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COMMUNITIES AND ECOSYSTEMS

By

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ABSTRACT

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Biotic pulses, bryotic pulses and the effects of mosses on communities and ecosystems

Chairperson: Dr. Ragan M. Callaway

  Non-vascular mosses are known to be ecologically influential and structure communities through their physiological and physical attributes. My dissertation had two objectives. The first goal was to understand how fundamental differences in the physiology of mosses might drive much of their ecological impact. Mosses frequently and rapidly fluctuate between dehydration and rehydration. During these transitions, the cellular integrity of moss is compromised and intra-cellular contents (carbohydrates, amino acids, and ionic compounds) are released. Thus during wet-dry transition periods, mosses have the potential to release pulses of nutrients which may have important effects on other species. However, few studies have explored these bryotic pulses in the context of communities and ecosystems. This first part of my dissertation worked to fill this gap by addressing: 1) How the unique physiology of mosses directly influenced ecosystem level processes? And 2) If bryotic pulses regulated biotic interactions and nutrient availability? The second objective of my dissertation was to understand the community level effects of mosses in semi-arid grasslands. Though mosses are abundant in many ecosystems ranging in climate from mesic to arid, our current understanding of their role in dryland systems is limited. I used a positive spatial association between native mosses and an exotic forb to investigate direct and indirect interactions between these co-occurring plants. I also considered the potentially widespread effects of moss as a component of biotic soil crusts on plant establishment in intermountain grasslands.

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Chapter 1: Bryotic pulses: desiccation greatly increases resource fluxes from mosses which alter CO_2 and N_2O efflux from soils

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Abstract

The general effects of mosses on nutrient cycling are well known. However, we know much less about how the desiccation and rehydration cycles that mosses undergo influence these processes. We examined the quantity of carbon and nitrogen in throughfall after precipitation passed through eight temperate moss species that were either hydrated continuously or desiccated and rehydrated. Desiccated-rehydrated mosses lost from 1.9 to 30.7 times more carbon than mosses that had not been desiccated in throughfall. Throughfall from desiccated-rehydrated mosses also contained nitrogen, whereas throughfall from hydrated mosses had little to no nitrogen. To explore the effects of these nutrients on soil microbial activity, we added the throughfall of four moss species from hydrated or desiccated-rehydrated mosses to soil and measured gases produced during carbon (CO_2) and nitrogen (N_2O) cycling. We also determined the cumulative priming effect to relate the net effect of the carbon added in moss throughfall on CO_2 production. Throughfall from hydrated mosses promoted a slight but positive priming effect, indicating an increase in organic matter decomposition, whereas throughfall from

desiccated-rehydrated mosses generated a negative priming effect for three of four moss species, indicating inhibition of decomposition. In addition, both types of throughfall generated a positive N_2O efflux but desiccated-rehydrated moss throughfall promoted a larger N_2O efflux than hydrated moss throughfall for three of four moss species. In sum, our results indicate that carbon and nitrogen lost from desiccated mosses during rehydration can influence soil carbon and nitrogen transformations. Our focus in this study was to understand the effect of moss desiccation-rehydration throughfall on soil gas production, but many other biogeochemical factors such as phosphorus availability, enzyme activity, and soil microbial communities may be influenced by bryotic pulses.

Introduction

Mosses modify their environments in many ways. They buffer soil temperature and moisture, decrease surface water runoff and enhance soil water retention (Pócs 1980; Veneklaas et al. 1990; Gornall et al. 2007; Blok et al. 2011). Mosses are often associated with higher soil organic matter, soil total carbon (C) and soil total nitrogen (N), which has been attributed to the direct effects of mosses on soil temperature and moisture (Lamontagne 1998; Sedia and Ehrenfeld 2005; Gornall et al. 2007; Turetsky et al. 2010; Zhao et al. 2014; Sun et al. 2017). Because of their high absorptive capacity, mosses also accumulate nutrients from precipitation and canopy throughfall, thereby acting as nutrient sponges (Lagerstrom et al. 2007; Turetsky et al. 2010; Street et al. 2013). However, our understanding of how mosses influence communities and ecosystems has been based on comparisons of processes ocurring either in the presence or absence of moss, which may or may not include the effects of desiccation and rehydration. Mosses are poikilohydrous; when water is not available they desiccate, equilibrating their

cellular water content with water levels of ambient air. When mosses rehydrate, their cellular integrity is compromised and intra-cellular contents (carbohydrates, inorganic nitrogen, amino acids, and ionic compounds) are lost (Carleton and Read 1991; Coxson 1991; Startsev and Lieffers 2006; Bach et al. 2009; Wilson and Coxson 1999). We know very little about these "bryotic" pulses of resources lost during moss rehydration and quantifying these pulses can 1) provide insight into the mechanism by which mosses affect their environment and other species, and 2) alter previous quantitative estimates of the overall effect of mosses on ecosystems.

Several studies have compared the leakiness of continuously hydrated mosses to that of desiccated-rehydrated mosses under controlled conditions, but generalizations to natural conditions are limited. Brown and Buck (1979) found that desiccated mosses lost intracellular potassium (K) during rehydration as a result of cellular membrane damage while hydrated mosses did not leak K into Petri dishes of water. Other research, also in glass containers, indicated that C and N losses were greater when previously desiccated mosses were rehydrated than losses from continuously hydrated mosses (Gupta 1977; Carleton and Read 1991). However, desiccated-rehydrated mosses were able to reassimilate lost nutrients when left in contact with the nutrient-laden water for multiple days (Gupta 1977; Brown and Buck 1979).

To our knowledge, two studies have measured nutrient releases from rehydrating mosses in a natural setting. In the tropics, precipitation that passed through previously desiccated epiphytic bryophytes (one moss and one liverwort species) had more C, K, and phosphorus than ambient rainfall and the composition and quantity of the throughfall C varied between bryophyte species (Coxson 1991; Coxson et al. 1992). Likewise in a boreal forest, throughfall from the ground-dwelling feather moss *Hylocomium splendens* contained higher C and K concentrations than ambient rainfall (Wilson and Coxson 1998). The corresponding flux of C with K in the

throughfall of mosses that were desiccated and rehydrated found in both studies (Coxson 1991; Coxson et al. 1992; Wilson and Coxson 1998) suggests that the source of this C was intracellular and associated with cellular membrane damage. However, to our knowledge there have been no comparisons of throughfall from hydrated versus desiccated-rehydrated mosses in experiments that do not allow reabsorption.

Mosses are well known for their positive effect on soil organic matter, C and N (Lamontagne 1998; Sedia and Ehrenfeld 2005; Gornall et al. 2007; Zhao et al. 2014; Sun et al. 2017), but desiccation and rehydration may play an important and underappreciated role in their effects on nutrient cycling. For example, bryotic pulses may stimulate organic matter decomposition by adding exogenous C. This increased rate of organic matter decomposition would be accompanied by higher rates of soil microbial respiration (quantified by soil CO_2 production) and result in a loss of native soil organic matter as a result of exogenous C inputs, a phenomenon termed the priming effect (Bingemann et al. 1953; Kuzyakov et al. 2000). Alternately, if moss-derived C inputs increase soil carbon, bryotic pulses would promote a "negative" priming effect (Lorenz et al. 2007; De Deyn et al. 2008; Tamura and Tharayil 2014). In addition to C, N can be lost from desiccated-rehydrated mosses (Gupta 1977; Carleton and Read 1991). The addition of C plus N to soils in bryotic pulses could stimulate inorganic N mineralization, nitrification, and the loss of N from soils as N_2O through the aerobic process of nitrifier denitrification. Thus, resources carried in throughfall from moss desiccation and rehydration have the potential to substantially influence soil biogeochemistry, but consequences for soil C and N remain unclear.

Here, we quantified the effects of moss desiccation and rehydration on C and N fluxes by comparing C and N in the throughfall of eight continuously hydrated versus desiccated and

rehydrated moss species. We also added the two forms of throughfall from four of these moss species to soil and monitored soil respiration (CO_2) and nitrous oxide (N_2O) efflux. We hypothesized that C and N in the throughfall of desiccated-rehydrated mosses would be at higher concentrations than throughfall from hydrated mosses, and that throughfall from desiccated-rehydrated mosses would drive greater increases in soil microbial activity related to C (CO_2) and N (N_2O) cycling.

Methods

Study sites and sampling regimes

Mosses were collected from forests in northwestern Oregon (45°41'00.5"N, 121°44'50.8"W) and west-central Montana (47°35'31.0"N, 115°13'44.9"W). Average annual temperatures near the Oregon site range from a high of 17.4˚C to a low of 7.5˚C with a mean annual rainfall of 95.3 cm (www.usclimatedata.com). The Montana site ranges in mean annual temperature from 14.5˚C to 0.9˚C and has a mean annual rainfall of 36.3 cm. Rainfall in both locations is highest in the spring and intermixed with multi-day periods of dry weather, thus moss desiccation-rehydration events should correspond with high levels of spring plant and soil microbial activity.

Throughfall analysis

We selected eight widespread moss species that co-occur and also vary in growth form and habitat preference. *Kindbergia oreganum* and *Ceratodon purpureus* were collected from Oregon on 9 April 2014. *Aulacomnium palustre*, *Dicranum scoparium*, *Racomitrium lanuginosum*, *Rhytidiadelphus triquetrus*, *Plagiomnium ciliare*, and *Syntrichia papillosissima*

were collected at the Montana site on 10 April 2014 (species identifications follow Flora of North America Editorial Committee 2007, 2014). All mosses were desiccated when harvested and within two days most of the plant debris and attached soil was removed by hand. Mosses were transplanted into 5 x 5 cm pots (n=16 per species) filled with a 2:1 planting medium of sand/Coir mixture (Down to Earth, Eugene, OR, USA) and placed into the University of Montana Diettart Research Greenhouse.

Mosses were misted for thirty minutes four times a day for two days to rehydrate the desiccated mosses and to remove atmospheric particulates. After 48 hours, eight pots of each species were removed from the misting table and placed on a nearby table to dry while the other eight pots were kept continuously hydrated for seven days. To collect throughfall, the moss was removed from a single pot and placed in a funnel attached by polyvinyl tubing to sterile 50 mL Falcon tubes and misted until 40 mL of throughfall was collected (adapted from Coxson 1991). The area of each moss sample was recorded after blotting surface water from mosses. We ran water through empty funnels as controls for background nutrient levels. Non-purgeable organic carbon and total N of throughfall were determined with a Shimadzu TOC-V TN Analyzer (Shimadzu Corporation, Kyoto, Japan). Total amounts of C and N for hydrated and desiccated-rehydrated mosses are presented by concentration in volumetric units (mg/L) and on an area basis (mg/cm^2).

Gas efflux from soil incubation

To explore the effects of nutrients released in the throughfall of hydrated or desiccated-rehydrated moss on soil CO_2 and N_2O effluxes, we collected and stored large quantities of throughfall in a similar manner as presented above and used this in a soil incubation experiment.

Between June and August 2017, we collected *Syntrichia papillosissima*, *Rhytidiadelphus triquetrus*, *Dicranum scoparium*, and *Racomitrium lanuginosum* from the Montana site. Mosses were collected dry, cleaned, and transplanted into the greenhouse within 48 hours. Mosses were planted in 5 x 5 cm pots (n=40 per species) and placed in a greenhouse where they were misted four times a day for nine days to maintain constant hydration. After nine days, half of the pots for each species (n=20 per species) were desiccated for six days while the other half remained continuously hydrated at the same misting rate. Throughfall was collected in the manner noted above and stored at 4°C. Storage of this throughfall may have altered its effects on gas efflux in ways that we do not understand; however, all samples were treated the same way.

Soil samples for the experiment were collected on 11 September, 2017, when soil was dry, from a forested area near Missoula, MT where all four species of moss co-occur (46°59'00.3"N, 114°01'35.1"W). Soil was collected to a depth of 12 cm, homogenized, and sieved through a 2 mm screen. For soil incubation, 45 1L Mason jars were prefilled with 5 mL of deionized water to ensure that soils maintained a constant moisture. Sixty g of moistened soil (~ 47 g dry weight) were measured into specimen cups which were placed inside the Mason jars. Forty of the specimen cups were treated with 25 mL of throughfall, with five jars assigned for each species-treatment (continuously hydrated vs. desiccated-rehydrated). Five specimen cup soil replicates were watered with 25 mL of deionized water as a control. Six additional jars were treated in the same way as the experimental jars and filled with 5 mL of deionized water to calibrate background gas levels. Mason jars were sealed with lids fitted with rubber septa for gas sampling and incubated at 22°C in the darkness. Fifteen mL gas samples were removed from the jar headspace at three time points (6, 24, and 48 hrs) and placed into 12 mL evacuated Exetainer vials (Labco Ltd., Buckinghamshire, UK). After each sample collection, lids were removed and

air was re-circulated for five minutes prior to resealing. Gas samples were analyzed for CO_2 and N_2O concentrations using a Shimadzu GC-2014 greenhouse gas analyzer (Shimadzu Scientific, Kyoto, Japan). Gas efflux was corrected for soil gravimetric water content and background gas levels and is presented on a dry weight basis.

Data analyses

Total organic carbon (TOC) concentration in hydrated and desiccated-rehydrated mosses was tested with a two-way ANOVA model, using adjusted TOC levels for moss area, with species, treatment (hydrated or desiccated-rehydrated) and their interaction as fixed factors. Total nitrogen (TN) concentrations could not be compared between treatments since so little N was detected in the throughfall of mosses that had not been desiccated. TN levels in the throughfall of desiccated-rehydrated mosses, adjusted for moss area, were compared across species with a one-way ANOVA model.

We calculated the cumulative priming effect as a measure of the net effect of C in the throughfall from mosses on CO_2 efflux. The cumulative priming effect was determined by subtracting the total CO_2 efflux of water treated soil (control) from throughfall treated soil and then subtracting this from the total amount of C added in the throughfall (from TOC values; mg C g soil^{-1}). This mass based approach is insensitive to pool substitutions, in which soil organic matter is decomposed by microbes but moss-derived C replaces the lost soil C, resulting in no net change in soil C. This method is not able to discern changes in microbial growth rates or turnover, which could influence our interpretation of priming. To overcome such limitations would involve adding isotopically labelled C to soil, which would require growing moss in a labeled CO_2 environment, an infeasible task in the present study. Therefore, we cautiously

interpret mass balance of C as an indicator of priming. Likewise, the impact of bryotic pulses on

N_2O efflux was determined by subtracting the total N_2O efflux of water treated soil from

throughfall treated soil (ng N g $soil^{-1}$). Two-way ANOVAs were used to evaluate the

independent and interactive effects of moss species and desiccation treatment on cumulative

priming and N_2O efflux. To determine if addition of throughfall treatments promoted differences

in cumulative priming within a study species, we used two-tailed t-tests were to evaluate if

means were different from zero. The effect of throughfall treatments on N_2O efflux within study

species were compared with one way ANOVAs. All analyses were performed in JMP, Version

11.0 (SAS Institute Inc., Cary, NC, 2013). Prior to analyses, distributions of means were checked

for normality using the Shapiro-Wilk test and TOC and TN concentrations were log-transformed

to satisfy assumptions of normality and homoscedasticity.

Results

Throughfall analysis

Desiccated-rehydrated mosses generated throughfall with 1.9 to 30.7 times more TOC, by

area, than hydrated mosses ($F_{1, 124}$ =304, *P*<0.0001, Figure 1), depending on the species. The

quantity of TOC in the throughfall of hydrated or desiccated-rehydrated mosses also varied a

great deal among species ($F_{7, 110}$=2.33, *P*=0.030). We could not quantitatively compare the

effects of desiccation and rehydration on throughfall TN, because TN was so low when mosses

were not desiccated. TN in the throughfall of desiccated-rehydrated moss did not differ among

species, but there was very high variation around the means for each species ($F_{6, 33}$=0.28,

P=0.941, Figure 1). Throughfall C/N from desiccated-rehydrated mosses ranged from 2.7-6.5, by

concentration (Table 1).

