Disturbance’s Effect on Nitrogen Fixation in Cryptobiotic Soil Crusts

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ABSTRACT:

In arid ecosystems nitrogen is frequently limiting. The harsh environment prevents legumes from taking root and providing biologically available nitrogen. Cryptobiotic soil crusts, symbiotic amalgamations of cyanobacteria, lichens and fungi, have evolved to fill this niche. Because these crusts must fix N anaerobically, they are brittle and can take years to establish. Disturbance can therefore have a large effect on their biological efficacy. We tested whether simulated disturbance and water addition would have a detectable effect on plant growth. Soil crusts were extracted from the National Bison Range in Moiese, MT. They were potted, half were disturbed and Western Wheatgrass (*Pascopyrum smithii*) stems were transplanted and clipped at the same height. Half of each treatment received water addition (150ml/pot/day). Results indicate that stem heights (cubed as a proxy for biomass) and actual weighed biomass were independent of treatment. The number of secondary (vegetatively reproduced) stems growing in pots was found to be dependent on water and marginally dependent on disturbance. An especially wet summer may be in part to blame for the unexpected results for the biomass experiments. An in situ study was also conducted to see biomass correlated with any one of several factors in the field, including percent cover of soil crusts. Field biomass was shown to correlate with plant cover and level of visual disturbance. The results of this study have implications for land managers of megafaunal grazers, as heavy disturbance of graze lands could prevent forage from regenerating annually.

INTRO:

Arid ecosystems provide a unique opportunity to study nutrient dynamics. While nitrogen is frequently limiting in terrestrial ecosystems, this is especially true in dry areas (Eldridge and Greene 1994). As a consequence of the natural nitrogen deficit in arid areas, unique taxa of niche species have developed to fill the nitrogen void.

Cryptobotic soil crusts have evolved as a fixer of nitrogen in dry to desert conditions (≤50cm annual precipitation). These symbiotic organisms are a combination of fungi, cyanobacteria, lichens and bryophytes, in differing proportions (Rosentreter et al. 2007). They can have a large impact, sometimes representing up to 70% of the biomass in systems while also fixing the majority of the nitrogen (up to 41 kg N ha^{-1} $year^{-1}$) (Belnap and Gardner 1993, Belnap 2002, and Evans and Ehleringer 1993). The cyanobacterial components of these crusts contain the N-fixing capabilities of these symbiotic communities. But because N-fixation must be performed anaerobically, these blue-green bacteria must create a dense but brittle network of filamental tubes in which to complete this task. These networks are present both on top of the soil and within the substrate itself. The filaments not only serve to fix nitrogen in harsh and arid environments, but as they infiltrate the soil, they can also significantly reinforce the substrate to erosive forces (Belnap and Gillette 1997). Crusts are by no means quick establishing flora. Because they can only grow in wet conditions, they can decades to centuries to fully form in arid lands (Belnap and Gillette 1997).

The delicate nature of soil crust filaments also means that they are quite susceptible to disturbance. Soil disturbance by anthropogenic causes, be they direct or latent, can therefore have a large impact on cryptobiotic soil crusts' abilities to fulfill their ecological role (Bowker et al 2005). One of the largest impacts is the trampling effect on these crusts during grazing by native ungulates (e.g. white tail deer, prong-horned antelope) and domestic animals (e.g. cattle [Bargner et al. 2006]). Soil crusts lose their abilities to both fix nitrogen and to stabilize substrate under heavy disturbance regimes (Bargner et al. 2006 and Belnap and Gardner 1993). The implications of these large impacts could be profound for areas like the National Bison Range in Moiese, Montana. As one of the nation's last publically held herds of the American bison (*Bison bison)*, the range's posterity is paramount both biologically and aesthetically. Land managers need to be mindful of the effects that large herbivores like bison can have on the very graze lands they require to survive.

We plan to study the effects of both disturbance and water addition on the nitrogen fixation of cryptobiotic soil crusts within the National Bison Range. As terrestrial N-fixation can be very difficult to determine, we plan to use plant growth as a proxy. We will use Western Wheatgrass (*Pascopyrum smithii*), to measure plant response to the treatments, as it is the heartiest of the grasses native to the local Palouse Prairie ecosystem.

