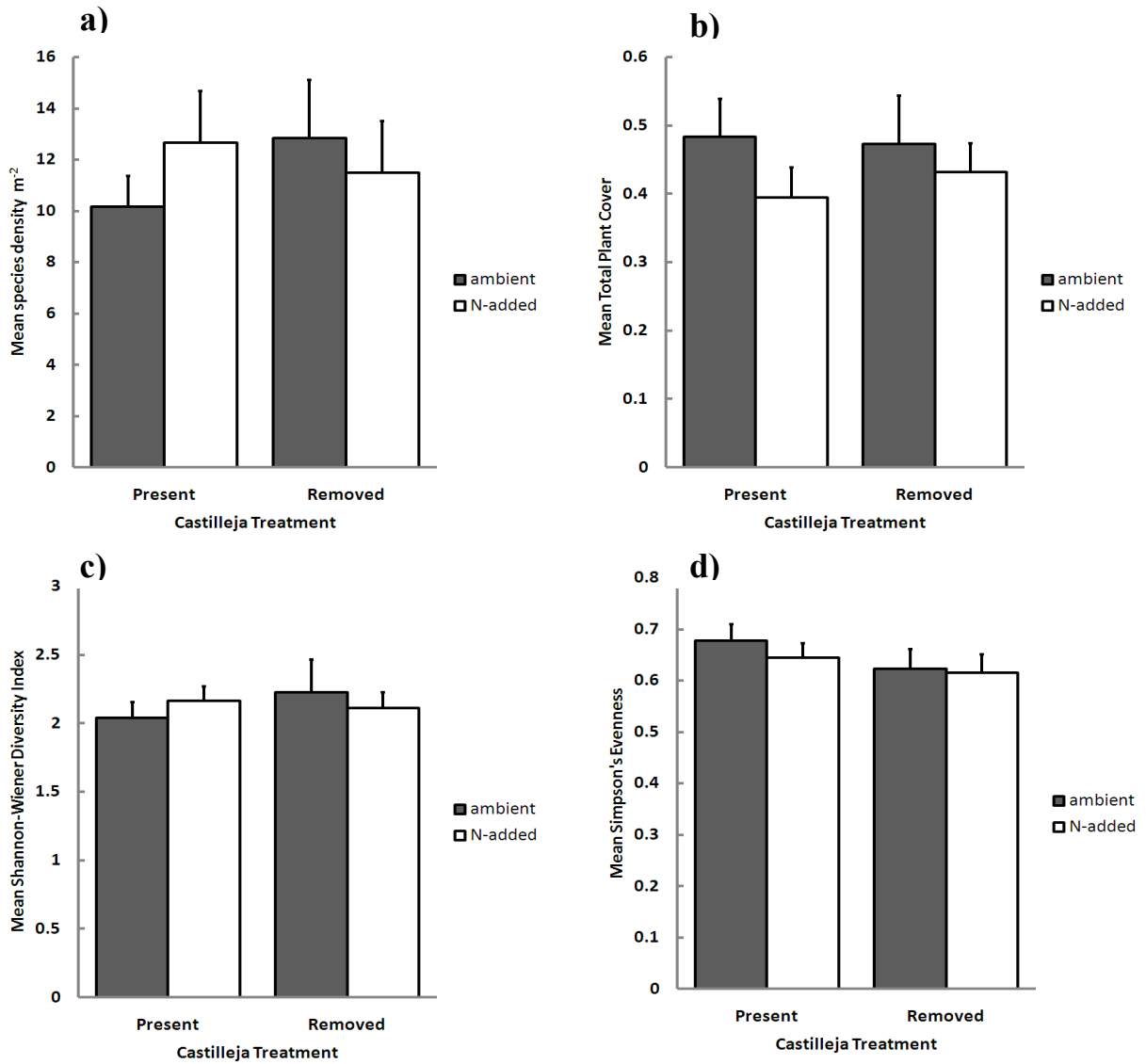


Figure 1. Effect of treatment type 23 days after treatment application on a) species richness ($F=0.5972_{3,23}$ $p=0.6242$), b) Mean total plant % cover ($F=0.5672_{3,23}$ $p=0.2541$), c) Mean Shannon-Wiener Diversity Index ($F=0.2617_{3,23}$ $p=0.9269$), and d) Mean Evenness ($F=0.6976_{3,23}$ $p=0.9410$), calculated as $(1/\sum p_i^2)(1/S)$ where p_i is the proportion of each species and S is the total number of species