Soil incubation gas efflux

Throughfall from the four moss species varied in their effect on soil CO_2 efflux ($F_{3, 32}$=5.44, *P*=0.004, Figure 2) with the throughfall of hydrated and desiccated-rehydrated mosses generally having opposite effects on soil carbon priming ($F_{1, 32}$=66.1, *P*<0.0001, Table 2). For two moss species (*Rhytidiadelphus* and *Dicranum*), soils exposed to throughfall from hydrated mosses promoted a slight but positive priming effect (*Rhytidiadelphus:* $t_{1, 4}$=0.013 and *Dicranum*: $t_{1, 4}$=0.012). Conversely, throughfall from two species of desiccated-rehydrated moss generated a negative priming effect (*Racomitrium*: $t_{1, 4}$<0.0001 and *Rhytidiadelphus:* $t_{1, 4}$=0.007). Throughfall from hydrated or desiccated-rehydrated *Syntrichia* had no effect on soil carbon priming ($t_{1, 4}$=0.948, $t_{1, 4}$=0.290, respectively).

Soil N_2O production from soils exposed to moss throughfall ranged between 124 and 257 ng N g soil^{-1} higher than soil exposed to water (Figure 2). For three of the four moss species (*Racomitrium*, *Rhytidiadelphus* and *Syntrichia*), soil N_2O production was significantly higher when soil was exposed to throughfall from mosses that were desiccated-rehydrated relative to soils that were exposed to throughfall of mosses that were hydrated (*Racomitrium*: $F_{1, 8}$=13.2, *P*=0.007, *Rhytidiadelphus*: $F_{1, 8}$=7.67, *P*=0.024, and *Syntrichia:* $F_{1, 8}$=4.71*, P*=0.062). *Dicranum* had statistically similar N_2O production between soils exposed to hydrated and desiccated-rehydrated throughfall ($F_{1, 8}$=0.037, *P*=0.852).

Discussion

Our results identify a potentially important mechanism by which mosses influence ecosystem processes and properties. Cellular damage in mosses, incurred during desiccation and

rehydration, resulted in the loss of far more C and N in throughfall, and this throughfall appeared have important effects on soil microbial activity and C and N fluxes. This reinforces and quantifies the importance of desiccation and rehydration as a mechanism driving the influence of mosses on ecosystems, and suggests that when evaluating the effects of mosses on soil C and N moss desiccation and rehydration should be considered.

Desiccation-rehydration and resource release

We found that the throughfall of desiccated-rehydrated mosses often contained an order of magnitude more C than the throughfall of hydrated mosses, and this effect varied among moss species. This variation among species may be due to how different moss species utilize C compounds for cellular protection throughout the desiccation and rehydration processes (see Hoekstra et al. 2001; Oliver et al. 2005; Green et al. 2011; Stark 2017). For example, C compounds are thought to replace water molecules in the cell membrane during desiccation to stabilize the membrane and prevent fracturing (Crowe et al. 1992; Smirnoff 1992; Hoekstra et al. 2001). Mosses also rely on C compounds for intracellular osmotic adjustment during the initial stages of drying, and as desiccation proceeds C compounds facilitate the transition of the cytoplasm to the more stable glass phase; preventing cellular collapse and protein denaturing (Koster 1991; Sun and Leopold 1997; Hoekstra et al. 2001). Finally, mosses rely on a variety of C compounds when hydrated and desiccated to scavenge reactive oxygen species which increase in abundance during water stress (Smirnoff and Cumbes 1989; Popp and Smirnoff 1995). Differences in the ways mosses species utilize carbon compounds to ensure survival may explain why they vary considerably in the types and amounts of C they contain (Robinson et al. 2000) and release. We do not know of comparisons of C compounds across species and environments,

but species from arid environments are thought to fine-tune the suite of morphological, physiological and molecular strategies used to survive repeated desiccation and rehydration cycles in a manner that minimizes the loss of cellular compounds during rehydration (Brown and Buck 1979). Whether such adaptations are the primary drivers of differences among moss species in the quality and quantity of C (and N) lost during rehydration in bryotic pulses is not known.

Our study included ground dwelling mosses from mesic habitats in western Oregon and xeric habitats in western Montana. *Kindbergia* and *Ceratodon* were both collected from Oregon and lost more N in throughfall when desiccated and rehydrated than the six species from Montana. However, *Kindbergia* lost 87% more C in throughfall when desiccated and rehydrated than *Ceratodon,* suggesting that factors other than climate influence the ability of individual species to avoid membrane damage and resource loss associated with desiccation and rehydration.

Variation among moss species in C and N loss following desiccation and rehydration may have less to do habitat and more to do with life-form, or the overall structure of a colony of individual shoots (*sensu* Mägdefrau 1982), which provides an indication of habitat differences related to moisture and light (Proctor 1990; Glime 2017). For instance, species with loose trailing life-forms are more common in moist forests; whereas tight cushion forming mosses are typical of exposed sites subject to frequent drying (Glime 2017). *Kindbergia* and *Ceratodon*, from our Oregon site, have very different life-forms. *Kindbergia* occurs in the understory of dense forests and has creeping stems that generate loose tufts of fronds (Ignatov 2014); whereas *Ceratodon* inhabits habitats from exposed roadsides to forest understory and forms short turfs (McIntosh 2007). Turf forming mosses like *Ceratodon* may be able to retain more nutrients in the

interspaces of shoots than loose feather mosses like *Kindbergia* and/or other traits may govern membrane damage to account for the differences in C loss measured here.

Carbon and N lost by mosses during desiccation and rehydration might be advantagous to the plant undergoing the process, in some conditions. *Racomitrium* and *Syntrichia* form dense turfs or cushions, both occur on dry and low nutrient soil (Mishler 2007; Ochyra and Bednarek-Ochyra 2007), yet desiccated-rehydrated *Racomitrium* lost 94% more C to throughfall than desiccated-rehydrated *Syntrichia*. Unlike *Syntrichia*, *Racomitrium* colonizes newly exposed ground during primary succession where it associates with epiphytic cyanobacteria that might benefit from pulses of C and in turn, potentially provide N for *Racomitrium* (Berg et al. 2013; Arróniz-Crespo et al. 2014).

Our study captured nutrient loss from mosses during just one simulated rain event, but natural desiccation-rehydration events may vary widely. Resource loss from mosses during rehydration is determined by the level of cellular damage incurred during desiccation and rehydration, thus very intense periods of desiccation may promote greater resource loss. The intensity of cellular damage is regulated by environmental factors that interact to influence the rate at which mosses desiccate, the length of time spent desiccated and the rate of rehydration (reviewed in Stark 2017). Thus, variation in the number, frequency, and intensity of dry-wet events experienced by mosses should alter the quantity of C and N entering ecosystems annually through bryotic pulses. Furthermore, locations where dry-wet cycles are more frequent should generate successive (and perhaps predictable) bryotic pulses.

Influence of bryotic pulses on soil microbial activity

Moss throughfall C and N concentrations altered soil microbial activity and greenhouse gas production. Throughfall with a high C/N ratio may be expected to cause a positive C priming effect in soil and inhibit the production of N_2O; whereas substrates with a low C/N ratio should increase N_2O production and cause negative C priming effects (Baggs et al. 2000; Sullivan and Hart 2013; Liang et al. 2015). Yet**,** in addition to the effect of resource stoichiometry, priming effects and N_2O production should be influenced by the chemistry of the C substrate (Morley and Baggs 2010; Wang et al. 2015; Luo et al. 2016).

Contrary to our predictions, we found that soils incubated with throughfall derived from continuously hydrated mosses resulted in more C emitted from soil as CO_2 than was added in throughfall. Therefore, we interpret this result as evidence that the cumulative priming effect of C addition from the throughfall of hydrated mosses was positive. In contrast, throughfall from desiccated-rehydrated mosses, which had higher C concentrations and C/N ratios, resulted in negative priming because the amount of CO_2 produced was less than the amount of C added in throughfall. This suggests that the quality or chemistry of C in bryotic pulses might affect particular groups of microbes differently based on their substrate preference (Six and Jastrow 2002; Wang et al. 2015; Luo et al. 2016). Longer term studies will be needed to determine how bryotic pulses influence soil C pools but negative priming effects could be a mechanism by which mosses increase soil organic matter accumulation (Lamontagne 1998; Sedia and Ehrenfeld 2005; Gornall et al. 2007; Zhao et al. 2014; Sun et al. 2017).

In general, moss throughfall had a positive effect on soil N_2O efflux, regardless of whether the moss was hydrated or desiccated-rehydrated. However, throughfall from desiccated-rehydrated mosses with higher C/N ratios promoted higher N_2O efflux than the throughfall of hydrated moss for three of our four moss species (*Racomitrium*, *Syntrichia* and *Rhytidadelphus*).

These results contrast with others which found that the addition of high C/N substrates reduced soil N_2O fluxes (Baggs et al. 2000; Liang et al. 2015). We also found that the highest rate of N_2O efflux occurred in the throughfall of desiccated-rehydrated *Syntrichia*, the species of moss that lost the least C and N during rehydration. It is likely that the quality of C in moss throughfall may be influencing N transformations and N_2O production in ways we don't understand. There are multiple N transformation pathways that generate N_2O (reviewed in Butterbach-Bahl et al. 2013) but two are likely relevant here. N_2O can be produced as a byproduct of the anaerobic process of denitrification or of the aerobic process of nitrifier denitrification, as both processes are regulated by N, C and water availability (Bremner 1997; Tiemann and Billings 2008). It is unlikely that our soil incubations were anaerobic, though small anaerobic pockets may have existed. Thus, nitrifier denitrification most likely accelerated N transformations as a result of the C and N added in the throughfall of desiccated-rehydrated and hydrated mosses. Mosses affect N accumulation (Bowden 1991), N storage (Oechel and Van Cleve 1986), N fixation (DeLuca et al. 2002; Deane-Coe et al. 2015) and N availability (Lindo and Gonzales 2010, Delgado-Baquerizo et al. 2016), but the role of nutrients lost by mosses following desiccation-rehydration on soil N pools and fluxes represents a new pathway by which mosses influence the soil environment.

Conclusions

Our results suggest that relatively large amounts of C and N released from mosses during desiccation-rehydration cycles are an important way by which mosses may alter soil C and N pools. The quantity of TOC in the throughfall of desiccated-rehydrated mosses was 1.9 to 30.7 times greater, by area, than the amount of TOC in the throughfall of continuously hydrated mosses. Throughfall TOC from desiccated-rehydrated mosses also varied substantially among

the eight species of mosses tested. Throughfall from hydrated mosses had little to no TN, whereas throughfall TN levels were consistent among species of desiccated-rehydrated mosses. The magnitude of C and N in these *single* simulated bryotic pulses was roughly equivalent to *annual* C and N fluxes in the throughfall of boreal trees (Blew et al. 1993; Mellec et al. 2010) and temperate deciduous forest litter (Carlisle et al. 1966; Qualls et al. 1991). Throughfall from desiccated-rehydrated moss differed from the throughfall of hydrated moss in its effect on soil gas production. We found that the throughfall of desiccated-rehydrated mosses generated a negative C priming effect while the throughfall of hydrated mosses generated a positive C priming effect. Additionally, throughfall from desiccated-rehydrated mosses promoted higher soil N_2O efflux than the throughfall of hydrated mosses. The intensity of the effects of throughfall from hydrated and desiccated-rehydrated mosses on soil gas production varied among moss species, and in a manner that suggests that the quality of C and N compounds carried in moss throughfall may drive variation in the effects of throughfall on soil biota. A better understanding of the biochemistry of bryotic pulses will provide more insight into the effects of mosses on ecosystems.

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Table 1. Mean total organic carbon, total nitrogen, and carbon-to-nitrogen ratio in the throughfall of mosses that were desiccated and rehydrated by concentration during a single simulated rain event.

Species	Total C (mg/L)	Total N (mg/L)	C-to-N
Kindbergia oreganum	179	1.65	108
Aulacomnium palustre	42.4	1.26	33.7
Ceratodon purpureus	23.2	1.80	12.9
Plagiomnium ciliare	20.0	1.37	14.6
Dicranum scoparium	27.1	1.01	26.8
Racomitrium langinosum	94.2	1.84	51.2
Rhytidiadelphus triquestrus	47.8	1.41	33.9
Syntrichia papillosissima	5.63	0.88	6.40

Table 2. Summary statistics generated from ANOVAs for the response variables: cumulative priming and N_2O efflux. For each response variable, we tested for the individual and interacting effects of species and treatment. Significant (* $P \leq 0.05$) values are in bold.

	Effects	*df*	*F-test*	*P-value*
Cumulative priming	Species	3 (32)	5.44	**0.004***
	Treatment	1 (32)	66.1	**<.0001***
	Species * Treatment	3 (32)	10.1	**<.0001***
N_2O efflux	Species	3 (32)	6.02	**0.002***
	Treatment	1 (32)	11.1	**0.002***
	Species * Treatment	3 (32)	1.85	0.158

Figure Legends

Figure 1. Total organic carbon and total nitrogen in throughfall of hydrated (open bars) or desiccated-rehydrated (filled bars) mosses by moss area. Data are means + SE.

