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Evaluating Nitrogen and Carbon Differences in Standing Litter from Normal and Prematurely Senesced Bromus tectorum Plants

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ABSTRACT

Evaluating Nitrogen and Carbon Differences in Standing Litter from Normal and Prematurely Senesced Bromus Tectorum Plants

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Bromus tectorum, an invasive winter annual, has displaced native vegetation throughout the western United States. *Bromus tectorum* litter influences nutrient cycling near the soil surface as well as plant establishment. Failed seed production of *B. tectorum* occasionally occurs in the field, with plants exhibiting weak flowering culms that turn straw-colored in spring when normal plants are green or purple in color. Because annual grasses transport most soluble carbon (C) and nitrogen (N) to reproductive organs, seed production failure results in significantly different fates for these nutrients compared with normal plants. As part of larger efforts to understand events leading to large-scale seedling emergence failure (termed *die-offs*), occasionally observed in near mono-cultures of *B. tectorum*, we here test the hypothesis that prematurely senesced litter associated with seed production failure has higher soluble C and N than normal litter. C and N concentrations of aboveground biomass were compared for normal and prematurely senesced *B. tectorum* plants. Two methods were used to cause premature senescence: fungal pathogen infection with *Clarireedia capillus-albis* and glyphosate herbicide application. In a related experiment, field sampling of normal and prematurely senesced plants under natural conditions was conducted to compare C and N levels *in-situ.* Herbicide-induced senescence resulted in 1.5 to 2 times greater soluble C concentrations, but fungal infection had no effect on soluble C under experiment conditions. Prematurely senesced litter had increased total N concentrations, resulting in lower C:N ratios. The C:N ratio for prematurely senesced plants (averaged across all studies) was 68:1, whereas mature normal plants averaged 243:1. These findings illustrate failed seed production associated with premature senescence results in *B. tectorum* litter with significantly higher N concentrations and can result in increased soluble C concentrations. Altered nutrient status may contribute to changes in soil microbial activity, including activity of soilborne pathogens found in die-offs.

Keywords: seed development, soilborne pathogens, source-sink relations

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INTRODUCTION

Bromus tectorum (cheatgrass, downy brome) is an invasive winter annual that has displaced native vegetation over hundreds of thousands of hectares. Success results from a variety of characteristics, including the ability to occupy sites of disturbance and adapt to a wide range of soil textures, sharp awns that aid in seed dispersal, high phenotypic plasticity that allows plants to produce at least some seeds over a range of weather scenarios, facultative germination in fall, continued growth throughout winter, and the development of fast-growing shoot and root systems (Knapp et al. 1996; Mealor et at. 2013; Norton et al. 2004). Additionally, *B. tectorum* begins growth earlier in spring than most native perennials, is an abundant seed producer, and is associated with greatly increased fire frequency (Brooks et al. 2004). Together, these features have shifted the biotic community composition after disturbances toward annual grass dominated systems and contribute to the difficulty of restoring native vegetation on invaded wildlands (Smith et al. 2008). During early development, *B. tectorum* vegetative organs accumulate soluble carbon (C) and nitrogen (N), which are involved in numerous cellular components and physiological processes (Fischer 2007). During later stages of the life cycle, photosynthesis decreases, and vegetative organs become net exporters of soluble C and N to developing seeds (Fischer 2007; Yan and Zhang 2005).

Plant biomass production and litter (above-ground dead plant material) decomposition are two of the main processes that drive nutrient cycling (King et al 2012). *Bromus tectorum* invasion has been shown to alter nutrient cycling by increasing total soil N in the soil (Belnap et al. 2005; Blank and Young 2008; Norton et al. 2004; Stark and Norton 2015). Other previous studies have demonstrated *B. tectorum* invasion alters below ground processes involved in nutrient cycling, soil structure, soil C and N pool sizes, changes in root exudates, mycorrhizal