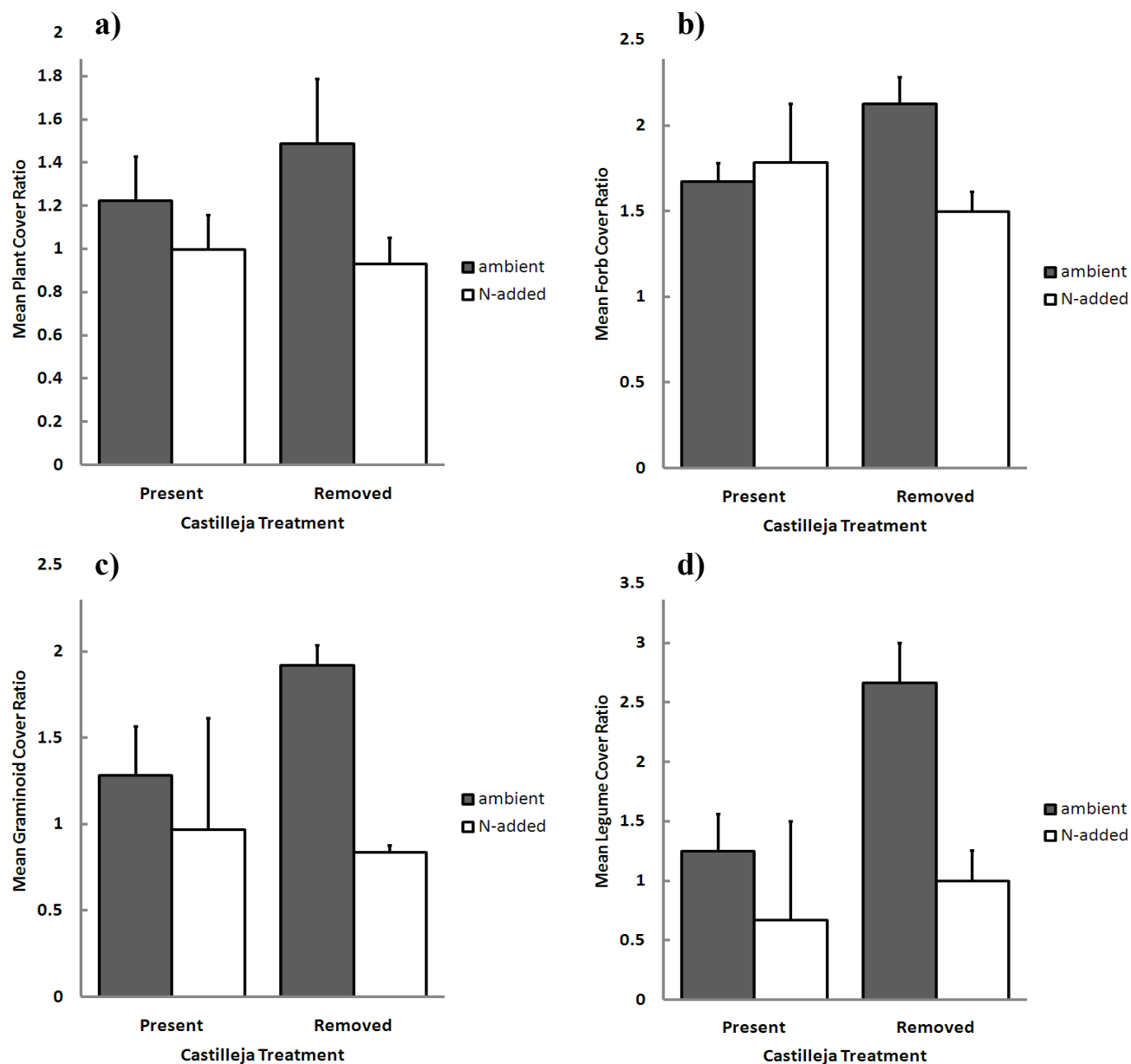


Figure 2. Effect of treatment type 23 days after treatment application where a value greater than 1 indicates growth over time, 1 indicates no growth, and a value less than 1 indicates negative growth. a) mean total plant cover ratio ($F=1.4651_{2,23}$ $p=0.2541$), b) mean non N-fixer forb cover ratio ($F=1.6658_{3,23}$ $p=0.2063$), c) mean graminoid cover ratio ($F=1.8118_{3,23}$ $p=0.1775$), and d) mean N-fixer cover ratio ($F=4.0022_{3,23}$ $p=0.0220$)

Table 1. Results of %VWC and Soil Temperature ANOVAs

DATE	^a SOIL MOISTURE (%VWC at 7cm)	^b SOIL TEMP (°C at 7cm)
7/9/12	F=0.7914 _{3,23} p=0.5129	F=1.3135 _{3,23} p=0.2977
7/13/12	F=1.1345 _{3,23} p=0.3591	F=0.8743 _{3,23} p=0.4709
7/17/12	F=0.5010 _{3,23} p=0.6859	F=0.7059 _{3,23} p=0.5597

^a On any given day, plot soil moisture measured as percent volumetric water content at 7cm did not vary by treatment

^b On any given day, plot soil temperature measured in degrees Celsius at 7cm did not vary by treatment

Table 2. Results of Species Richness, Diversity, and Evenness ANOVAs

MEASUREMENT	TOTAL PLANT % COVER	^c SPECIES RICHNESS	^d DIVERSITY	^e EVENNESS
Pre-treatment	F=1.5846 _{3,23} p=0.2244	F=0.5078 _{3,23} p=0.6813	F=0.1525 _{2,23} p=0.9269	F=0.1302 _{3,23} p=0.9410
^a Post-treatment 1	F=0.2775 _{3,23} p=0.8409	F=0.2978 _{3,23} p=0.8265	F=0.0931 _{3,23} p=0.9630	F=0.7128 _{3,23} p=0.5558
^b Post-treatment 2	F=0.5672 _{3,23} p=0.6430	F=0.5972 _{3,23} p=0.6242	F=0.2617 _{3,23} p=0.7394	F=0.6976 _{3,23} p=0.5644

^a Post-treatment 1 indicates percent cover measurement taken 10 days after treatment application

^b Post-treatment 2 indicates percent cover measurement taken 23 days after treatment application

^c Total number of species found in plot does not vary by treatment before treatment application nor 10 and 23 days after

^d Shannon-Wiener Diversity (diversity=) does not vary by treatment before treatment application nor 10 and 23 days after

^e Evenness (simpson's evenness= $(1/\sum p_i^2) (1/S)$) does not vary by treatment before treatment application nor 10 and 23 days after