Hypotheses:

Given the profound effect disturbance can have on N-fixation, we predict that our simulated large herbivore disturbance will have a significant detrimental effect on plant growth. We predict the undisturbed soil crust to produce significantly higher amounts of plant growth/biomass. Additionally, we predict that the watered treatments will produce significantly higher biomass than the unwatered treatments. There could very well be a significant interaction between water and disturbance, or a result contrary to expected with water as a factor; Bargner et al. 2006 has described the process by which disturbed soils tend to leach nutrients (i.e. N) faster their undisturbed counterparts.

METHODS:

We used plant growth as a proxy for the fixation of nitrogen by cryptobiotic soil crusts. Soil crusts were procured from the National Bison range in Moiese, Montana. Crusts were placed into cylindrical pots (15.2cm diameter x15.2cm high), with soil from where they came, all from the same area of the National Bison Range. Soils were divided into four treatment types: undisturbed, unwatered; undisturbed, water added; disturbed, unwatered; and disturbed, water added. Ten replicates per treatment type resulted in a population size (n) of 40.

Disturbance was simulated by dropping a large mass (21.5kg) with a flat bottom surface onto the potted crusts from a height of 40cm. Because the mass was too small to disturb the entire crust surface with one drop, it was dropped three times per pot to completely disturb the surface. Five holes were drilled in each of the pots and Western Wheatgrass (*Pascopyrum smithii*) stems were transplanted into the holes. After being given a few days to establish, the stems were all cut to the same height of 20cm. 150ml of water was added to the "water added" treatments every day. Plant growth for each stem was measured and recorded every fifth day for 15 days. Any secondary growth, in addition to the five main stem heights, was cubed (to proxy biomass) and then averaged within each pot and recorded as the response. At the end of the experiment, the stems were again cut to 20cm and together the main and secondary stem clippings were dried, massed and recorded as biomass.

In addition to the manipulative experiment on cryptobiotic soil crusts, an in situ observational study was conducted on the National Bison Range to characterize the microclimates surrounding the soil crusts in myriad environments. Sites were haphazardly selected within the National Bison Range for their potential for cryptobiotic crusts. At each site, four .25m^2 quadrats were thrown every 10m in a random direction along a 40m transect. At each quadrat, soil crust percent cover, litter percent cover, bare ground percent cover, grass percent cover, forb percent cover, and hoof-print (i.e. ungulate) percent cover were recorded. An overall disturbance index (between 1-10) was visually estimated using cues like droppings, grazing and wallowing for 1m surrounding the center of the quadrat to cover an area 3.14m^2/quadrat.

RESULTS:

A 2^2 ANOVA was run in R with water and disturbance as factors, 10 replicates per treatment and average stem heights cubed as the response. Stem heights were cubed to more accurately depict biomass. A Shapiro-Wilk normality test indicated that the stem heights data violated the assumptions of the ANOVA, even after being cubed and several further attempts at normalization transformations. Two non-parametric Kruskal-Wallis tests were run on the cubed stem heights with disturbance and water as factors. The results indicate that neither disturbance nor water had a significant effect on height ($p=0.6$ and $p=1$, respectively [Figure 1]).

The clipped and weighed biomass from the manipulative experiment was run in the same 2^2 ANOVA. The results for the effect of disturbance and water were highly non-significant (p=0.75 and p=0.79 respectively). A Shapiro-Wilk test again indicated that the data violated the assumptions of the model. Because the results didn't approach significance, no further tests were performed (results not shown).

The number of secondary stems within each pot was summed and used as the response variable for another 2^2 ANOVA with disturbance and water as factors. Because, as per our hypotheses, we have a priori reason to believe that disturbance and water will affect the response in only one direction, we ran only one-tailed tests. The Shapiro-Wilk test confirmed that the data met the assumptions of the ANOVA (p=0.42). The results of the ANOVA indicated that the number of secondary stems is dependent on water (p=0.033), and demonstrated a marginally significant trend under disturbance (p=0.064). Both the directions of the trends were as predicted, with higher water and less disturbance yielding more stems (Figure 2). There was no significant interaction between the factors (p=0.25).