associations, and microbial species (Belnap and Phillips 2001; Ehrenfeld 2003; Mealor et al. 2013; Norton et al. 2004; Whisenant et al. 1999). Microorganisms are the major decomposers in grassland systems and changes in litter composition, such as lower C:N ratios (total C to total N) can increase decomposition rates (McKee et al. 2016). Decomposition rates affect the rate of return to the soil for compounds such as sugars, which provide energy to the microbial community. Soluble components such as labile C released from litter and decaying roots during decomposition and leaching events drive the growth and activity of opportunistic microorganisms (Hooker et al. 2012). Opportunistic microorganisms include soilborne pathogens such as *Fusarium sp., Pyrenophora semeniperda, Ustilago bullata,* and *Tiletia bromi,* which have been reported in *B. tectorum* stands (Berg and McClaugherty 2008; Meyer et al. 2016). *Bromus tectorum* biomass contributes an abundant source of litter annually, providing nutrients for soil microorganisms (Mealor et al. 2013; Meyer et al. 2016). Additionally, intact *B. tectorum* litter contributes to wetter soil conditions through reduced evaporation from the soil surface (Baughman et al. 2016; Belnap et al 2001; Sheley et al. 2009). B*romus. tectorum* litter typically has higher C:N ratios (150:1) compared to native bunchgrass litter (50 to 75:1), resulting in slower litter decomposition rates and prolonged litter surface cover compared to native perennials (Bansal et al. 2014; Evans et al. 2001; Norton et al. 2004). Litter is also affected by temperature and precipitation, with increases contributing to faster decomposition rates (Fraser and Hockin 2012). Increased disease cycles have also been correlated with dense litter cover, with litter acting as an inoculum source, providing conditions conducive to disease transmission, increasing available moisture, and decreasing temperature fluctuations (Beckstead et al. 2011; Berg and McClaugherty 2014; Ehlert et al. 2014; Evans et al. 2001; Sheley et al. 2009).

Litter composition of *B. tectorum* stands influences many processes at the soil-litter interface and we are interested in the effect of premature senescence on *B. tectorum* litter composition. *Bromus tectorum* tissues contain both structural and non-structural carbohydrates that undergo significant changes during flowering and seed development (Raven et al. 2005; Yu et al. 2015). During normal maturation of annual plants, non-structural carbohydrates including glucose, fructose, sucrose, fructans and starch are translocated to developing seeds (Raven et al. 2005). However, with premature senescence, seed production is prevented so that non-structural carbohydrates remain in vegetative tissues. These non-structural carbohydrates in prematurely senesced tissues may become available to soil microbes as litter decomposes and also during leaching that occurs following precipitation events (Berg and McClaughtery 2008; Raven et al. 2005; Yu et al. 2015). In one study, soluble C (sucrose, glucose, fructose) accounted for approximately 57% of total nonstructural carbohydrate concentrations in meadow brome grasses (Jensen et al. 2014). This ranged from 6 to 13% of dry mass, with pre-seed filling soluble C concentrations measuring approximately 13% in May and decreasing to 6% post- seed filling in August (Jensen et al. 2014). These major soluble carbohydrates are readily transferred from plant tissues to developing seeds, but carbohydrates left in litter are susceptible to a variety of different outcomes including dissolution, leaching, and consumption by microorganisms (Berg and McClaughtery 2014; Distelfeld et al. 2014).

Nitrogen is another essential element in plants. In cereal grains, N accumulates in vegetative tissues prior to anthesis (flowering). Leaves and stems then redistribute most N to seeds, providing the major N source during grain filling. The amount of N transported to seeds largely determines grain yield and crop protein content (Berg and McClaughtery 2014; Kitchey et al. 2007; Masclaux-Daubresse et al. 2010; Park et al. 2014; Vasquez et al. 2008). For example, in

winter wheat cultivars 60 to 95% of the N in grain at harvest came from the remobilization of pre-anthesis N stored in stems (Kitchey et al. 2007).

In our studies we have frequently observed prematurely senesced *B. tectorum* plants *in-situ*. These plants have structurally weak flowering culms and fail to produce seeds. They turn straw-colored in spring several weeks before normal plants. Failed seed production in *B. tectorum* has not been extensively studied but may result from aphid infestation, herbicide application, or fungal pathogens such as the newly described crown-rot pathogen *Clarireedia capillus-albis* that causes 'bleach blonde syndrome' (Meyer et al. 2016; JF Pearce, unpublished data). This disease is manifested as stunting of growth, premature maturation, and sterility (Franke et al. 2014; Meyer et al. 2016; JF Pearce, unpublished data).