Figure 2. Cumulative priming effect (a) and N_2O efflux (b) evolved over a 48 hour incubation of throughfall amended soil. Throughfall was collected as mosses were rehydrated from a desiccated state (filled circles) or after passing through hydrated mosses (open circles). The effects of throughfall from four species of moss were compared. Data are means ± SE.
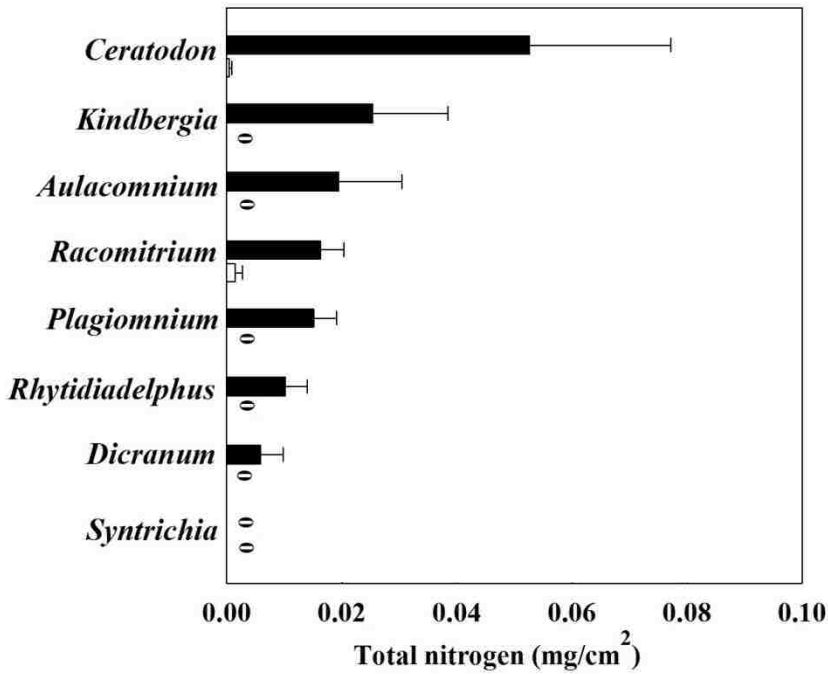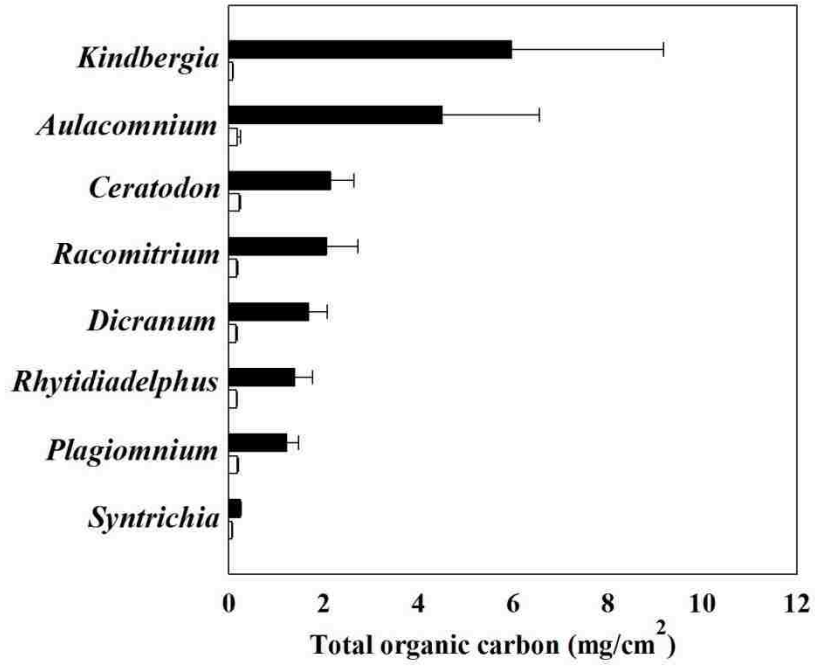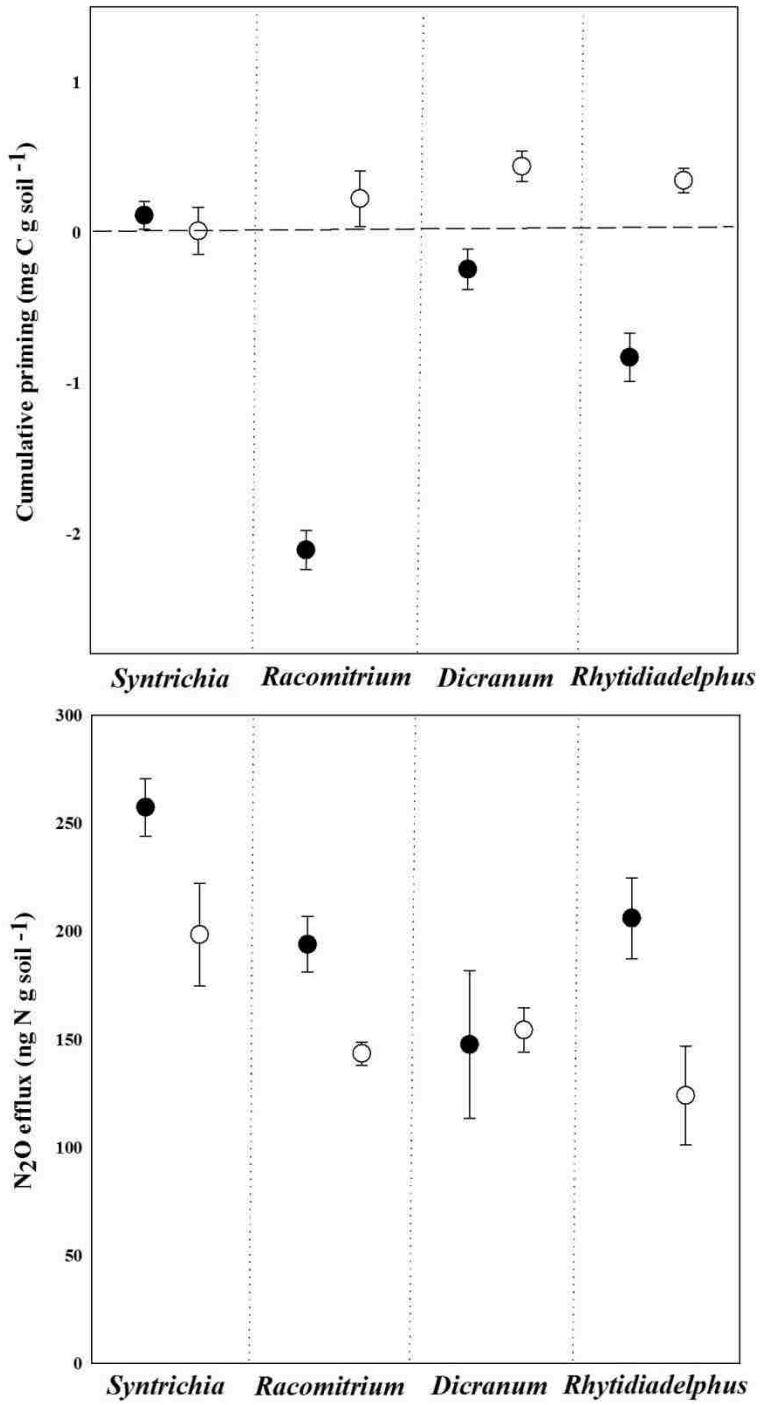
Figure 1.

Total organic carbon (mg/cm²) bar chart

Figure 1.

Figure 2.

Chapter 2: Presence of an invasive forb and native moss modifies the effect of water pulses on soil nitrogen availability

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Summary

1. Interactions among plants can be affected by the frequency and magnitude of resource pulses; which may also increase the abundance of exotic invasive plant species relative to native species. We explored the effects of resource pulses in the context of observations that *Centaurea stoebe*, an exotic forb, was positively associated with native mosses, which produce pulses of nutrients through desiccation and rehydration.

2. We conducted field surveys to evaluate the cover of *Centaurea* and native mosses and established a field removal experiment testing interactions between *Centaurea* and mosses in the context of water pulses, and measured changes in soil N. Experimental plots were treated with one large or three small water pulses, equal in total magnitude, in each of two years when mosses were dry. In a greenhouse experiment, we compared interactions between *Centaurea* seedlings and mosses to evaluate differences related to life-stage.

3. *Centaurea* and mosses were positively associated in our surveys and across both water pulse treatments, moss biomass decreased when *Centaurea* was removed, indicating that the invader facilitated mosses. Mosses did not affect adult *Centaurea* in the field, but *Centaurea* seedlings were facilitated by mosses in the greenhouse. Water pulse type affected soil N concentrations,

but did not affect the biomass of *Centaurea*, mosses or their interactions. However, interactions between *Centaurea* and native mosses altered the effects of water pulses on N availability.

4. *Synthesis.* These results highlight a unique set of positive interactions between an exotic forb and native mosses, but we found no evidence that water pulses affected these interactions. However, pulses had important effects on soil N availability when both *C. stoebe* and mosses co-occurred, suggesting that complex interactions between resource pulses and species interactions can affect ecosystem processes.

Keywords: competition, facilitation, exotic species, moss, nitrogen, water pulse

Introduction

Interactions among native and invasive species, and the effects of invasive plants on ecosystem processes, may be tempered by the frequency and magnitude of resource pulses (Davis *et al*. 2000; James *et al*. 2006; Radford 2013). Variation in the timing of resource delivery can affect competition among plant species (Chesson *et al*. 2004; Lamb *et al*. 2012) and subsequently plant colonization (Davis *et al*. 2000). This may be due in part to how the frequency and duration of resource pulses decouple biogeochemical processes and thus creates environments that favor some species over others (Loik *et al*. 2004; Collins *et al*. 2008). If combined with variation among species in their ability to respond to resource pulses, fluctuation in the timing of resource supply can also influence species coexistence (Chesson *et al*. 2004).

Invasive plant species commonly outcompete natives for resources (Reinhart & Callaway 2006; Wilsey & Polley 2006; Maron & Marler 2008; Aschehoug et al. 2016) and have strong negative impacts on native species and communities (Vilá *et al*. 2011; Wardle *et al*. 2011). Yet, we have observed that mono-dominant stands of the exotic invader *Centaurea stoebe* are

frequently positively associated with unusually dense native moss communities, suggesting that *Centaurea* might facilitate mosses, or vice versa. We know of no example of *Centaurea* facilitating other species, but mosses can have positive (Cross 1981; Ren *et al*. 2010; Casanova-Katny & Cavieres 2012; Sand-Jensen *et al*. 2015) or negative (Harmon & Franklin 1989; Van Tooren 1990; Gornall *et al*. 2011) effects on vascular plant species and vascular plants can suppress or facilitate mosses (Levine 2000; Ingerpuu *et al*. 2005).

Mosses are noteworthy for their effects on resource availability, often punching far above their biomass in their effects on resource pools and fluxes in terrestrial ecosystems. In part, this is because mosses can act as "nutrient catchment systems" in ecosystems due to their exceptional absorptive capacity, which allows them to accumulate nutrients from precipitation, canopy throughfall, and cyanobacterial associations (reviewed in Cornelissen *et al*. 2007). Additionally, unlike most vascular plants, mosses are poikilohydric, rapidly fluctuating between extreme states of desiccation and rehydration. During desiccation, their cellular integrity can be compromised prompting the release of large amounts of their intra-cellular contents (carbohydrates, amino acids, and ionic compounds) upon rehydration (Brown and Buck 1979; Carleton & Read 1991; Coxson 1991; M.L. Slate, *unpublished data*). Thus, during dry-wet transition periods mosses have the potential to release substantial pulses of labile resources and provide a particularly interesting system in which to study the effects of resource pulses on interactions and ecosystems.

Pulsed resources are likely to have strong effects on vascular plants if they coincide with periods of rapid plant growth (James & Richards 2005; Wijesinghe *et al*. 2005; James *et al*. 2006) and microbial activity (Austin *et al*. 2004; Loik *et al*. 2004; Collins *et al*. 2008). In arid and semi-arid systems, plant growth and microbial activity depend on precipitation events, which

concurrently release labile nutrients from re-hydrating mosses. Thus, when moss rehydration

coincides with periods of plant growth and microbial activity, rehyration-induced resource fluxes

from mosses may influence above and belowground communities.

Here, we investigated interactions between native mosses (primarily *Syntrichia*

papillosissima) and the exotic invasive forb, *Centaurea stoebe,* and the effect of these

interactions on soil N and water content. We also considered the effects of water pulses of

different magnitude and frequency on *Syntrichia* and *Centaurea*, their interactions, and their

effects on ecosystem properties. We conducted field surveys and a two-year field removal

experiment in locations where *Syntrichia* and *Centaurea* co-occurred to examine: 1) the effects

of *Syntrichia* and *Centaurea* on each other, on soil water and soil N, and 2) the impact of water

pulses, of different magnitude and frequency, on plant growth, plant interactions and soil water

and N. Interactions between mosses and vascular plants are known to vary during different life

stages (Sohlberg & Bliss 1987; Casanova-Katny & Cavieres 2012). To account for this, we

established a greenhouse experiment to determine the influence of *Syntrichia* on *Centaurea*

seedlings. We hypothesized that the unique co-occurrence of *Syntrichia* and *Centaurea* would

be explained by facilitation. Mechanistically, we expected to find that large water pulses would

carry resources released by mosses during rehydration into soil and stimulate changes in N

availability that positively affected *Centaurea* growth.

Methods

Study species

Centaurea stoebe L. (spotted knapweed, *Centaurea* hereafter) is native to Europe but is

now widely invasive across North America. *Centaurea* has strong competitive effects on North

American natives (Ridenour & Callaway 2001; Aschehoug *et al*. 2012), is generally tolerant to competition from these natives (Callaway *et al*. 2011), and represents an acute threat to grassland diversity (Sheley *et al*. 1998; Ridenour & Callaway 2001; Ortega & Pearson 2005).

Syntrichia papillosissima (Coppey) is native to the western United States where it is widespread throughout the Great Basin and shrub-steppe ecosystems (Mishler 2007). *Syntrichia* occurs primarily on low nutrient soil where it forms dense cushions interspersed among native shrubs and bunchgrasses (Mishler 2007; M.L. Slate, *personal observation*).

Field studies

We conducted field surveys and experiments in the Missoula and Blackfoot valleys of western Montana where *Centaurea* is an aggressive invader of native grasslands. Precipitation in this region averages 325 mm a year and falls primarily in May and June with a mean annual average temperature of 4ºC (www.ncdc.noaa.gov). Soils consisted of fine to gravelly, loamy Mollisols and Alfisols (www.websoilsurvey.sc.egov.usda.gov).

Field surveys − We selected sites in the Blackfoot Valley, Montana where both *Centaurea* and *Syntrichia* co-occur. Historically all study sites were vegetated by intermountain prairie. In August 2013, we located four 10-m transects at each of three different sites (Site 1: 47°00'14.2"N 113°22'19.4"W, Site 2: 46°58'35.2"N 113°04'04.1"W, Site 3: 46°57'57.4"N 112°58'09.9"W)**.** We sampled the percent cover of *Centaurea* and *Syntrichia*-dominated moss communities (henceforth *Syntrichia*) in circular 30-cm diameter plots (n = 120) at each meter on transects when mosses were dry. When measuring cover, we also measured the depth of *Syntrichia*. A paired *t*-test was used to compare *Syntrichia* depth when *Centaurea* cover was ≤ 30% and >

30%. The strength of the relationship between *Centaurea* cover and *Syntrichia* cover was measured with Pearson's correlation coefficient (*r*) using SigmaPlot, Version 13 (Systat Software, San Jose, CA).

Field experiment − We designed a field experiment to test the effects of 1) *Centaurea* on *Syntrichia*, 2) *Syntrichia* on *Centaurea*, and 3) *Centaurea* and *Syntrichia* on soils. We also added water to plots at two different magnitudes and frequencies to explore the effects of water pulse variation on *Centaurea* and *Syntrichia*, their interactions, and their effects on soil nutrient availability.

In June 2013, we initiated a full factorial removal experiment in a *Centaurea*-invaded grassland in Bonner, MT (46.8778ºN, 113.8889ºW). At this site, the cover of *Centaurea* and *Syntrichia*, the latter being primarily in the understory of *Centaurea*, were both greater than 90%. The dominant moss species at our study site was *Syntrichia papillosissima* though threads of other congeners and genera were also present; these represented less than 5% of the total moss biomass. *Syntrichia* depth at this location was on average 2.6 cm.

We established sixty-four 1 x 1 m plots divided into four treatments (each treatment n=16): 1) *Centaurea* removed, 2) *Syntrichia* removed, 3) both *Centaurea* and *Syntrichia* removed, and 4) un-manipulated control. In July and August of 2013 and 2014, we treated half of the plots in each of the four treatments above with different pulse magnitudes and frequencies of water. One treatment was three small 3.7 L pulses of water which, when distributed over a 1 x 1 m plot, equaled 0.6 cm of precipitation, a common size for summer rain events. Our second treatment was a large single water pulse of the same total amount; one 11.1 L pulse of water equal to 1.8 cm of precipitation. Water pulses were applied in 2013 on July 8 (large and small

pulse), August 5 (small), and August 16 (small) and in 2014 on July 3 (large and small), July 18 (small), and August 2 (small). Water pulses were applied when *Syntrichia* was dry. The total rainfall that fell naturally during this time period was 2.46 cm in 2013 and 1.09 cm in 2014, thus we roughly doubled the total precipitation during the experiment. To determine soil water content and inorganic soil N, comprised of ammonium (NH_4^+) and nitrate (NO_3^-), we collected soil samples on July 4, 2014 (24 hours after the July 3 large and small water pulses) and immediately prior to harvesting plant biomass on August 12. At each sample date, three 5-7 cm deep soil samples (~50 g) from each plot were collected and combined. We determined gravimetric water content (GWC) by mass for all soil samples within 48 hours. To extract soil NH_4^+ and NO_3^- we added approximately 15 mL of soil to 50 mL of 2 M KCl, shook for an hour and allowed the mixture to settle overnight at 4ºC. The solution was filtered and stored at -20ºC until colorimetric determination of NO_3^- and NH_4^+ (Solórzano 1969; Doane & Horwath 2003) using a Synergy 2 Microplate Reader (BioTek, Winooski, VT, USA). Nitrogen data were corrected for soil moisture content. The aboveground biomass of *Centaurea* and *Syntrichia* in each plot was harvested by clipping plants at the soil surface on 13 August, 2014. Plants were then dried at 60ºC and weighed.

We tested the effects of *Syntrichia* presence and water pulse type (large or small) on *Centaurea* using two-way ANOVAs, with *Syntrichia*, water pulse type and their interaction modeled as fixed factors and *Centaurea* biomass as the dependent variable. We also tested the effects of *Centaurea* presence and water pulse type on *Syntrichia* using two-way ANOVAs, with *Centaurea*, water pulse type and their interaction modeled as fixed factors and *Syntrichia* biomass as the dependent variable. To understand how interactions between our study species and water pulses influenced soil nutrient and water availability, we tested the effects of water

pulse type and *Syntrichia* or *Centaurea* presence, individually, as fixed factors and inorganic N

(NO_3^- or NH_4^+) or GWC as dependent variables in two-way ANOVAs. We ran separate

ANOVAs for each combination of variables at the 24 hour time point and the final time point

(twelve analyses). One-way ANOVAs were used to compare the lone effect of water pulse type

on inorganic N (NO_3^- or NH_4^+) or GWC for planting treatments at each time point. Finally, eight

separate two-way ANOVAs were used to test the interacting effects of water pulses and

Syntrichia or *Centaurea* when growing alone or together on inorganic N (NO_3^- or NH_4^+) or

GWC at each time point. In cases where pulse type did not have a significant effect, data for the

two pulse treatments were combined and results are presented for one-way ANOVAs only.