The in situ data was analyzed in SYSTAT 13 and R. A correlation matrix was performed and bare ground percent cover was highly correlated with cryptobiotic soil crust percent cover. This in light of the fact that bare ground, crust, and litter percent cover added to 100 resulted in bare ground being discounted from subsequent analysis. Individual regressions were run on soil crust percent cover, litter percent cover, bare ground percent cover, grass percent cover, forb percent cover, and hoof-print percent cover and disturbance index as variables (results not shown). Only the disturbance index was found to significantly regress with biomass (p=0.014 Figure 3). A Shapiro-Wilk test indicated that the data was normally distributed (p=0.17). A variable was created that combined grass and forb percent cover. This plant cover regressed strongly with biomass (p=0.004 Figure 4), but didn't quite meet the assumptions of regression (p=0.034). A regression that combined plant cover and disturbance showed that, once combined, plant cover still significantly correlated with biomass (p=0.02) but disturbance index was only marginally significant (p=0.09) with only 17% of the variance was explained by the line (r^2=0.17 results not shown).

DISCUSSION:

It was a climatically atypical summer in the Moiese Valley of western Montana, just north of Missoula. The precipitation was above average for the summer during the time this study was conducted (NOAA 2010). Specifically, there were several large rain events during the manipulative experiment, which may have confounded the effects of water treatment. The absolute lack of significance for the water factor in the stem heights ANOVA (p=1) is still somewhat surprising (Figure 1). Water is usually limiting in the cool desert that comprises the area. The fact that there was abnormally high precipitation could have been masking the effect of water addition as the soils may have already been at their saturation point.

The lack of difference in stem heights with disturbance as a factor may too be in part attributable to the abnormal precipitation throughout the valley. Cryptobiotic soil crusts are not only valuable to soils and plants because they fix nitrogen, but also because they retain water up to eight times better than soils without them (Belnap and Gardner 1993). The water logging of this experiment could then also be hamstringing any ability to detect disparities in growth due to increased moisture in undisturbed soils. Both the disturbance factor and the water factor for the manipulative experiment could have been affected by the fact that neither stem growth nor stem growth cubed is a true measure of biomass. Specifically, some of the secondary stems were initially flat, more like a leaf than a stem. The 2-D leafs therefore did not have any appreciable volume and cubing them in the context of discrete-looking 3-D stems in other pots probably wasn't a perfect representation of biomass, though it did correlate with the measured biomass (p=0.01).

As the measured biomass and the cubed stem height highly correlated (p=0.01), it is not surprising that the same ANOVA yielded similar non-significant results for disturbance and water (p=0.75 and p=0.79, respectively). A longer-term experiment might have made differences between treatments more detectable. Main stem growth visually seemed rather low. Secondary stem growth was much more robust and thus this experiment may have tested vegetative reproductive ability of stems more than their biomass. Replicating this experiment earlier on in the growing season my remedy this phenomenon, as Western Wheatgrass' main growing season is in early summer (USDA 2002).

As secondary stem growth seemed to be at least in part what this experiment was measuring, the number of secondary stems in any pot were summed and averaged. The results of the same 2^2 ANOVA indicate that number of secondary stems is indeed dependent on water ($p=0.033$) and trends towards significant dependence on crust disturbance ($p=0.064$ Figure 2). Because of the a priori predictions of our hypotheses, only a one-tailed test was run. The direction of response for both of the factors was as predicted: additional water and less disturbance both yielded more vegetative reproduction. These results are consistent with our hypotheses but inconsistent with our other results. It may be that because these experiments were run in late summer, vegetative reproduction is the only appreciable response that can be elicited form Western Wheatgrass. Nonetheless, if this experiment were to be repeated at this time of year and only consider secondary growth as response, more pots with fewer stems in each might maximize time constraints as the results did not change whether summed secondary stems or averaged secondary stems from each pot were used in the analysis. Additionally, a p-value that was lower than our α-level may have been detectable with more replication.