As part of a larger effort to understand die-off events, we tested the hypothesis that premature senescence of *B. tectorum* leads to increased soluble C and N levels in leaves and stems as compared with normal plants. Our study included a combination of greenhouse and field studies to observe the impact of senescence timing on litter and examine whether aboveground litter collected from prematurely senesced *B. tectorum* plants with failed seed production differs from normal plants in C and N concentration. In addition, we conducted an *in-situ* population sampling experiment that compared normal and prematurely-senesced wild plants where the cause of aborted lifecycle was unknown.

MATERIALS AND METHODS

General Experimental Approach

For all studies, we compared aboveground *B. tectorum* tissues from prematurely senesced plants that failed to produce seed with those from normal mature plants that completed their life cycle. For field experiments, samples were taken at least one meter from plot edges to avoid potential edge effects. Harvested samples included all plant material that was 0.5 cm above the soil level. After collecting, samples were placed in paper bags, transported to the laboratory and seeds (where present) were removed and analyzed separately. Measurements in each experiment included soluble C, total C, neutral-detergent-soluble carbohydrates (NDSC), and total N. C:N ratios were also calculated. Soluble C represents the dissolved organic carbon and NDSC represents all detergent soluble carbon materials including sugars (monosaccharides and oligosaccharides), starch, fructans, pectins, organic acids, proteins, and nucleic acids, but excluding cellulose, hemicellulose, and lignin (Hall 2000; Scarbrough et al.2004; Soest et al. 1991).

Premature Senescence Through Fungal Infection

The fungal infection experiment was conducted by JF Pearce (unpublished data) and evaluated pathogenicity of the crown-root fungal pathogen *Clarireedia capillus-albis*, known to cause premature senescence in *B. tectorum*, on germinating seedlings. A growth medium of sand, vermiculite, peat moss, and Agsorb (Agsorb Products Group, Chicago, IL) were mixed (4.5: 3: 3: 1.5 by volume) in 220 L batches. Non-dormant *B. tectorum* seeds were planted in the growth medium in cylindrical containers measuring 4 cm wide by 21 cm deep. For each mixed batch, a complete fertilizer formula (19: 6: 12) was added during mixing with nitrogen (34 grams),

phosphorus (18 grams), and potassium (9.5 grams) (Osmacote, The Scotts Company, Marysville, OH) and each batch of medium was steamed at 60°C for 45 min. Containers were infected with *C. capillus-albis* through an incorporation of dry inoculum (inoculum grown on oats and rice hulls as described by JF Pearce (unpublished data) into the top 2.5 cm of soil. After planting, the soil was moistened to field capacity and all containers were vernalized at 4°C in a growth chamber for 6 weeks to induce flowering and watered every 14 days. After vernalizing, containers were transferred to a greenhouse at 27 to 30°C and watered weekly until maturation. In this case, the comparison was between *B. tectorum* plants infected with *C. capillus-albis* and mature uninfected plants. Infected plants were harvested after senescence, while uninfected plants were harvested after seed production but before seed dispersal. After harvesting, seeds were removed (where present), plants were placed in brown paper bags, oven-dried at 60°C for 24 hours and weighed. We then analyzed the harvested samples for soluble C, total C, NDSC, and total N as previously described, and C:N ratios were calculated from these values.

Imposed Premature Senescence Through Herbicide Application

Parallel field experiments were conducted at two locations: a Mohave Desert site located in Southern Nevada (elevation 854 m; mean July temperatures of 40°C; 39.9741°N, 114.5003°W), and a cold desert site located in Northern Utah (elevation 1,446 m; mean July temperature of 34°C; 40.2211°N, 112.7444°W). *Bromus tectorum* dominated both locations. The sites were harvested in 2017, but sampling dates differed due to earlier maturation at the Nevada site.