Analyses were followed with Tukey HSD comparisons when appropriate. All analyses were

performed in JMP, Version 11.0 (SAS Institute Inc., Cary, NC, 2013). Distributions of means

were checked for normality using the Shapiro-Wilk test and *Centaurea* biomass, NO_3^- and NH_4^+

data were log transformed to satisfy assumptions of normal distribution of means and

homoscedasticity in all analyses. Water pulse type did not affect the biomass of *Centaurea* or

Syntrichia, either when grown alone or together. Therefore, biomass data from both water pulse

treatments were pooled for comparisons of plant biomass among the planting treatments

Greenhouse experiment

We conducted a greenhouse experiment to evaluate the effects of *Syntrichia* on

Centaurea seedlings. In October 2013, we filled seventy-eight 500 mL pots with a mixture of

50% native soil: 50% silica sand. Fifty-two pots were planted with one two-week-old *Centaurea*

seedling. Half of these pots were also planted with field-harvested *Syntrichia* (collected from an

area directly adjacent to the field experiment) surrounding the *Centaurea* seedlings (n = 13 for

each). We included 13 pots with bare soil and 13 pots with *Syntrichia* but no *Centaurea* as experimental controls. All pots were placed under a misting system and misted for 10 minutes 4-6 times a day so that *Syntrichia* would remain hydrated, and also placed in pans with ~3 cm of standing water. This ensured that *Centaurea* seedlings were well watered. Twelve weeks after planting, total *Centaurea* biomass was harvested, dried at 60ºC, and weighed. At this time, soil samples were collected and inorganic N levels were determined following the methods described above. A reciprocal experiment to test the effect of *Centaurea* seedlings on *Syntrichia* would have been ideal but was logistically unfeasible. Moss growth is much slower than that of vascular plants creating a temporal mismatch so that *Centaurea* would no longer be in the seedling stage once *Syntrichia* established. We also encountered logistical limitations when trying to replicate our experimental field design with water pulses on dried mosses. Many mosses can move soil water externally from the base of their shoot to the shoot tip (Mägdefrau 1935; Ayres *et al*. 2006). Thus, the amount of water required to prevent *Centaurea* seedling mortality (even when watered from below) also inhibited *Syntrichia* from drying. Since our overall interest was to understand the relationship between *Syntrichia* and *Centaurea* at the seedling stage, we engineered our greenhouse experiment so that *Syntrichia* hydration would be constant so that any impact on *Centaurea* could only be related to moss presence.

We tested the effects of *Syntrichia* on *Centaurea*, with *Syntrichia* presence modeled as a fixed factor and *Centaurea* biomass as a dependent variable in a one-way ANOVA. We also tested the effects of *Syntrichia* and *Centaurea* on soil N, with *Syntrichia* and *Centaurea* modeled as fixed factors and inorganic N (NO_3^- or NH_4^+) as dependent variables in two separate two-way ANOVAs using JMP, Version 11.0 (SAS Institute Inc., Cary, NC, 2013). Analyses were followed with Tukey HSD post hoc comparisons when appropriate. Prior to statistical analyses,

distributions of means were checked for normality using the Shapiro-Wilk test and *Centaurea* biomass was log transformed to satisfy the assumptions of a normal distribution of means and homoscedasticity.

Results

Field Surveys

The cover of *Centaurea* and *Syntrichia* were positively correlated across study sites ($r = 0.784$, $P < 0.001$; Fig 1). This pattern was consistent among sites (Site 1: $r = 0.35$, $P = 0.027$; Site 2: $r = 0.46$, $P = 0.003$; Site 3: $r = 0.77$, $P < 0.001$). *Syntrichia* depth increased with *Centaurea* cover (*t*-test; $F_{1, 219} = 134.2$, $P < 0.001$). Across all three study sites, at points where *Centaurea* cover was $\leq 30\%$ *Syntrichia* depth was 0.91 ± 0.05cm. When *Centaurea* cover was $> 30\%$, the average *Syntrichia* depth was 2.21 ± 0.13cm.

Field experiment

Plots with *Centaurea* had 51% more *Syntrichia* biomass after two years than plots where *Centaurea* had been removed (Fig 2; Table 1). However, *Syntrichia* removal did not affect *Centaurea* biomass (Fig 2; Table 1).

Syntrichia, *Centaurea,* and water pulse type affected soil N and soil moisture in the short term (Table 2; Figure 3). In plots where plants were present, overall NO_3^- availability was lower and NH_4^+ availability was higher 24 hours after water pulses were added. When *Syntrichia* was present, soils had 67% less NO_3^- and 28% more NH_4^+ than plots where *Syntrichia* was removed (Table 2). *Centaurea stoebe* presence produced the same general pattern, with 86% less NO_3^- and 22% more NH_4^+ than in plots where *Centaurea* was removed (Table 2). When *Centaurea*

and *Syntrichia* co-occurred and were watered with large water pulses, soil NO_3^- was 91% higher than in plots where they were watered with small water pulses 24 hours previously (Table 3). Conversely, NO_3^- availability did not differ in plots watered with large or small water pulses when only *Centaurea* or *Syntrichia* were present 24 hours after the water pulse treatments (*Centaurea*: $F_{1, 11}$= 0.000, P=0.999; *Syntrichia*: $F_{1, 14}$=0.036, P=0.853). Not surprisingly, larger water pulses generated 21% higher soil moisture levels compared to smaller water pulses 24 hours after they were watered (Table 2). Soil moisture levels were 27% greater in plots where *Syntrichia* was present and 21% greater in plots where *Centaurea* was present 24 hours after water additions as compared to plots where *Syntrichia* or *Centaurea* had been removed (Table 2).

Syntrichia, *Centaurea* and water pulses also had long-term effects on soil N and soil moisture, evident at the end of the two-year experiment (Table 2; Figure 3). In plots where plants were present, overall NO_3^- and NH_4^+ availability were 62% and 68% lower and soil moisture was 50% higher than in plots where plants were absent by the end of the experiment. Plots with *Syntrichia* had 45% less NO_3^- and 30% less NH_4^+ but 34% higher GWC than plots where *Syntrichia* was removed (Table 2, Fig. 3). Plots with *Centaurea* had 60% less NO_3^-, 69% lower NH_4^+, but 43% higher GWC than plots where *Centaurea* was removed by the end of the experiment (Table 2). Across all treatments combined, large water pulses decreased NH_4^+ by 38% relative to multiple small water pulses over two field seasons (Table 2).

Greenhouse experiment

In contrast to the field experiment which focused on mature *Centaurea* plants in a heterogeneous natural environment, in greenhouse conditions pots with *Syntrichia* had 21% more

Centaurea biomass than pots without *Syntrichia* (Fig 4; $F_{1,50} = 5.64$, $P = 0.021$). There were no

differences in soil NH_4^+ or NO_3^- in pots with *Centaurea* and no *Syntrichia*, and pots with

Centaurea and *Syntrichia* (NH_4^+, $F_{1,15} = 0.114$, $P = 0.740$, NO_3^-, $F_{1,15} = 0.047$, $P = 0.831$; data

not shown).

Discussion

Interactions between the exotic invasive *Centaurea stoebe* and native *Syntrichia*-

dominated moss communities were positive, if considering both the interactions between mature

Centaurea and *Syntrichia* in the field and those between *Centaurea* seedlings and *Syntrichia* in

the greenhouse. Water pulses did not affect interactions between *Centaurea* and *Syntrichia* over

the course of the two-year experiment, but interactions between *Centaurea* and *Syntrichia*

substantially altered the effects of water pulses on short term N availability. Our general pulse

effects are consistent with those reported by others, who found that even small water pulses

influenced short-term N availability (Birch 1958; Cui & Caldwell 1997; Saetre & Stark 2005).

However, our finding that particular combinations of plant species altered the effects of water

pulses on soil N is unique. The large majority of studies on resource pulses have focused on the

direct effects of abiotic pulses on soil nutrient availability or the response of different plant

species to pulsed resources, but to our knowledge, modification of the effect of resource pulses

by plants on soil nutrient availability is novel.

Our study reveals a unique set of positive interactions between the exotic invasive

Centaurea and native *Syntrichia*-dominated moss communities. We found that *Centaurea*

facilitated *Syntrichia* in our field experiment. *Syntrichia* facilitated *Centaurea* seedlings in the

greenhouse and but not mature plants in the field. Furthermore, the distribution and abundance

of *Syntrichia* and *Centaurea* were strongly positively correlated in the field. Exotics usually suppress natives, but can facilitate them (Rodriguez 2006; Wolkovich *et al*. 2009), and natives commonly facilitate exotics (Callaway et al. 1991; Maron & Connors 1996; Cavieres *et al*. 2008; Becerra and Bustamante 2011). However, our results provide one of the few examples of bidirectional facilitation (see Schöb *et al*. 2014) between an exotic invader and native species. Holzapfel & Mahall (1999) found that positive and negative effects occurred simultaneously and reciprocally between a native shrub and invasive grasses.

We did not explore other mechanisms driving facilitative interactions between our target species, but there are at least two ways that *Centaurea* could have facilitated *Syntrichia*-dominated moss communities. First, *Centaurea* appears to generate substantially more shade than the scattered bunchgrasses and forbs it replaces, with the invader forming nearly closed canopies. Shade may benefit mosses by prolonging hydration and decreasing oxidative damage incurred by dry mosses in full sun (Beckett *et al*. 2004; Sand-Jensen 2015). Second, *Centaurea* has deep taproots capable of accessing water sources and redistributing them to the upper portion of the soil profile (Pankey 2009). Whether this provides an ecologically relevant amount of water to mosses is unknown but mosses can take up soil water through external conductance via capillarity (Mägdefrau 1935; Ayres *et al*. 2006). Thus, through shade or hydraulic redistribution *Centaurea* might have facilitated moss growth by prolonging hydration times and/or minimizing oxidative damage.

We only found significant facilitative effects of *Syntrichia* on *Centaurea* in the greenhouse. Mosses facilitate vascular plants by increasing soil water availability (Špačková *et al*. 1998; Gornall *et al*. 2007; Jeschke & Kiehl 2008), dampening fluctuations in soil temperature (Gornall *et al*. 2007; Jeschke & Kiehl 2008), and increasing substrate stability (Groeneveld &

Rochefort 2005). Mosses often increase soil nutrients, potentially facilitating co-occurring vascular plants (Sohlberg & Bliss 1984; Sedia & Ehrenfeld 2005; Gornall *et al*. 2007; Crowley & Bedford 2011). Casanova-Katny & Cavieres (2012) found that the facilitative effect of mosses on the grass *Deschampia antarctica* depended on the latter's life stage. Seedlings planted in moss grew faster but did not differ in their survival from those growing outside the moss carpet. Likewise, Sohlberg & Bliss (1987) found that adult plants did equally well in and outside of mosses, but young seedlings survived better inside moss clumps than outside (also see Jeschke & Kiehl 2008). This may explain why moss had a strong positive effect on *Centaurea* seedling biomass in the greenhouse but no significant effect on established *Centaurea* plants in the field. Given that each experiment was performed in different settings, other drivers may also have promoted these outcomes. Interestingly, *Centaurea* facilitation in the greenhouse could not be attributed to differences in N or water availability since N levels did not differ between the *Centaurea* only and *Centaurea* and moss pots and water levels were kept constant. Mosses have been associated with greater AMF colonization rates in neighboring vascular plants (Crowley & Bedford 2011) and *Centaurea* forms intense mycorrhizal associations that may intensify the invader's competitive effect on native grasses and establishment (Marler *et al*. 1999a; Marler *et al*. 1999b).

Our results show that variation in water pulse magnitude and frequency can affect soil N. However, these effects were altered by plant species. Plant community composition commonly affects soil microbial composition, which then can drive positive or negative feedbacks that in turn may be mediated by resource fluctuations (Ehrenfeld *et al*. 2005). Furthermore, mosses may influence nutrient availability via the release of labile carbon and N when they rehydrate from a desiccated state (Carleton & Read 1991; Coxson 1991; Wilson & Coxson 1999; M.L.

Slate, *unpublished data*). Carbon and N lost from mosses in throughfall following rehydration can be orders of magnitude greater than the amount of carbon and N released from non-desiccated mosses, depending on the species (M.L. Slate, *unpublished data*).

Desiccated mosses can absorb many times their weight in water during rehydration. Small water pulses in this study may have been more fully absorbed by *Syntrichia* and less accessible to soil microbes and plants than larger water pulses. Conversely, large water pulses are more likely to rehydrate and move extra-, intra- or inter-cellular compounds through moss canopies and into the soil. The fate of these introduced compounds is a result of what remains following microbially mediated nutrient transformations and uptake by plants and microbes. Thus, in the current study 24 hours after large water pulses passed through *Syntrichia* and interacted with both the soil microbial community and *Centaurea*, NO_3^- availability was 17x greater than in the small pulse plots, supporting part of our hypothesis. Yet when *Syntrichia* grew alone and was watered with large or small water pulses, inorganic N availability did not vary in the short or long term. Previous work in similar intermountain grasslands, found that *Centaurea* presence was associated with an increase in ammonia oxidizing bacteria (AOB) abundance, potentially increasing the capacity of soil to generate NO_3^- when conditions are favorable (McLeod *et al*. 2016; see also Hawkes *et al.* 2005). The addition of labile carbon compounds and organic and inorganic nutrients increases microbial biomass, activity, and rapid turnover of soil microbial community (Chen *et al*. 2007; Cleveland *et al*. 2007; Eilers *et al*. 2010). Thus carbon and nutrient-laden moss throughfall, in combination with a favorable N cycling community associated with *Centaurea* may have resulted in the quick conversion of NH_4^+ to NO_3^- in the soil and increase of soil NO_3^- availability.

In contrast to our predictions, water pulses of different magnitude and frequency did not affect the biomass of *Centaurea* and *Syntrichia* when they were grown alone, nor did they affect interactions between them. The ability of plants to respond to short-duration nutrient pulses is thought to be important in shaping plant community composition but determined by a combination of plant phenology and maximum growth rate (Bilbrough & Caldwell 1997; Lamb & Cahill 2006). Thus, scenarios with smaller seedlings, only involving vascular plants, lower total resource availability, or more contrasting water pulse frequencies may have generated substantially different results. Our two-year field experiment may also have been too short for testing the effects of pulse frequency on plant growth or interactions, or may have been performed too late in a well-established plant invasion.

In conclusion, we found an intriguing set of positive interactions between the exotic invasive *Centaurea stoebe* and native *Syntrichia*-dominated moss communities. Our results also have important implications for understanding the effects of precipitation pulses on soil N availability in semi-arid systems as interactions between *Centaurea* and *Syntrichia* modified the effects of water pulses on short-term soil N availability. Whether this effect was due to a rehydration related pulse of nutrients from *Syntrichia* encountering a novel soil microbial community promoted by *Centaurea* is unknown, but such changes in resource availability may affect plant communities over time.

Acknowledgments

Author contributions

MLS and RMC designed the study, MLS performed the research and analyzed data, MLM conducted soil analyses and interpretation of results; and all authors contributed to writing.

Data Accessibility

Data associated with this study will be deposited in the Dryad repository.

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Figure Legends

Figure 1. Correlation between the cover of the native moss *Syntrichia papillosissima* and the exotic invasive forb *Centaurea stoebe* at three sampling sites in the Blackfoot Valley, MT. Sites are designated by: squares (site 1), circles (site 2), and triangles (site 3) (n = 120).

Figure 2. Total biomass of a) *Syntrichia*-dominated moss communities or b) *Centaurea stoebe* when grown alone (white bar) or with each other (black bar) in our field experiment. Error bars represent 1 SE. (* $P \leq 0.05$; n = 16)

Figure 3. Inorganic soil NO_3^- and NH_4^+, and soil gravimetric water content (GWC) in each of four removal treatments crossed with either multiple low volume or a single high volume water pulses. The total volume of water added in the two different pulse frequencies was the same. Soil samples were taken 24 hours after the first water pulse of the year, and at the end (*Final*) of the two year field experiment. Error bars represent 1 SE. (n = 8)

Figure 4. Total biomass of *Centaurea stoebe* with *Syntrichia* (black bar) or without *Syntrichia* in our greenhouse experiment. Error bars represent 1 SE. (* $P \leq 0.05$; n = 13)

Table 1. ANOVA *F*-statistics and *P*-values for the effects of *Syntrichia* and water pulse type on aboveground *Centaurea* biomass, and the effects of *Centaurea* and water pulse type on *Syntrichia* biomass (* $P \leq 0.05$; in bold).