The data from the in situ observations was somewhat contrary to expectation. The fact that cryptobiotic soil crust percent cover didn’t correlate with biomass was not predicted. This could be because some of those soil crusts that were observed and counted in percent cover were also disturbed. The disturbed crusts would not be able to fix nitrogen as well and biomass would subsequently be reduced (Bowker et al 2005). The fact that plant cover (a combination of grass and forb percent coverage’s) correlates with biomass ($p=0.004$) ensures that the observational data should still be lent some credence. However, it was not normally distributed ($p=0.034$). Though disturbance strongly correlated with biomass ($p=0.014$), it was also likely the least empirical variable of those recorded in the observational study, as evaluation of multiple factors simultaneously is inherently more difficult in coming to a value. The disturbance index correlation’s directionality was consistent with prediction that disturbance and biomass would vary indirectly (Figure 3). The combination of disturbance and plant cover yielded a fairly significant line ($p=0.09$ and $p=0.02$, respectively), but only explained a small amount of the variance ($r^2=0.17$). While these two variables were the best predictors of biomass, they were less than ideal. Other factors may have been at play. Future work should include soil moisture in an observational study such as this one, as the high precipitation this summer was likely influencing all of our results.

The results of this study have implications for land managers of areas with high disturbance and/or grazing. Our data indicates that vegetative reproduction in plants will be significantly lower when soil crusts are disturbed. This is particularly important for management in late summer when vegetative growth is the only appreciable growth happening in grasses like *Pascopyrum smithii*. Not only will disturbance affect the N-availability in this N-limited species (USDA 2002) by reducing cryptobiotic crusts' ability to fix nitrogen, but disturbance will also affect moisture availability. Though there were no significant interactions between disturbance and water in the abnormally wet summer in which this study was conducted, normal years may well demonstrate interplay between disturbance, nitrogen availability and water, as crusts are the dominant sink of available water in arid ecosystems (Belnap and Gardner 1993). An increase in disturbance could easily cause water to be limiting instead of nitrogen. Places like the National Bison Range need to account for the disturbance of crust when evaluate regeneration times for graze lands. Considering the time scales necessary for establishment and reestablishment of soil crusts, grazers should be managed to minimize impact on crusts whenever possible.

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FIGURES:

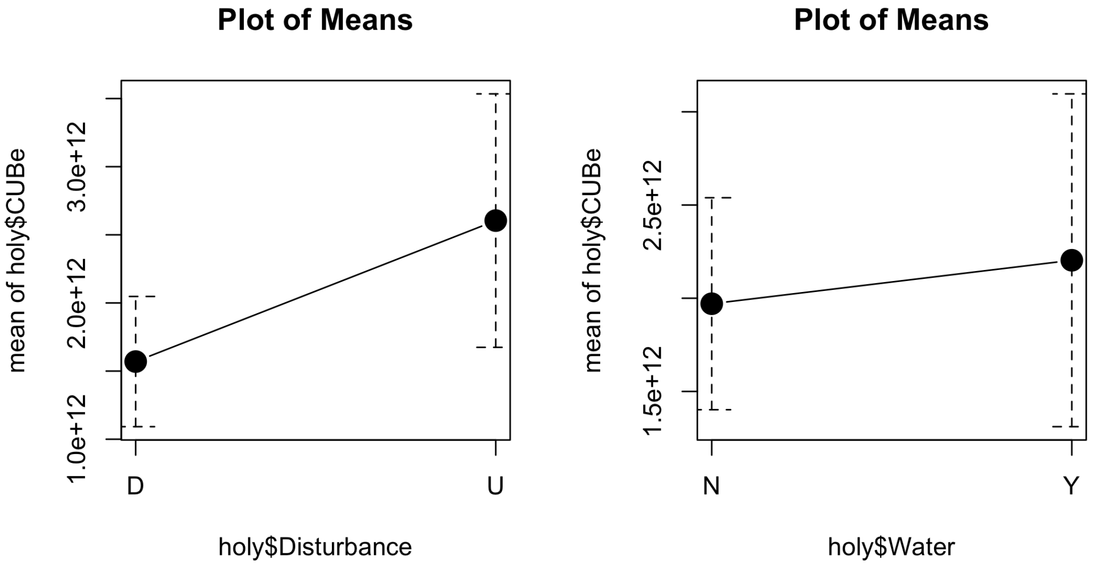

Figure 1. Plot of each stem height (including secondary's) cubed and then averaged. A non-parametric Kruskal-Wallis showed that neither disturbance (p=0.6) nor water (p=1.0) had an effect, though the means trend in the way expected.