The experimental design at both sites consisted of a fully randomized split-plot with three replicate blocks. Blocks were 9 m by 9 m and split in half to be 9 m by 4.5 m. Three replicate blocks were installed at each site and included 0.1 m^2 subplots available for sample collection.

Three subplots were randomly chosen and harvested for each sample date from each of the three blocks at both sites. Plants were allowed to emerge and actively grow until just before bolting of flowering culms, when half of each block was treated with glyphosate herbicide following manufacturer recommendations (Roundup Pro Max, Monsanto, St. Louis, MO). The other half of each block was left untreated as a control.

At the Nevada site, fertility islands were present in subplots due to shrubs recently killed by wildfires (Allen et al. 2011; Ravi et al. 2007). To account for possibly increased C and N associated with fertility islands around dead shrubs, samples were taken from both fertility islands and interspaces and analyzed separately following the same procedures as indicated earlier.

Aboveground plant samples were harvested in 0.1 m^2 plots and samples were collected March 14, April 24, and May 26 in Nevada and April 26, May 24, July 13, and October 6 2017 at the Utah site. Samples were stored in brown paper bags, transported to the laboratory and oven dried at 60°C for 24 hours. Samples were then sorted, and non-*B. tectorum* debris was removed. Biomass of each sample was recorded by weighing. Biomass measurements were multiplied by the percentage of C and N concentrations to calculate available C and N per unit area for herbicide-treated and untreated control plants.

In Situ Population Sampling

In this part of the study we compared aboveground tissues between normal plants and those that had prematurely senesced naturally (i.e., without treatment). Field samples of *B. tectorum* plants were collected from wild populations at eight locations throughout northern Utah: Whiterocks (40°19.680′N 112°46.680′W), Spanish Fork (40.1150° N, 111.6549° W), Dugway

(40.2211° N, 112.7444° W), Birdseye (39.9244° N, 111.5496° W), Stansbury Island (40.8383° N, 112.5030° W), Kaysville (41.0352° N, 111.9386° W), Provo (40.2338° N, 111.6585° W), and Garden City (41.9469° N, 111.3935° W).

For all collection sites, normal *B. tectorum* plants were harvested prior to bolting when plants were green in color, and again when plants had reached full maturity, completed seed dispersal, and turned straw colored. Prematurely senesced plants with failed seed production were collected when vegetative tissue became straw colored. At the time prematurely senesced plants were harvested, surrounding normal plants were still green or greenish-purple. Plant samples were analyzed for soluble C, total C, NDSC, total N, and C:N ratios as previously described. Specific causes of premature senescence were not determined, but we have previously observed fungal infection, crown aphids, and herbicide treatments to cause premature senescence *in-situ*.

Statistical Analysis

Data were analyzed using JMP version 14.0 (SAS Institute, Cary, NC). For the *C. capillus-albis* infection and *in-situ* population sampling studies, we analyzed differences between prematurely senesced and normal plants for soluble C, total C, NDSC, total N, and C:N ratios as a mixed model one-way analysis of variance (ANOVA). Treatment (infected or prematurely senesced vs. uninfected or natural senescence) was the fixed effect, and pairwise comparisons of prematurely senesced and normal plants were made using a Least Significant Difference means separation test.

To determine the statistical significance of treatments for the herbicide application experiment, data were analyzed using Mixed Model Analysis of Variance and Restricted Maximum Likelihood (REML) models (JMP 14.0, 2018). Spatial and temporal correlations and

repeated field measures were included through the REML models. The experiment was analyzed by site. Blocks were arrayed in a randomized plot design with plot as the random effect, while treatment (herbicide-treated vs. untreated control plants) and sample date were treated as fixed effects. Response variables included soluble C, total C, NDSC, total N, available C and N inputs, and C:N ratio. Subsamples within blocks were pooled for analysis, providing three replicates per sample date per treatment per block. When fertility islands were present in sampling plots, fertile islands were sampled and analyzed as an independent variable but produced no treatment effect; therefore, samples were pooled across fertile islands and interspaces to double replication at the Nevada site. Pairwise mean separations were made for analyzing the temporal effect of month on *B. tectorum* litter using least significant difference multiple means separation tests.