Source of variation	df	*F*	*P*-value
***Centaurea stoebe* biomass**			
Syntrichia	1	2.022	0.167
pulse	1	0.413	0.526
Syntrichia x pulse	1	0.049	0.827
***Syntrichia* biomass**			
Centaurea	1	19.37	**<0.001***
pulse	1	0.222	0.641
Centaurea x pulse	1	0.350	0.559

Table 2. ANOVA F-statistics and P-values for the effect of *Syntrichia*, *Centaurea* and water pulse type on NH_4^+, NO_3^-, and gravimetric water content (GWC) in the field experiment. Separate ANOVAs were performed for each response variable at the two sampling dates (24 hours & Final; * $P < 0.05$; in bold).

Source of variation	df	F	P-value	Source of variation	df	F	P-value
NH_4^+ 24 hour				***NH_4^+ Final***			
Syntrichia	1	14.82	**<0.001***	*Syntrichia*	1	22.66	**<0.001***
Centaurea	1	14.14	**<0.001***	*Centaurea*	1	60.02	**<0.001***
pulse	1	0.001	0.991	pulse	1	4.979	**0.029***
Centaurea x pulse	1	1.546	0.291	*Centaurea* x pulse	1	1.067	0.308
Syntrichia x pulse	1	0.965	0.332	*Syntrichia* x pulse	1	1.218	0.276
NO_3^- 24 hour				***NO_3^- Final***			
Syntrichia	1	61.01	**<0.001***	*Syntrichia*	1	25.67	**<0.001***
Centaurea	1	164.7	**<0.001***	*Centaurea*	1	53.55	**<0.001***
pulse	1	0.113	0.738	pulse	1	0.130	0.720
Centaurea x pulse	1	4.336	**0.044***	*Centaurea* x pulse	1	0.029	0.867
Syntrichia x pulse	1	2.167	0.149	*Syntrichia* x pulse	1	0.049	0.827
GWC 24 hour				***GWC Final***			
Syntrichia	1	35.40	**<0.001***	*Syntrichia*	1	36.90	**<0.001***
Centaurea	1	21.68	**<0.001***	*Centaurea*	1	55.81	**<0.001***
pulse	1	7.423	**0.008***	pulse	1	0.009	0.977
Centaurea x pulse	1	0.002	0.897	*Centaurea* x pulse	1	0.100	0.754
Syntrichia x pulse	1	0.722	0.400	*Syntrichia* x pulse	1	0.269	0.607

Table 3. ANOVA *F*-statistics and *P*-values for the interacting effects of water pulse type and *Syntrichia* and *Centaurea* when growing alone or together on NH_4^+ and NO_3^- in the field experiment. Eight separate ANOVAs were performed for each response variable at each of two sampling dates (24 hours & Final; * *P* < 0.05; in bold).

| Source of variation	df	*F*	*P*-value	Source of variation	df	*F*	*P*-value
NH_4^+ 24 hour				**NH_4^+ Final**			
Syntrichia	1	3.617	0.068	*Syntrichia*	1	6.310	**0.018***
Syntrichia + Centaurea	1	0.146	0.706	*Syntrichia + Centaurea*	1	0.327	0.572
Centaurea	1	5.182	**0.031***	*Centaurea*	1	3.210	0.084
Centaurea + Syntrichia	1	1.095	0.305	*Centaurea + Syntrichia*	1	0.781	0.385
NO_3^- 24 hour				**NO_3^- Final**			
Syntrichia	1	7.428	**0.012***	*Syntrichia*	1	17.29	**<0.001***
Syntrichia + Centaurea	1	2.066	0.164	*Syntrichia + Centaurea*	1	0.874	0.358
Centaurea	1	2.062	0.165	*Centaurea*	1	2.976	0.096
Centaurea + Syntrichia	1	1.478	**0.046***	*Centaurea + Syntrichia*	1	0.061	0.807

Figure 1.

$R^2 = 0.615$
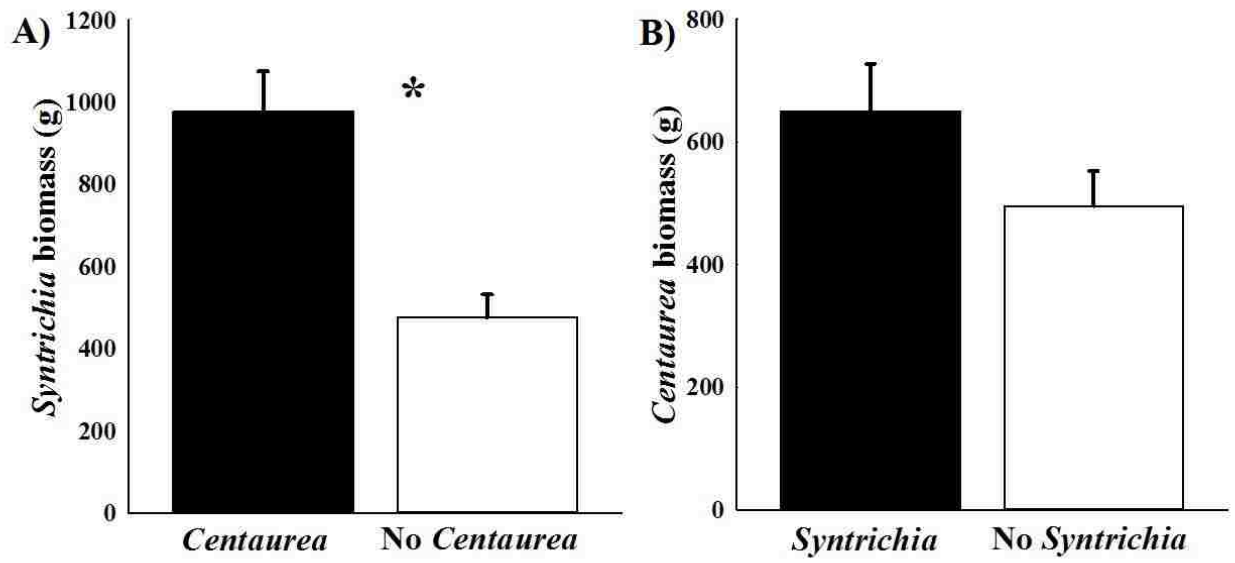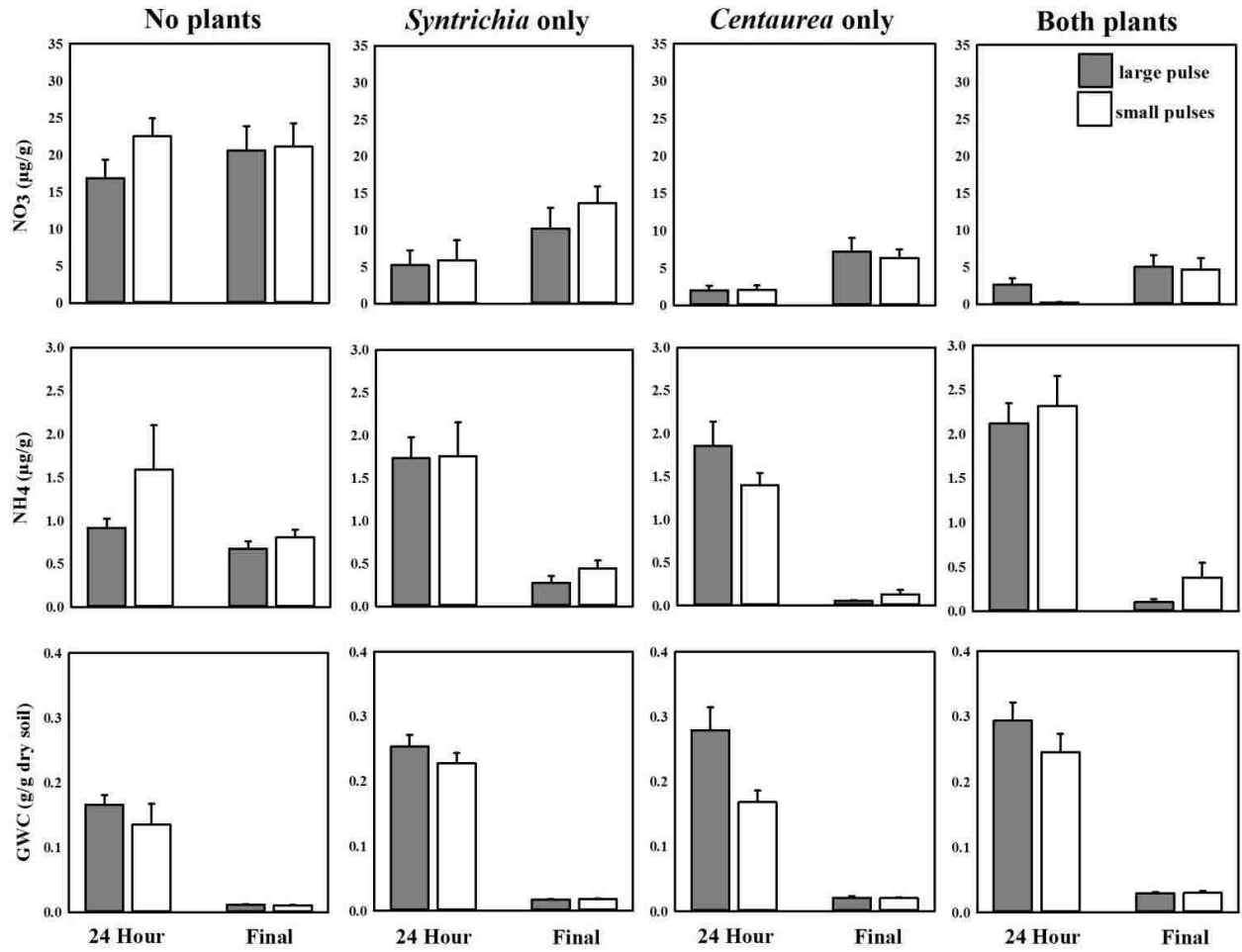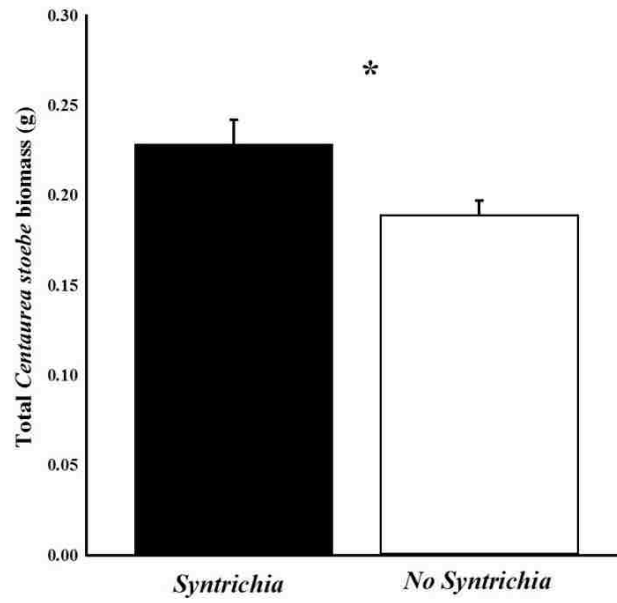$P < 0.001$
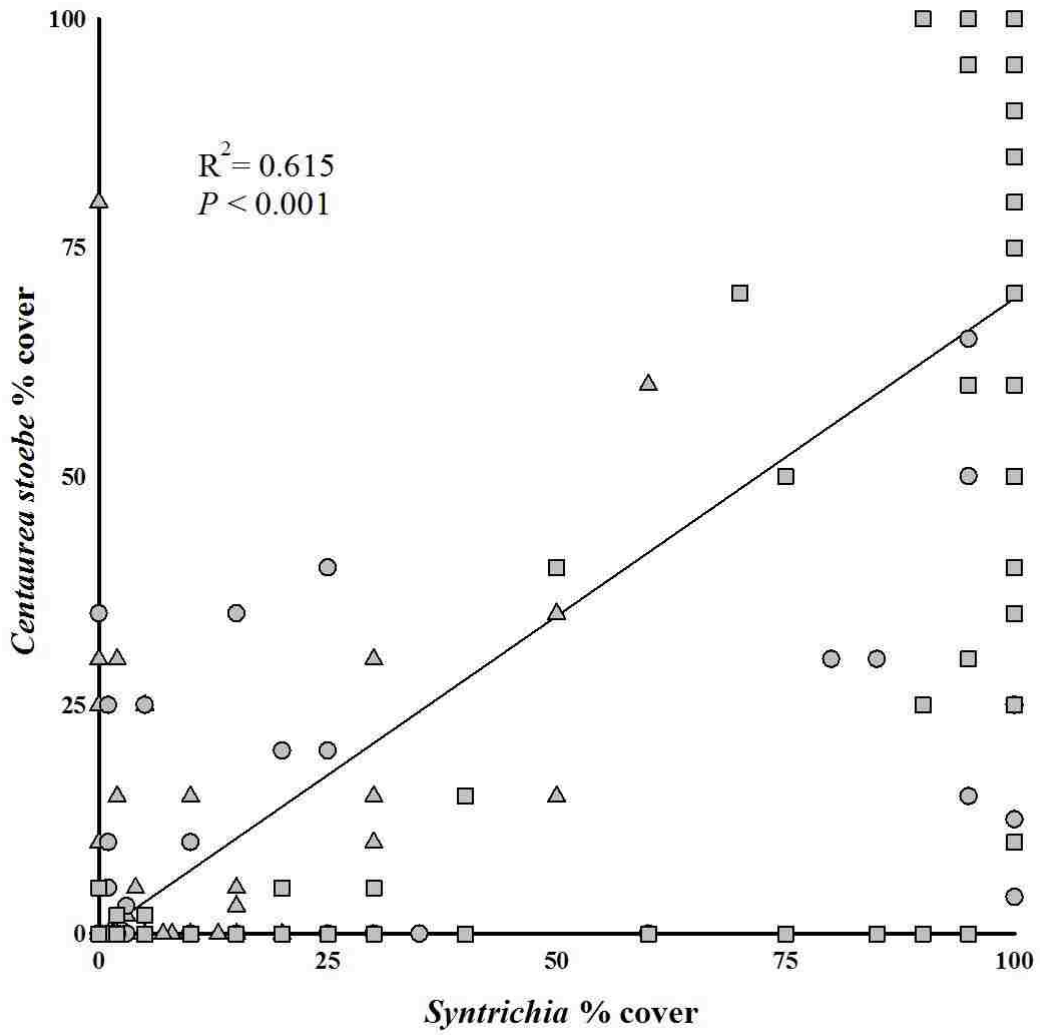
Figure 2.

Figure 3.

Figure 4.

Chapter 3: Life in interstitial space: biocrusts inhibit exotic but not native plant establishment in semi-arid grasslands

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ABSTRACT

Exotic plant species commonly exploit disturbances more successfully than native plants, but why this happens is not clear. Biocrusts are an important component of semi-arid ecosystems that are often treated as part of the "interstitial space" among vascular plants, but biocrusts can strongly inhibit plant establishment and thus may provide a source of biotic resistance to plant invaders. We conducted a two-year field experiment to examine how intact versus disturbed biocrusts influenced the establishment of four native and four exotic plant species in Intermountain bunchgrass systems. In field experiments, biocrusts had no overall effect on the establishment of native seedlings, but they inhibited the establishment of exotic seedlings. In a greenhouse experiment designed to measure the effect of biocrusts on germination, biocrusts inhibited both native and exotic species, but only in a high water treatment. Our results suggest that intact biocrusts in intermountain grasslands may suppress the establishment of exotic plants more than native plants, and that the underlying mechanism may be linked to water availability.

Thus in intermountain grasslands, biocrusts may represent and underappreciated source of biotic resistance to exotic plant invasions.

Key words: *biotic soil crusts, disturbance, grasslands, exotic invasive species, germination, establishment, invasion, biotic resistance*

INTRODUCTION

Exotic plant invasions often negatively impact native plant communities, and the success of exotic plants has been linked to disturbance, perhaps more than any other factor (D'Antonio et al. 1999, MacDougall et al. 2014, Jauni et al. 2015). Many studies have shown that disturbance strongly favors the establishment of exotic over native plant species (e.g. Hobbs and Huenneke 1992, Maron et al. 2012), but few studies have explored disturbance to ecosystems beyond that to soils and plant communities. One important, yet understudied component of semi-arid systems that has been exposed to widespread and intense disturbance is biocrust. Biocrusts cover much of the interstitial space among plants in arid and semi-arid systems (Daubenmire 1942, Belnap et al. 2001) and are complex associations of lichen, moss, cyanobacteria and other microorganisms that can stabilize soil (Belnap and Gardner 1993), regulate water and nutrient cycling (Harper and Pendelton 1993, Pendelton et al. 2003, Chamizo et al. 2012), and buffer soil temperatures (Gornall et al. 2009). Biocrusts can also influence the establishment of vascular plants via physical and chemical effects on seed germination and seedling establishment (reviewed in Zhang et al. 2016), thus whether or not they are intact may affect plant communities.