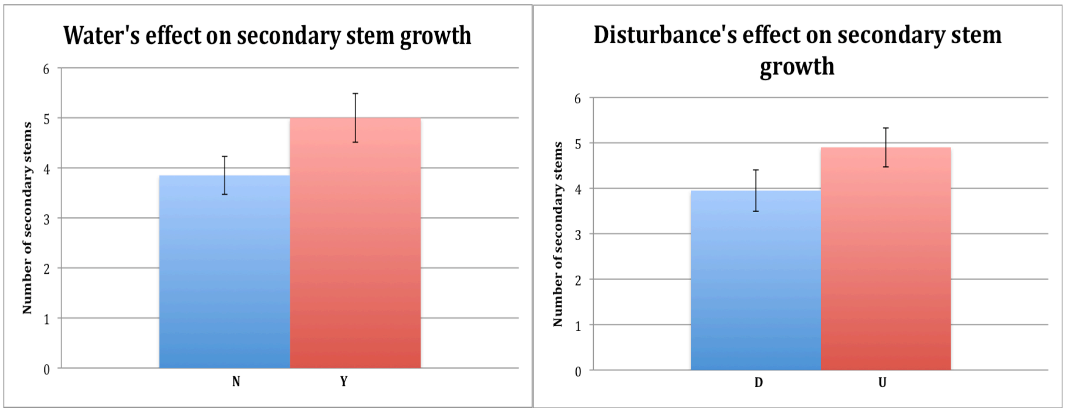

Figure 2. The number of secondary growth stems is higher with added water (p=0.033) and showed a marginally significant trend with undisturbed crusts (p=0.064). These results were as expected.