Laboratory Analyses

Plant samples were oven dried at 60°C for 24 hours and biomass determined by weighing. Samples were ground with a plant tissue grinder (Wiley Mini-Mill, Thomas Scientific, Swedesboro, NJ) to a uniform consistency until they passed through a 2 mm sieve. To analyze soluble C, which represents the dissolved organic carbon readily lost from litter, 0.1gram samples of oven dried and ground tissue samples were individually placed into 50 ml conical tubes. The volume in each tube was brought to 50 ml with ultrapure laboratory grade deionized water (Milli-Q Reference Water Purification System, Burlington, MA, USA). Tubes were capped and placed in an end-over-end shaker (E6010- Fixed Speed Reciprocal Shaker, Eberbach Corporation, Ann Arbor, MI) for 1 hour at 20°C. After shaking, the suspension was filtered through a 0.45 μm membrane filter. Filtrate samples were diluted and preserved with hydrochloric acid to pH 2.5 as per instrument specifications, then stored in the dark at 2° to 4°C

until analysis. The extracted soluble C solutions were oxidized and combusted using a 680°C combustion catalytic oxidation instrument (Shimadzu TOC L-Series, Kyoto Japan).

For NDSC analysis, ground plant samples were weighed out to approximately 0.5 grams and the weights recorded. Samples were placed in individual fiber filter bags and sealed with a 120 V heat sealer (Ankom Technology, Macedon, NY USA). Samples were then digested in a detergent solution using a fiber analyzer, resulting in insoluble residues primarily composed of structural carbohydrates (cellulose, hemicellulose, and lignin) (Ankom Technology, Model A200, Method 6, Macedon NY). The NDSC measurement represents the water-soluble carbohydrates (dissolved organic carbon) and neutral detergent soluble compounds (monosaccharides, oligosaccharides, starch, fructans, proteins, nucleic acids, organic acids, proteins, and pectins) that were removed during digestion (Hall 2000; Scarbrough et al.; Soest et al. 1991). NDSC was included in this study primarily to account for starch in plant tissues. To measure NDSC, we first measured the structural components of the plants (cellulose, hemicellulose, and lignin), recorded as percent on a scale of 0 to 100%. We then calculated NDSC as 100% minus the structural component (cellulose, hemicellulose, lignin) to determine the soluble versus the insoluble structural fraction for each plant (Scarbrough et al. 2004).

Total C and N were included in this study to determine C:N ratios, which influence plant litter performance, specifically decomposition rates. For total C and N analysis, approximately 0.5 grams of oven dried and ground plant tissue samples were wrapped in a tin capsule and burned in pure oxygen (LECO Corporation, St. Joseph, MI). Total C and N were measured with an elemental dry combustion-based analyzer following manufacturer guidelines, and the C:N ratios were calculated from the measured values (CHN 628, Leco Tru-SPEC, LECO Corporation, St. Joseph, MI).

RESULTS AND DISCUSSION

Premature Senescence Through Fungal Pathogen Infection

Clarireedia capillus-albis infection was successful at causing premature senescence, thereby preventing seed production as reported by JF Pearce (unpublished data). Infection resulted in a significant increase in soluble C concentrations for vegetative tissues compared to seeds (Figure 1A; Table 1). However, for vegetative tissues infection had no impact on the concentration of plant soluble C in aboveground biomass, accounting for 11% of total C in tissues (Figure 1A). Soluble C concentration in seeds harvested from control plants was 0.6% lower than either infected or uninfected vegetative tissues (Table 1). Total C concentrations were approximately 44% for both infected and uninfected plants and did not differ among tissue types (Figure 1B). These data illustrate that *C. capillus-albis* infection did not change tissue soluble or total C concentrations despite premature senescence. While no published data is available for soluble C concentrations of *B. tectorum* litter, Jensen et al. (2014) reported soluble C concentrations ranging from 6 to 13% dry weight for meadow brome, a related *Bromus* grass, which is similar to the present experiment for both infected and uninfected plant tissues. Evans et al. (2001) measured the effects of *B. tectorum* litter dynamics on arid grasslands and reported total C concentrations for *B. tectorum* litter to be 41%, which is also consistent with the present experiment. Although fungal infection did not alter the soluble or total C concentrations of prematurely senesced plants in this experiment, *B. tectorum* invasion can alter the soil biota, specifically fungal and bacterial actinomycete numbers or species in the soil (Belnap and Phillips 2001) through increased nutrient cycling rates from annual litter production and root turnover (Belnap et al. 2001; Norton et al. 2004, Whisenant et al. 1999). Together, these changes will also

influence decomposition rates and nutrient availability for other plants and the microbial community.