Most studies examining the effects of biocrusts on vascular plant establishment have been conducted in arid ecosystems where biocrusts are composed of cyanobacteria and/or lichen.

Evidence from these ecosystems indicates that cyano-lichen biocrusts generally inhibit plant germination, with disturbance to biocrusts often improving the germination of both native and exotic plants (Deines et al. 2007, Song et al. 2017). These inhibitory effects on germination have been primarily attributed to the negative impact of intact cyano-lichen biocrusts on seed water availability (Eldridge and Greene 1994, Hawkes 2004, Escudero et al. 2007, Serpe et al. 2008, Zhang et al. 2010, Godínez-Alvarez et al. 2012). In addition, seeds that do germinate on intact cyano-lichen biocrusts often do not survive because these biocrusts physically and chemically inhibit root penetration (Zaady et al. 1997, Eldridge et al. 2000, Prasse and Bornkamm 2000, Beyschlag et al. 2007, Serpe et al. 2008, Song et al. 2017).

In semi-arid ecosystems, biocrusts composed of lichens and mosses appear to function differently than the cyano-lichen biocrusts in arid systems. Intact lichen-moss biocrusts vary in the direction of their effect on native plant germination from positive to negative, sometimes within a single system (Deines et al. 2007, Hernandez and Sandquist 2011, Godínez-Alvarez et al. 2012). In contrast, intact lichen-moss biocrusts, in general, negatively influence exotic plant germination (Eldridge and Simpson 2002, Hernandez and Sandquist 2011, but see Deines et al. 2007). However, the majority of these studies were performed in controlled conditions (Eldridge and Simpson 2002, Deines et al. 2007, Godínez-Alvarez et al. 2012), or if carried out in a field setting, spanned a relatively small area (Hernandez and Sandquist 2011. In addition, most former studies measured the responses of no more than four plant species, with few studies comparing multiple native and exotic species. Finally, to our knowledge, no field study has followed the effect of lichen-moss biocrusts on native and exotic plant recruitment from the germination to establishment stage, despite the fact that interactions between cyano-lichen biocrusts and plants vary mechanistically at different plant-stages (Prasse and Bornkamm 2000, Serpe et al. 2008,

Zhang et al. 2010, Godinez-Alvarez et al. 2012, Song et al. 2017). Positive correlations between native plants and lichen-moss biocrust cover, and negative correlations between exotic plants and biocrust cover, suggest that intact biocrusts may help semi-arid systems resist plant invasions and this warrants further research (Lesica and Shelly 1992, Stohlgren et al. 2001, Scott and Morgan 2012).

Some ecosystems or regions appear to be more susceptible to disturbance-based exotic invasion than others but how this relates to biocrust disturbance is unknown. Circumstantial evidence suggests that at least in some cases biocrust disturbance may have preceded exotic invasion. For example, prior to the introduction of large livestock in semi-arid grasslands west of the Continental divide, lichen-moss biocrusts were said to account for over 50% of the land cover and be distributed on all ground surfaces unoccupied by vascular plants (Mack and Thompson 1982). Widespread grazing, however, has caused extensive and long lasting damage and loss of biocrusts (Poulton 1955, Daubenmire 1970, Krannitz 2008) that in many locations coincides with the proliferation of exotic plants (Mack 1981, Krannitz 2008). Field experiments in this system have demonstrated that disturbance strongly favors the establishment of exotic plant species over natives (Maron et al. 2012, Pearson et al. 2018), but these studies did not explicitly consider the effects of disturbance to biocrusts.

Here, we investigated the role of lichen-moss biocrusts on native and exotic plant recruitment over two years and across four sites in intermountain grasslands. We conducted field and greenhouse experiments where we added the seeds of four native and four exotic species to biocrusts that were intact, disturbed or removed to examine: 1) whether biocrusts affect the recruitment of native and exotic species, 2) if the effects of biocrusts varied at different points in

the ontogeny of native and exotic plants (germination, emergence, and establishment), and 3)

whether individual native and exotic species differed in their response to biocrusts.

METHODS

Field Experiment

Our field experiments were located in bluebunch wheatgrass (*Pseudoroegneria spicata*)

habitats of west-central Montana (Mueggler and Stewart 1980), because disturbances in these

habitats shift native communities toward exotic plant dominance (Pearson et al. 2018). Without

invasion, these communities are comprised of scattered native perennial grasses, forbs and

shrubs with interstitial spaces dominated by lichen-moss biocrust communities. Biocrust cover in

our study sites was predominately lichens from the genera *Peltigera* and *Cladonia* and mosses

from the genus *Syntrichia*. Regional annual rainfall within our study area averages 32.5 cm a

year and occurs primarily in May and June with limited rainfall from July through mid-

September (www.ncdc.noaa.gov). Our two study years varied in total spring rainfall, with 14.4

cm of precipitation falling between January and June in 2016 versus 25.6 cm during the same

period in 2017.

We conducted seed addition experiments in 2015-2016 and 2016-2017 to evaluate how

biocrust, biocrust disturbance, and biocrust removal affected emergence and establishment of

eight common native and exotic plant species (Table A1). In October of 2015, we selected four

field sites with each site separated by at least 70 km and all sites collectively spanning ~20,000

km^2, (Site 1: 47.3706° N, 114.2571° W; Site 2: 46°54'43.4"N 113°58'07.1"W; Site 3:

46°39'37.2"N 113°14'49.9"W; Site 4: 46°59'28.2"N 113°05'44.5"W). At each site, we ran four

20 m line transects in grasslands that were moderately disturbed but contained some intact

lichen-moss biocrusts. Each transect was separated by at least 20 m. Five (2015-2016) or eight (2016-2017; see below) 1 x 1 m quadrats were placed along each transect where biocrusts were abundant enough to establish treatments, with new transects and quadrats established annually. Each quadrat on each transect was assigned to one plant species, with all species represented on each transect one time. Three 10 x 10 cm subplots with biocrusts were located in each quadrat. One subplot was disturbed by striking the ground with a mallet 20 times to simulate trampling. Biocrusts were fully removed from a second subplot without disturbing the underlying soil. Biocrusts were left intact in the third subplot. In October 2015 and 2016, we added twenty seeds of each plant species superficially to the three subplots within their respective quadrat. In 2015, we sowed three native species, *Achillea millefolium, Cerastium arvense,* and *Poa secunda*, and two exotic species, *Centaurea stoebe* and *Poa bulbosa* at each site. In 2016, we established new transects at each site and sowed the same species as well as the native *Pseudoroegneria spicata* and the exotics *Bromus tectorum* and *Potentilla recta*. Thus, for each species and each treatment at each of the four sites, there were four replications. Because species varied substantially in the timing of their germination, we visited the plots two times each year in the spring to record emergence, defined as the maximum number of seedlings on either date. We visited the plots a third time to record establishment, defined as the number of surviving seedlings, at the beginning of the dry season. In 2016, we recorded emergence on 10 March and 14 April and establishment on 10 May yet could only measure establishment at three of our four sites. In 2017, we recorded emergence on 7 April and 5 May and establishment on 1 July. Emergence and establishment were measured much later in 2017 because of a more persistent snowpack and rainy spring.

Seeds were collected in the field in the same growing season that they were sown, and from >30 individuals of each species in the Missoula Valley, MT (46.8787° N, 113.9966° W).

Seeds from all plants for a species were combined and mixed before sowing. *Bromus tectorum* seeds were placed in a warm and dry location for one month to end dormancy prior to sowing (Serpe et al. 2008). All species exhibited ≥85% germination on filter paper in Petri dishes.

Greenhouse Experiment

The long distances between field sites prevented us from visiting sites frequently enough to measure germination in the field so we conducted a greenhouse experiment to examine the potential effects of lichen-moss biocrusts on the germination of our study species. Dry plugs of intact biocrusts were collected with a bulb planter (6.5 cm wide and 7.5 cm deep) from two of our field sites on 14 and 15 June, 2016 and placed into 6.5 x 6.5 cm square plastic Ziploc containers that allowed drainage. Containers were randomly assigned to treatments as follows: intact biocrusts, disturbed biocrusts (crumbled by hand) and bare soil (biocrusts removed). It is worth noting that the process of removing biocrusts for the greenhouse experiment appeared to disturb the underlying soil more than the process of removing biocrusts in the field experiment. Initially, all containers were misted twice a day (two minutes per misting) for ten days to allow settling from harvesting and watering and germination and removal of preexisting seeds and seedlings in the collected biocrusts (Zhang and Belnap 2015). On 25 June, seeds from the same eight study species (Table A1) used in our field trials were sown on the biocrust or soil surface (n = 16 for each species and each treatment). Because of differences in seed size and container size constraints, 15 grass seeds versus 20 forb seeds were sown. Containers were randomly assigned to one of two watering treatments: a high water treatment (misting for one minute a day) or a low water treatment (misting one minute every three days) (n = 8 per species per disturbance treatment by water treatment combination). Containers were rotated randomly on the tables

within their respective watering treatments every three days. Seedlings first appeared on 29 June and were counted daily for 12 days, after which very few new seedlings emerged. This allowed us to determine the average first day of germination and the percentage of sown seeds that germinated for each species.

Statistical Analyses

Unless otherwise noted, all analyses were conducted using R version 3.4.2 (R Development Core Team, Vienna, Austria) using the MASS (Venables and Ripley 2002), lme4 (Bates et al. 2015) and lsmeans (Lenth 2016) packages. To test the effects of plant origin (native vs. exotic) and biocrust treatment (intact biocrust, disturbed biocrust, or removed biocrust) on the number of seedlings, we used generalized linear mixed-effects models (one GLMM for emergence and one for establishment). Since we used different numbers of species in each of our two study years (2015-2016 or 2016-2017), we ran two separate GLMMs for each year (four GLMMs total). In all models, species was nested in origin and treated as a random factor. We also analyzed the number of emerged and established seedlings for each plant species individually (16 separate GLMMs). For those species with two years of data, both years of data were included. Site (four sites) was included as a random factor for blocking with transect (four per site) nested within site for all models. We accounted for zero inflation with a negative binomial distribution and examined residuals with normality tests which in all cases were reasonable. When factors had a significant effect on a response variable ($P<0.05$), we used Tukey HSD post-hoc comparisons to evaluate those relationships. Data from experimental subplots that were disturbed in ways other than our removed/disturbed treatments were excluded from the final analyses (0 subplots affected in 2016; 9 subplots affected in 2017). There were

only a few cases where results differed between disturbed and removed biocrust treatments for origin or species, so these two treatments were pooled in our final analyses (see Appendix A for analyses with these two treatments separated).

We used a GLMM to determine how plant origin (native or exotic), biocrust treatment (intact, disturbed, or bare soil), and water treatment (high or low water) influenced the average first day of seed germination in our greenhouse experiment. When factors had a significant effect on a response variable ($P<0.05$), we used Tukey HSD post-hoc comparisons to evaluate those relationships. To assess the effect of plant origin, biocrust treatment, and water treatment on percentage seed germination (number of seeds germinated/ number of seeds added), we ran a lognormal Type III ANOVA model using the GLIMMIX module (SAS, version 9.2) to account for the skewness of the data. Post-hoc differences among treatment means were evaluated with multiple comparisons and adjusted for the number of comparisons. Site where crusts were collected ($n = 2$) was included as a random variable in all greenhouse models. The disturbed and bare soil treatments were again pooled as above (see Appendix A for model results for each treatment separated).

 RESULTS

Field Experiment

Emergence – Overall differences between native and exotic seedling emergence within each study year were only significant in 2016 when 83% more exotic than native seedlings emerged (Tables 1 and A2). In 2017, 43% fewer exotic seedlings emerged on intact biocrusts than on removed/disturbed biocrusts (Tukey HSD, $Z_{1,\ 127}$=-4.19, $P<0.001$), but in 2016 there was no difference in exotic seedling emergence on intact vs. removed/disturbed biocrusts (Tukey

HSD, $Z_{1, 64}$=-0.03, P=0.97). Likewise, there was no difference in the number of native seedlings that emerged on intact or removed/disturbed biocrusts in 2016 (Tukey HSD, $Z_{1, 96}$=-1.35, P=0.18) or in 2017 (Tukey HSD, $Z_{1, 129}$=-1.90, P=0.06, Figs 1 and A1). For individual species, there was lower seedling emergence on intact biocrusts than removed/disturbed biocrusts for the native forb *C. arvense* (two years of data), the exotic forb *P. recta* (data from 2017 only), and the exotic grass *B. tectorum* (data from 2017 only, Tables 2 and A3, Figs. 2 and A2).

Establishment – In 2016, 50% more exotic seedlings established on removed/disturbed biocrusts than on intact biocrusts (Tukey HSD, $Z_{1, 64}$=-1.95, P=0.05). In 2017, 81% more exotic seedlings established on removed/disturbed biocrusts than intact biocrusts (Tukey HSD, $Z_{1, 124}$=-3.97, P<0.001, Figs. 1 and A1). There was no difference in the number of native seedlings that established on intact or removed/disturbed biocrusts for either year (Tukey HSD, 2016: $Z_{1, 72}$=1.14, P=0.25, 2017: $Z_{1, 126}$=-0.54, P=0.59). In 2016, 76% more exotic than native seedlings established on removed/disturbed biocrusts (Tukey HSD, $Z_{1,68}$=2.82, P=0.005) but there was no difference in the number of exotic and native seedlings that established on removed/disturbed biocrusts in 2017 (Tukey HSD, $Z_{1, 126}$=-0.37, P=0.72). Eighty-three percent more native than exotic seedlings established on intact biocrusts in 2017 (Tukey HSD, $Z_{1, 126}$=-2.10, P=0.04). For individual species, establishment for all exotic seedlings was inhibited by intact biocrusts (Table 2, Fig. 2). In contrast, intact biocrusts only inhibited seedling establishment of one native, *Cerastium,* which had 47% fewer seedlings on intact biocrusts than on removed/disturbed biocrusts (Tables 2 and A3).

Greenhouse experiment

Percentage germination – The germination percentage of native and exotic species differed by biocrust treatment and water availability (Tables 3 and A4). Seed germination percentages of native and exotic species were 94% and 91% lower on intact biocrusts than on removed/disturbed biocrusts under high water conditions, respectively (LS Means, native: $t_{1,98}$=5.71, P<0.0001, exotic: $t_{1,98}$=4.61, P<0.0001, Figs. 3 and A3). Conversely, under low water conditions there was no difference in native or exotic seed germination percentages on intact or removed/disturbed biocrusts (LS Means, native: $t_{1,\,97}$=0.25, P=0.80, exotic: $t_{1,\,98}$=0.64, P=0.52). The germination percentage for native species was 64% higher than that of exotic species under high water conditions on removed/disturbed biocrusts (LS Means, $t_{1,\,128}$ =-2.51, P=0.01).

First day of germination – The average first day of germination was influenced by plant origin, biocrust treatment, and water availability (Tables 3 and A4). Water availability did not influence the timing of germination for native or exotic seeds when sown onto intact biocrusts (Tukey HSD, native: $Z_{1,\,189}$= -0.19, P=0.998, exotic: $Z_{1,\,190}$=1.92, P= 0.219, Figs. 3 and A3). However, native seeds germinated about three days earlier on intact biocrusts than removed/disturbed biocrusts under high water conditions (Tukey HSD, $Z_{1,\,189}$= 2.43, P=0.061). In contrast, the timing of germination for exotics did not differ between biocrust treatments under high water conditions (Tukey HSD, $Z_{1,\,190}$=1.44, P= 0.476). There was no difference in the timing of native or exotic seed germination on intact or removed/disturbed biocrusts under low water conditions (Tables 3 and A4).