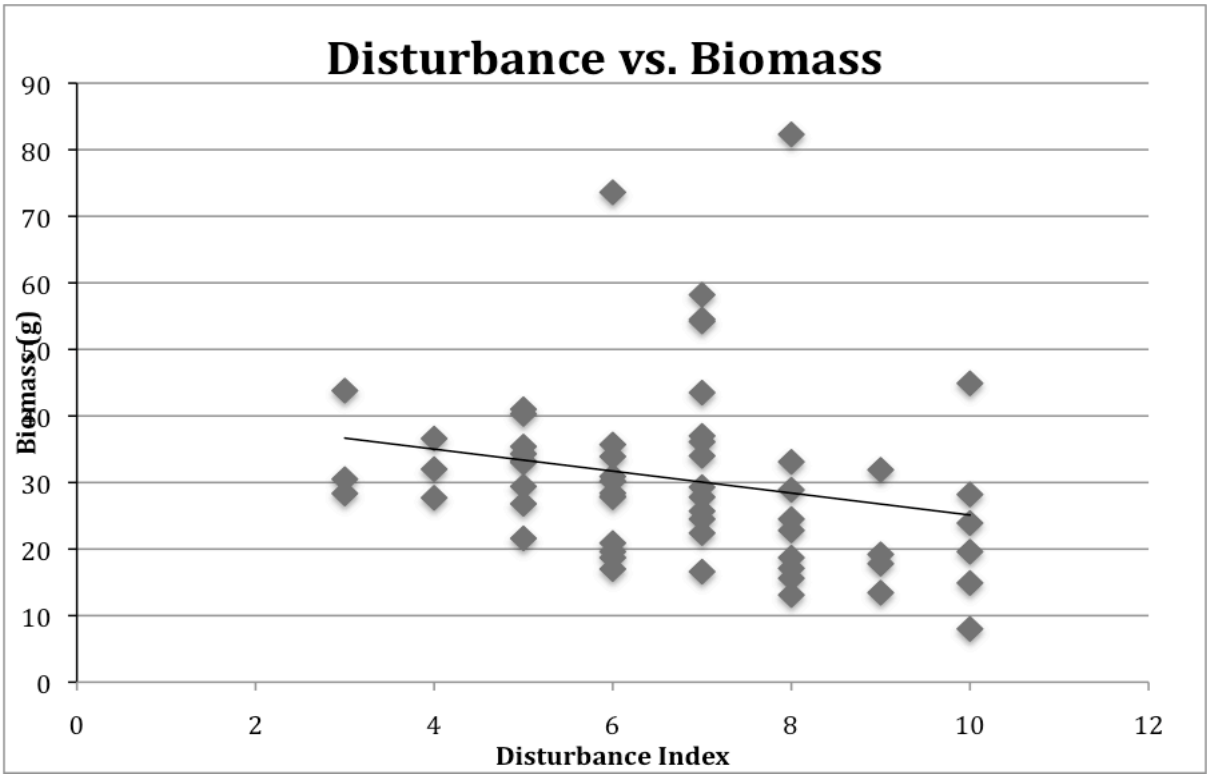

Figure 3. Regression of Biomass with Disturbance as an explanatory variable (p=0.014). The trend is as predicted: biomass decreases with increasing disturbance.

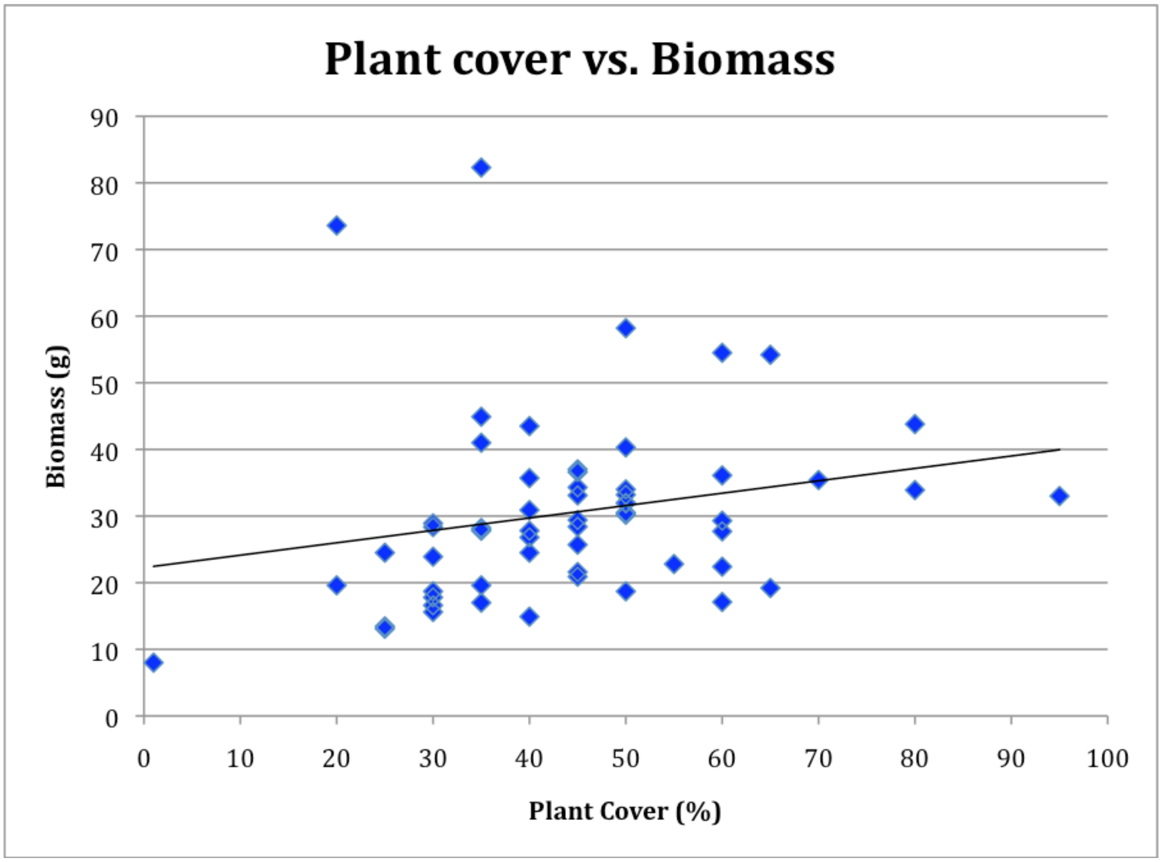

Figure 4. Regression of Biomass with plant cover as an explanatory variable (p=0.004). Results were not normal, even after transformation (p=0.034), but the large sample size (n=59) allows us to still accept the result. Increased plant cover predicted higher biomass, which was as expected.