NDSC concentrations (organic acids, sugars, starch, fructans, nucleic acids, pectins, proteins, and nucleic acids) were greater for infected plants compared to uninfected control plants. NDSC accounted for 79% of total C in infected plants and 67% in uninfected plant tissues (Figure 1C; Table 1). The NDSC concentrations measured in uninfected control plants were expected, as structural carbohydrates (cellulose, hemicellulose, lignin) in *B. tectorum* are typically around 38% at maturity (Ganskopp and Bohnert 2006). NDSC for control seeds (45%) was greater than for both infected and uninfected plant tissues (30 to 35%) ($p=0.0017$). These data support the well-known source: sink pathway that soluble C is translocated to seeds where it is converted and stored as starch, the primary storage compound in seeds. NDSC was higher in seeds than plant tissues at maturity, as previously reported by Yang and Zhang (2005).

In contrast with soluble and total C, total N was nine times greater in vegetative tissue from *C. capillus-albis* infected plants than from uninfected plants ($p=0.004$; Figure 1D; Table 1). As expected, seeds were much higher in total N than either infected or uninfected plant tissues. Butterly et al. (2015) measured total N in *B. tectorum* litter and reported an average value of 0.25%, which is near the high end for total N herein (0.01 to 0.25%; Figure 1D). Results from other monocarpic winter annual grasses are also relevant, including winter wheat cultivars which have been measured at maturity and reported to have 60 to 95% of the N in seeds at harvest originating from the remobilization of pre-anthesis N translocated from maturing stems (Atkinson and Brown 2016; Kitchey et al. 2007). Our data are consistent with studies that show N translocation to developing seeds in monocarpic species, which have been extensively studied (e.g. Distelfeld et al. 2014; Fischer 2007; Kitchey et al. 2007). Furthermore, the early

developmental N accumulation measured in prematurely senesced plants in our experiment led to increased N concentrations in tissues prior to lifecycle disruption. The higher tissue N concentrations measured in our infected plants illustrate disruption of normal metabolic pathways resulting in changed litter composition and the potential for modifications in soil ecology during decomposition. Previous studies (Morris et al. 2015; Norton et al. 2004; Reitstetter et al. 2017; Stark and Norton 2014) reported that *B. tectorum* invasion increased soil N availability through root systems that release N coupled with annual root turnover and that the soil organic matter was far more labile. These conditions result in a more rapid turnover rate and easily degradable carbohydrates, in *B. tectorum* dominated areas, with net N mineralization rates more than double that occurring beneath sage-brush plants (Zou et al. 2005). Our results suggest that prematurely senesced litter will contribute additional increased available N to the soil compared to the already elevated levels associated with the transition to near monocultures of *B. tectorum*. Increased available N could lead to larger soil N pools and faster mineralization rates, resulting in additional organic N converted to ammonium and nitrate, which are susceptible to leaching losses in the soil, but also readily available for plant uptake (Johnson et al. 2005).

Imposed Premature Senescence Through Herbicide Application

At the Nevada site, herbicide-treated plants had higher concentrations of soluble C compared to untreated control plants across all sample dates ($p=0.0525$; Figure 2A; Table 2). The soluble C concentrations for plant samples harvested prior to herbicide application averaged 12%. Soluble C concentrations for herbicide-treated plants harvested in April and May were similar at 10 to 11%, while concentrations in untreated control plants dropped to 7% in April and to 5% in May. By May, soluble C concentrations in untreated control plants decreased to less than half (5%) the

value of herbicide treated plants (11%). However, total C concentration was similar for all plants across all sample dates (42% for herbicide-treated; 43% for untreated control) (Figure 2B; Table 2). Soluble C accounted for 25% of herbicide-treated and 11% of untreated control plant tissue total C composition, indicating higher soluble concentrations in herbicide-treated tissues.