DISCUSSION

Our key finding is that lichen-moss biocrusts may play a role in the resistance of intermountain grasslands to exotic plant invasions. By extension, widespread disturbance to

biocrusts in the semi-arid west may exacerbate exotic invasion (see Mack 1981, Mack and Thompson 1982). It is well documented that disturbance in western grasslands is followed by increases in exotic plant abundance (Knapp 1996, Chambers and Wisdom 2009, Chambers et al 2014, Pearson et al. 2018) and often the transformation of native perennial grassland to mono-dominant stands of exotic forbs and annual grasses (Ortega and Pearson 2005, Kulmatiski 2006, Chambers et al. 2014, Pearson et al. 2016). Such widespread shifts in plant community composition are due, in part, to invaders outcompeting native plant species (e.g., Levine et al. 2003, Callaway 2011, Vilà et al. 2011, Aschehoug and Callaway 2015), and to disturbance creating opportunities for invaders while decreasing competitive resistance from natives (see Besaw et al. 2011). Our results suggest that disturbance to biotic crusts must be considered more carefully.

Serendipitously, we were able to consider the responses of native and exotic species to biocrusts under high and low precipitation conditions because the two years of the experiment differed in spring rainfall. Biocrust effects on native species were consistent and generally neutral in both years with the exception of *Cerastium*, which was suppressed. In contrast, in the wet year of 2017 lichen-moss biocrusts inhibited the emergence and establishment of all exotic plants, considered as a group. In 2016, biocrusts did not inhibit exotic plant emergence but did inhibit exotic plant establishment. However, differences between study years may have occurred for reasons other than precipitation. First, we used one native (*Pseudoroegneria*) and two exotic species (*Potentilla* and *Bromus*) in 2017 that were not included in 2016. *Potentilla* and *Bromus* were the only exotic species for which emergence was inhibited in 2017, and if these species had been included in 2016 perhaps we would have found inhibition in both years. Second, emergence and establishment were measured later in 2017 than in 2016 because of a more persistent

snowpack and rainy spring. This difference in timing could mean that we captured or missed peaks in seedling emergence between the years, but we measured establishment roughly at the onset of the dry season in both years.

Our field experiments provided different lines evidence for biocrusts resisting invasion in 2016 versus 2017. In 2016, exotics established far better on disturbed biocrusts than on intact biocrusts, and this did not occur for natives. Importantly, this may have been due to inhibition by intact biocrusts or by legacy nutrient increases to soil from biocrusts that were made available by disturbance and disproportionately utilized by exotics (e.g. Ferrenberg et al. 2017). In 2017, biocrust disturbance increased the establishment of exotics, but five times fewer exotics established on intact crusts than natives, suggesting that exotics may simply perform worse than natives in the presence of biocrusts in some conditions.

Interactions between lichen-moss biocrusts and vascular plants probably vary at different points in a plant's ontogeny (Deines et al. 2007, Godinez-Alvarez et al. 2012). Lichen-moss biocrusts are thought to inhibit seed germination and seedling emergence through secondary compounds and/or competition for water (Serpe et al. 2008, Michael et al. 2011), but as seedlings grow biocrusts can also create a physical barrier that keeps roots from contacting soil (Deines et al. 2007). Few studies have compared the effects of biocrusts on native versus exotic vascular plants from seed germination through seedling establishment and, to our knowledge, none have considered the influence of water availability on these effects. In the greenhouse, we found that intact biocrusts inhibited the germination of native and exotic species under high but not low water conditions. Likewise in our field experiment, native and exotic seedling emergence was inhibited by biocrusts only during a wet spring. Lichens and mosses are thought to leak secondary compounds from damaged cells when rehydrating from a desiccated state

(Michel 2011), and perhaps the influence of secondary compounds on seeds and seedlings was more common under high water conditions. Alternatively, biocrusts might compete more strongly for water with seeds and seedlings in high water conditions. Lichens and mosses do not have roots but instead compete with seeds and seedlings for water by tightly holding onto to water once hydrated (Ostus and Zobel 2004, Serpe et al. 2006, Serpe et al. 2008). Very little is known about how or when lichen-moss biocrusts inhibit seedling root penetration. Deines et al. (2007) found that lichen-moss biocrusts prevented the roots of the exotic grass *Bromus tectorum* from accessing the soil, but the roots of the native grass *Vulpia microstachys* were not inhibited. Clearly more work is needed to understand if lichen-moss biocrusts physically inhibit other exotic plant seedlings and how water availability might influence these outcomes. Finally, though not a form of inhibition, biocrusts improve soil fertility (Brotherson and Rushforth 1983, Barger et al. 2016) and disturbing biocrusts may benefit some species by allowing access to these more fertile soils (Ferrenberg et al. 2017). Any increase in access to resources after biocrust disturbance may have stronger beneficial effects when water is plentiful (Hawkes 2004, Delgado-Baquerizo et al. 2016).

Biocrust effects on native and exotic seed germination in our greenhouse study were influenced by water availability. Biocrusts inhibited the percentage of native and exotic seeds that germinated in high water conditions and native seeds germinated about three days earlier on intact biocrusts than on disturbed biocrusts while the timing of exotic seed germination did not vary. In contrast, under low water conditions, biocrusts did not impact the percentage of native or exotic seeds that germinated or the timing of seed germination. Deines et al. (2007) similarly noted that lichen-moss biocrusts did not influence the germination rate or the timing of

germination of a native and exotic grass. Thus, lichen-moss biocrusts may have variable effects on seed germination that differ for native and exotic species in wet or dry years.

Lichen-moss biocrusts had species-specific effects in our field study. Emergence and establishment of one native species was inhibited by biocrusts, the widespread ruderal forb *Cerastium*. Ruderal plant species like *Cerastium* often benefit from disturbance (*sensu* Grime 1979), such as our biocrust removal and disturbance treatments. Similarly, in Californian coastal sage scrub, the ruderal, *Lotus scoparius*, appeared to be inhibited by intact biocrusts, whereas a later successional native species, *Artemisia californica*, was facilitated by biocrusts (Hernandez and Sandquist 2011). In our field study, the emergence of two exotic species, *Centaurea* and *P. bulbosa*, was not inhibited by biocrusts, whereas emergence of *Potentilla* and *Bromus* were inhibited by biocrusts. It is likely that species-specific effects of biocrusts on plant recruitment result from interactions among biocrusts and seed and seedling traits (e.g., seed water holding capacity or root growth rate) which may or may not vary by origin but should be determined by the evolutionary history of an individual species. These species-specific differences may be important in terms plant community assembly in intermountain grasslands.

In sum, lichen-moss biocrusts appear to provide some level of biotic resistance to exotic invasion in semi-arid grasslands. In this context, it is important to note that exotic plant litter itself can eliminate biocrusts independently of disturbance (Serpe et al. 2013). Hence, exotic plant establishment could, even at an early stage, promote a negative feedback in biocrust systems that would benefit the proliferation of more exotic species. Land use strategies that minimize biocrust disturbance may play a key role in maintaining ecosystems dominated by native vascular plants.

ACKNOWLEDGEMENTS

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TABLE 1. Results from mixed models used to compare the effects of origin and biocrust treatment (Trtmt) by year on the number of seedlings in the early (*emergence*) and later part (*establishment*) of the growing season in our field experiment. Significant (*P*≤0.05) values are in bold and marginally significant values are annotated with an *m* (*P*≤0.1).

		Emergence			Establishment		
	Effect	**df**	*F*	*P-value*	**df**	*F*	*P-value*
2016	Origin	1 (239)	5.30	**0.021**	1 (179)	5.02	**0.025**
	Treatment	1 (239)	0.66	0.416	1 (179)	0.25	0.619
	Origin * Trtmt	1 (239)	1.17	0.279	1 (179)	4.86	**0.028**
2017	Origin	1 (382)	0.07	0.788	1 (373)	0.65	0.421
	Treatment	1 (382)	18.2	**<0.001**	1 (373)	7.40	**0.007**
	Origin * Trtmt	1 (382)	2.96	0.085^{m}	1 (373)	8.71	**0.003**

TABLE 2. Results from mixed models (16 separate models) used to compare the effect of biocrusts on seedling emergence and establishment for each study species in the field experiment. Species with no asterisk were added to experimental plots in both years while species with asterisks were only a part of the 2017 study. Significant ($P{\leq}0.05$) values are in bold and marginally significant values are annotated with an m ($P{\leq}0.1$).

		Emergence			Establishment		
		df	F	P-value	df	F	P-value
Native species	Cerastium	1 (90)	3.79	**0.051**	1 (81)	6.02	**0.014**
	Achillea	1 (90)	2.68	0.101	1 (75)	0.46	0.497
	P. secunda	1 (90)	0.62	0.433	1 (81)	2.36	0.125
	* Pseudoroegneria	1 (42)	0.04	0.846	1 (45)	1.26	0.263
Exotic species	* Potentilla	1 (42)	16.5	**<0.001**	1 (42)	8.12	**0.004**
	Centaurea	1 (90)	2.15	0.138	1 (78)	8.44	**0.004**
	* Bromus	1 (41)	3.38	0.060^{m}	1 (41)	15.8	**<0.001**
	P. bulbosa	1 (90)	2.44	0.118	1 (78)	3.40	0.061^{m}

TABLE 3. Results from type III tests (germination percentage) or mixed models (germination day) used to compare the fixed effects of plant origin, biocrust treatments, and water treatments on seed germination percentage and first day of germination in our greenhouse experiment. Significant ($P \leq 0.05$) values are in bold and marginally significant values are annotated with an *m* ($P \leq 0.1$).

	Germination percentage			Germination day		
Effect	**df**	*F*	*P-value*	**df**	*F*	*P-value*
Origin	1 (373)	6.27	**0.013**	1 (373)	5.50	**0.019**
Biocrust treatment	1 (373)	31.3	**<0.001**	1 (373)	4.54	**0.033**
Water treatment	1 (373)	47.2	**<0.001**	1 (373)	22.3	**<0.001**
Biocrust * Origin	1 (373)	0.12	0.725	1 (373)	0.01	0.942
Water * Origin	1 (373)	0.24	0.623	1 (373)	0.99	0.318
Water * Biocrust	1 (373)	22.1	**<0.001**	1 (373)	3.20	0.073[m]
Water * Biocrust * Origin	1 (373)	0.54	0.462	1 (373)	1.35	0.246

FIGURE LEGEND

FIGURE 1. Seedling emergence and establishment for native and exotic plant species on intact or removed/disturbed biocrusts in northwestern Montana grasslands in 2016 and 2017. Bars represent least squared means +SE. Letters indicate differences among treatments and are based on Tukey HSD post-hoc comparisons.

FIGURE 2. Mean seedling emergence and establishment for four native and four exotic plant species in 10 x 10 cm subplots with intact or removed/disturbed biocrusts from a two year field experiment. Data are two year averages for all but *Pseudoroegneria*, *Bromus*, and *Potentilla* for which we only had one year of data. Bars represent least squared means +SE. Asterisks indicate differences between treatments.