The NDSC (soluble and non-structural compounds) in plant tissues decreased with maturity for untreated control plants at the Nevada site ($p=0.0001$; Figure 2C; Table 2). This is a typical pattern observed in annual grasses as plants translocate soluble C from stems to seeds, and the non-soluble fiber content (cellulose, hemicellulose, lignin) increases with maturity to support cell wall structure of stems and florets (Chen 2016; Cook and Harris 1952; Ganskopp and Bohnert 2006; Mealor et al. 2013). Additionally, NDSC was greater in herbicide-treated plants (44%) than in untreated control plants (26%) in May ($p=0.0058$; Table 2), providing support for the hypothesis that premature senescence causes a buildup of sugars in vegetative tissues. NDSC accounted for approximately 90% (herbicide-treated) and 68% (untreated control) of total C, again indicating greater soluble concentrations in herbicide-treated plants, and untreated control plants were similar to fungal infection control plants (67%). Our NDSC values decreased for untreated control plants over time from 46% in March to 25% in May and remained constant for herbicide-treated plants. Decreased NDSC concentrations with maturity were also reported by Ganskopp and Bohnert (2006) for healthy *B. tectorum* plants. NDSC concentrations at maturity in their study ranged from 25 to 38%. These values are quite similar to the present experiment (30 to 44%) for mature, untreated *B. tectorum* litter. Higher soluble C and NDSC values in herbicide-treated plants suggest that premature senescence likely results in more readily available C to soil microbes during decomposition or leaching events. Additionally, NDSC concentrations measured in herbicide-treated plants were similar to the pre-treatment immature

green plants harvested in early spring. Herbicide-treated plants contained higher energy (carbohydrates) values compared to untreated control plants, which demonstrates prevention of seed production disrupts the normal translocation pathway for these nutrients (Table 2).

Total N concentration was 3.5 times greater for herbicide-treated plants compared to untreated control plants at the Nevada site for both sample dates. A pairwise comparison of N concentration between treatments showed total N was significantly greater in herbicide-treated plants compared to untreated control plants for both sample dates ($p=0.0001$; Figure 2D). This difference is attributable to the completion of seed filling in untreated control plants, with total N being remobilized to developing seeds. Srivalli and Khanna-Chopra (2004) measured effects of reproductive spikelet removal from wheat plants on N remobilization. They reported removal led to lower proteolytic activities, which are responsible for N remobilization, thereby slowing N remobilization and leading to N accumulation in the flag leaf. We speculate that premature senescence due to herbicide treatment has a similar effect on proteolytic activities, based on increased N accumulation in aboveground biomass.

At the Utah site, herbicide-treated plants had higher soluble C compared to untreated control plants for all sample dates ($p=0.048$; Figure 3A). By October, soluble C in herbicide-treated plants was 1.5 times higher than untreated control plants. For comparison, Frank et al. (2010) analyzed other annual pasture grasses for soluble carbohydrates and reported ranges from 2.0-13% (dry matter basis). Their results were similar to the present experiments, where overall soluble C measured 11% (herbicide-treated) and 5% (untreated control) by May at the Nevada site and 3% (herbicide-treated) and 2% (untreated control) at the Utah site by October. Results from both sites show increased soluble C concentrations for herbicide-treated plants compared to

untreated control plants. The increased soluble C is likely associated with more rapid decomposition by microbes (Hooker and Stark 2008; Parton et al. 1987; Zou et al. 2005).