FIGURE 3. Seed germination percentage (upper) and first day of germination (lower) for native and exotic species on intact or removed/disturbed biocrusts in a greenhouse experiment. Seeds were watered daily (high) or every three days (low). Bars represent least squared means +SE. Letters indicate differences in LS-means.
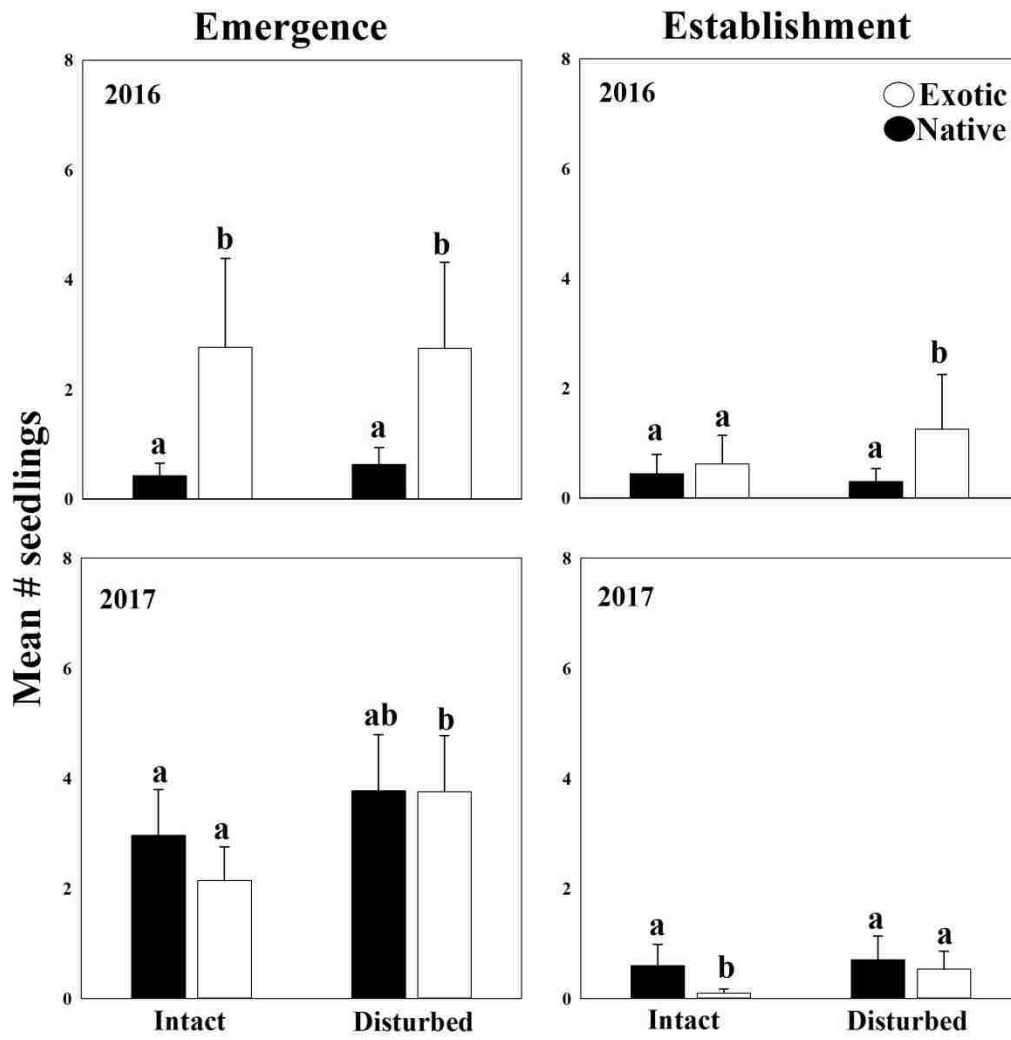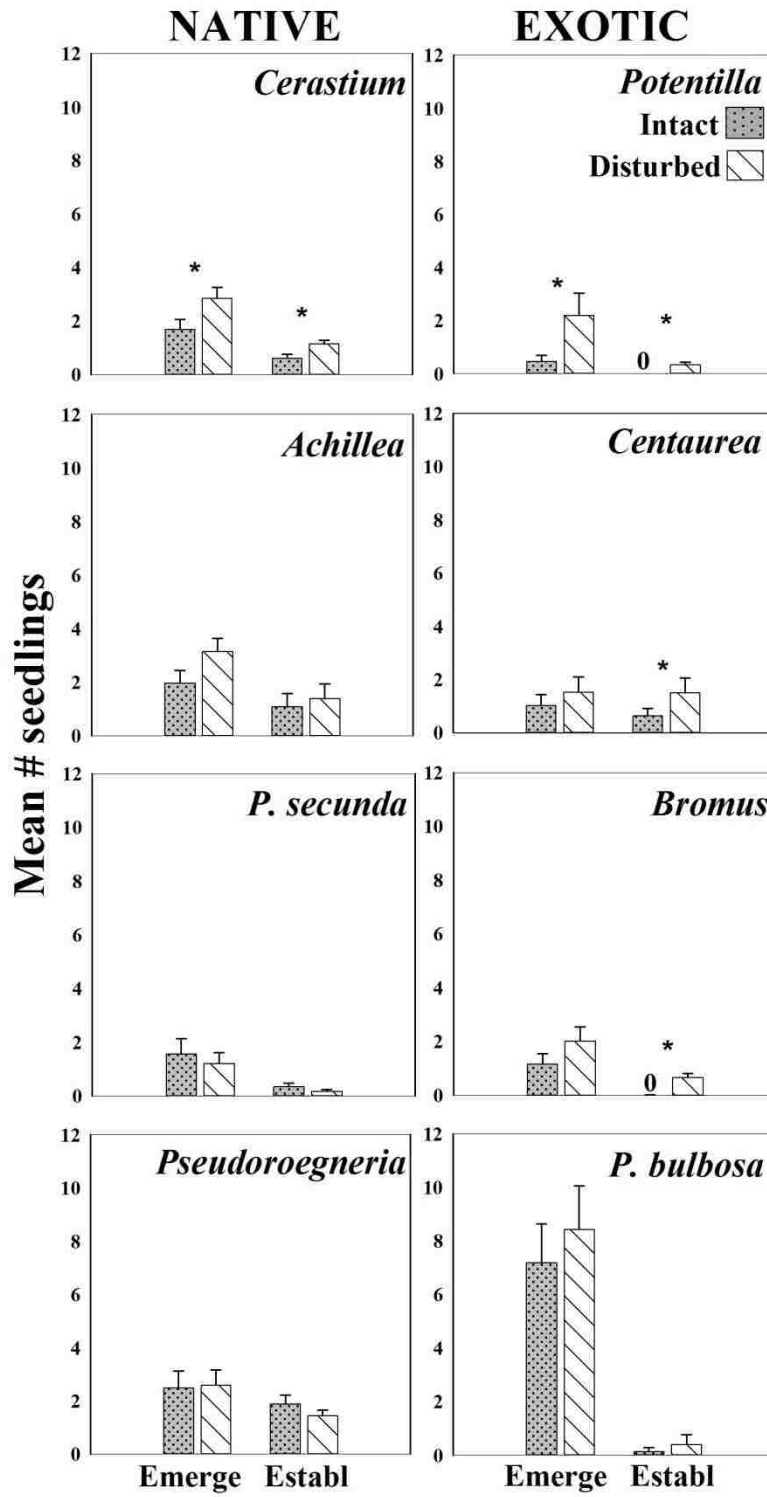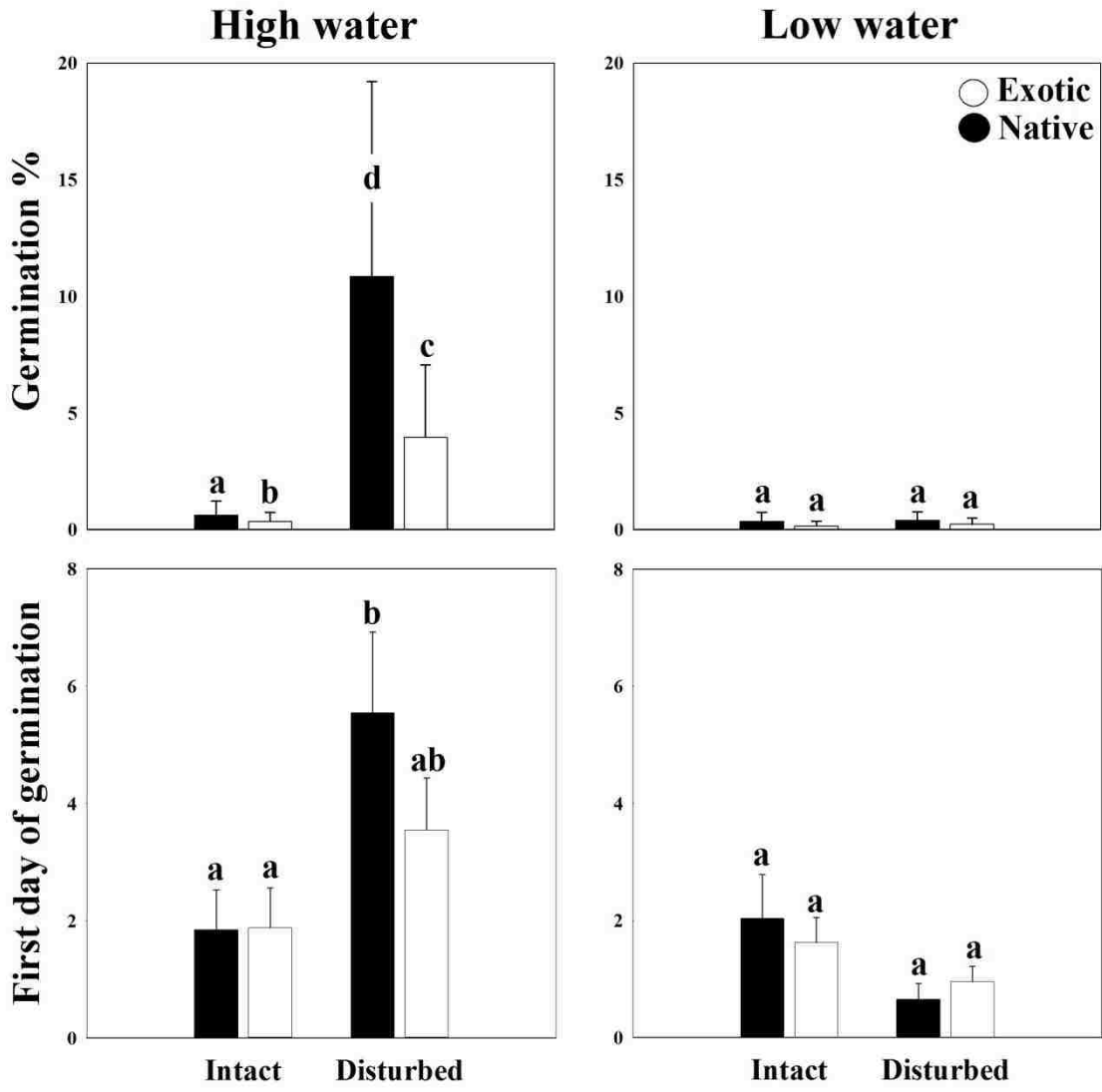
FIGURE 1.

FIGURE 2.

FIGURE 3.

SUPPLEMENTAL MATERIAL

Appendix A

List of study species.

Figures and model results of field and greenhouse experiments with all three treatments for

comparison.

APPENDIX A.

List of study species.

Figures and model results of field and greenhouse experiments including all three treatments for comparison.

TABLE A1. Vascular plant species included in field and greenhouse studies. Species with no asterisk were used in both years while species with asterisks were used only in 2017.

	Native	Exotic	Family
Forb	*Achillea millefolium*	*Centaurea stoebe*	Asteraceae
	Cerastium arvense	** Potentilla recta*	Caryophyllaceae/ Rosaceae
Grass	*Poa secunda*	*Poa bulbosa*	Poaceae
	* Pseudoroegneria spicata	** Bromus tectorum*	Poaceae

TABLE A2. Results from mixed models used to determine the effects of plant origin and biocrust treatment (Trtmt) by year on the number of seedlings in the early (*emergence*) and later part (*establishment*) of the growing season in our field experiment. Significant ($P \leq 0.05$) values are in bold and marginally significant values are annotated with an *m* ($P \leq 0.1$).

		Emergence			Establishment		
Effect		**df**	*F*	*P-value*	**df**	*F*	*P-value*
2016	Origin	1 (239)	5.31	**0.021**	1 (179)	4.85	**0.028**
	Biocrust treatment	2 (238)	2.78	0.252	1 (179)	2.98	0.226
	Origin * Biocrust	2 (238)	2.03	0.362	1 (179)	4.95	0.084^{m}
2017	Origin	1 (382)	0.07	0.787	1 (373)	0.66	0.417
	Biocrust treatment	2 (381)	19.3	**<0.001**	1 (373)	8.03	**0.018**
	Origin * Biocrust	2 (381)	3.54	0.170	1 (373)	9.76	**0.008**

FIGURE A1. Mean number of seedlings that emerged or established on intact, disturbed, or removed biocrusts in 2016 and 2017. Bars represent least squared means +SE. Letters indicate differences among treatments and are based on Tukey HSD post-hoc comparisons.
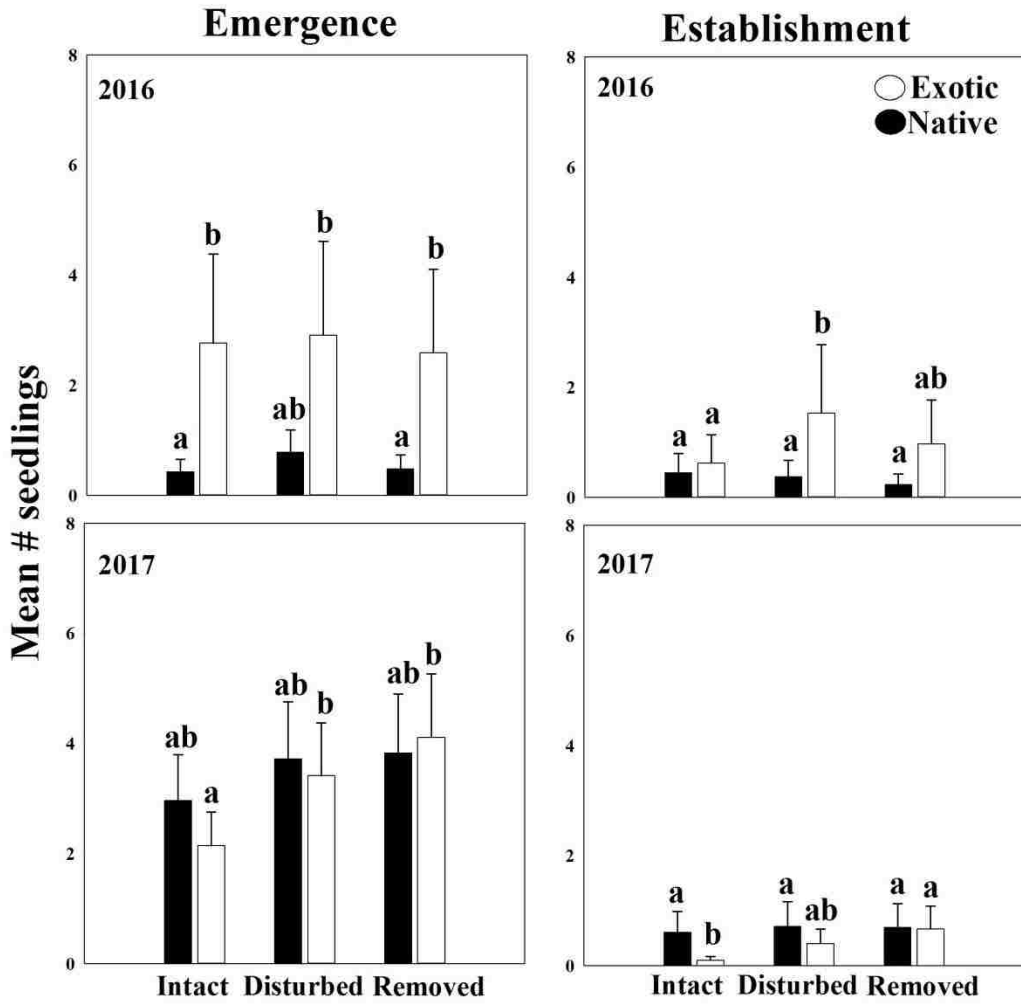
TABLE A3. Results from mixed models (16 separate models) used to compare the effect of biocrust treatments on emergence and early establishment of each study species in our two year field experiment. Species with no asterisk were added to experimental plots in both years while species with asterisks were only a part of the 2017 study. Significant ($P \leq 0.05$) values are in bold.

		Emergence			Establishment		
		df	*F*	*P-value*	df	*F*	*P-value*
Native species	*Cerastium*	2 (90)	2.24	0.107	2 (81)	7.59	**0.023**
	Achillea	2 (90)	1.34	0.261	2 (75)	0.28	0.757
	P. secunda	2 (90)	0.32	0.723	2 (81)	4.00	0.135
	* *Pseudoroegneria*	2 (42)	2.06	0.124	2 (45)	6.94	**0.031**
Exotic species	* *Potentilla*	2 (42)	14.6	**<0.001**	2 (42)	9.76	**0.008**
	Centaurea	2 (90)	1.08	0.333	2 (78)	4.34	**0.013**
	* *Bromus*	2 (41)	1.71	0.168	2 (41)	17.5	**<0.001**
	P. bulbosa	2 (90)	1.23	0.291	2 (78)	1.80	0.161

FIGURE A2. Emergence (Emerge) and early establishment (Establ) of four native and four exotic plant species on intact, disturbed, or removed biocrusts. Data for all species other than *Pseudoroegneria*, *Bromus*, and *Potentilla* are two year averages. Bars represent least squared means +SE. Letters indicate differences in LS-means.
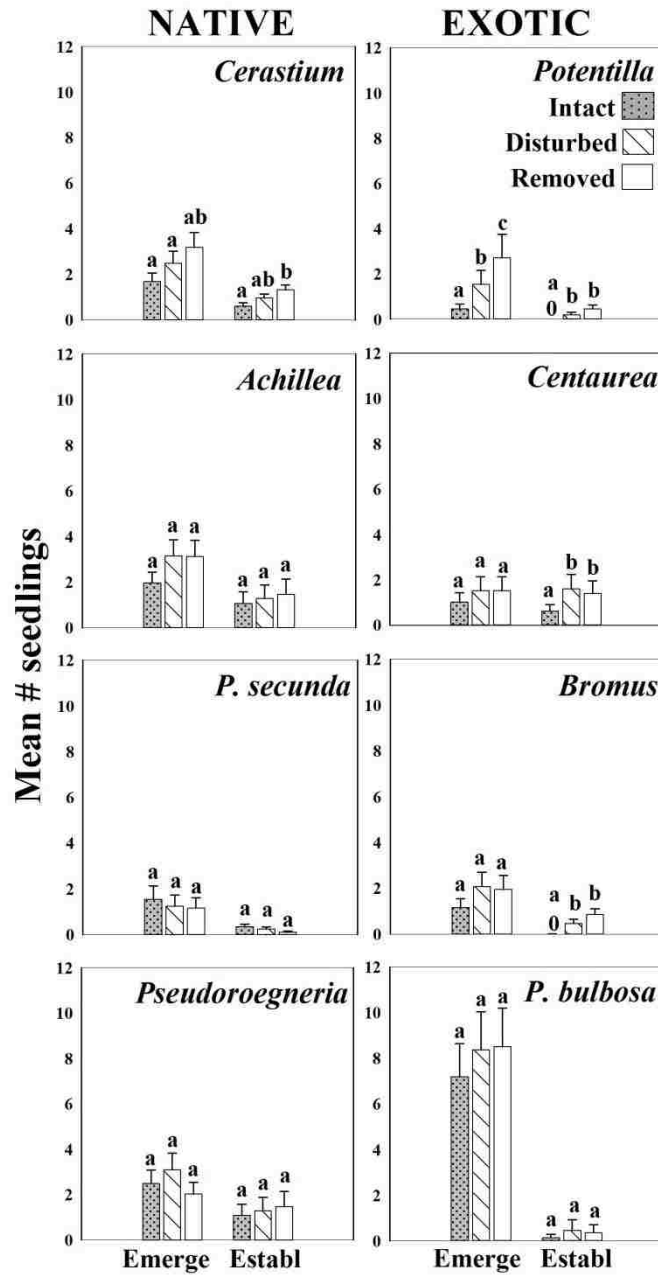
TABLE A4. Results from type III tests (germination percentage) or mixed models (germination day) used to compare the effects of all three biocrust treatments (Dist), origin, and water treatments (Water) on seed germination percentage and first day of germination in the greenhouse. Significant ($P \leq 0.05$) values are in bold.

Effect	Germination percentage			Germination day		
	df	*F*	*P-value*	df	*F*	*P-value*
Biocrust treatment	2 (369)	25.0	**<0.001**	2 (369)	10.9	**0.004**
Origin	1 (369)	8.05	**0.005**	1 (369)	5.55	**0.018**
Water treatment	1 (369)	83.4	**<0.001**	1 (369)	27.7	**<0.001**
Biocrust * Origin	2 (369)	0.18	0.835	2 (369)	0.02	0.988
Water * Origin	1 (369)	0.64	0.425	1 (369)	1.02	0.312
Water * Biocrust	2 (369)	11.5	**<0.001**	2 (369)	9.85	**0.007**
Water * Biocrust * Origin	2 (369)	0.65	0.522	2 (369)	1.46	0.483

FIGURE A3. Seed germination percentage (top) and first day of germination (bottom) for native and exotic species on intact, disturbed or removed biocrusts in the greenhouse. Seeds received a high or low water treatment. Bars represent least squared means +SE. Letters indicate differences in LS-means.