Total C concentrations were similar for all experimental treatments across all sample dates (Figure 3B; Table 3). Total C included both soluble C and NDSC measurements, in addition to insoluble structural carbohydrates (e.g., cellulose, hemicellulose, and lignin) that we did not measure. The soluble C concentrations measured in plant tissues accounted for 5 to 7% of total C in herbicide-treated and untreated control plants, and NDSC accounted for 88 to 90% of total C. We acknowledge that NDSC is only a crude estimate of total digestible carbohydrates as it also includes nucleic acids, organic acids, and proteins; this fact decreases the percent of total C accounted for by this test (Hall 2000). NDSC values at the Utah site remained constant across treatments for all sample dates (Figure 3C; Table 3). Although NDSC was not greater in herbicide-treated plants at the Utah site, as it was at the Nevada site, this may be attributable to specific site conditions such as temperature and precipitation. Ganskopp and Bohnert (2006) reported high variability between years and months when measuring NDSC of *B. tectorum and* concluded that this variation was associated with differences in precipitation timing and quantity.

Herbicide-treated plants had higher soluble C concentrations throughout sampling, likely due to a lack of developing seeds that act as nutrient sinks during plant maturation. Soluble C in plant stems and leaves becomes the main energy source for the soil microbial community during decomposition. Furthermore, litter with increased soluble C concentrations can release soluble C during leaching that follows precipitation events. These changes in litter C and N concentrations can influence the growth and species composition of fungal and bacterial populations under *B. tectorum* stands (Adair and Burke et al. 2010; Bansal et al. 2014; Jiang et al. 2014).

Total N was two times greater in herbicide-treated plants compared to untreated control plants by the final sample date in October at the Utah site ($p=0.0018$; Figure 3D; Table 3). The difference in N between herbicide-treated and untreated control plants is consistent with findings of Kitchey et al. (2007), who reported only a small fraction (<30%) of seed N comes from post-flowering N soil uptake, with the main proportion of N (71%) stored in stems prior to anthesis. Nitrogen that is stored in these stems and leaves is then translocated to developing seeds as plants mature. Beckstead and Auspurger (2004) measured *B. tectorum* N concentrations at bolting, flowering, and maturity to compare nitrogen, litter, and water addition or reduction at different life stages. They found that litter N concentrations decreased from 2.3% at bolting to 0.4% at maturity. These results are similar to those reported herein, with N concentrations decreasing with maturation for normal plants (1.8 to 0.3% Nevada; 1.0 to 0.4% Utah) and remaining constant for prematurely senesced plants with no seeds acting as sinks during nutrient remobilization.

Litter collected from both the Nevada and Utah field experiments had higher soluble C and total N in prematurely senesced plants compared to normal plants. Soluble C was greater in herbicide-treated plants compared to normal plants in the field, but this result was not observed for fungal pathogen-infected plants under greenhouse conditions. Increased soluble C from litter accelerates microbial populations, as C is the necessary energy source to support growth and activity of soil microbes. Soluble C also leads to increased microbial N demand (Hooker and Stark 2008) and possibly favors higher fungi to bacteria ratio in the soil (Jiang et al. 2014). Fungal pathogens present in soils occasionally become suppressed through fungistasis, i.e., the inhibition of fungal spore germination or growth of hyphae in soils due to competition for available nutrients or unfavorable abiotic conditions (Garbeva et al. 2011; Meyer et al. 2016). A

pulse of soluble C associated with prematurely senesced litter could terminate fungistasis, allowing suppressed pathogenic fungi to increase activity in the soil, or even provide these fungi with the required energy to produce defensive compounds that provide protection against antagonistic microorganisms (Garbeva et al. 2011; Meyer et al. 2016).

Both herbicide-treated and *C. capillus-albis* infected plants had higher N levels in vegetative tissue than did normal healthy plants. Root systems of *B. tectorum* are typically very dense, fine (<1mm) and shallow compared to native perennial grasses. The decaying root system left behind creates very porous soil, increasing root decomposition through enhanced air and water movement (Norton et al. 2004). Large N pools reported beneath *B. tectorum* stands have been attributed to both annual root turnover as well as litter contribution and cover (Hooker et al. 2012). Our data suggest that prematurely senesced litter provides an additional source for increased total N concentrations compared to normal *B. tectorum* stands, either during decomposition or leaching from above-ground litter that occurs following precipitation. This N pulse, in conjunction with or independent from increased soluble C, likely contributes to accelerated cycling of labile C and N (Hooker and Stark 2012).

In Situ Population Sampling

Field collections examined differences in tissue C and N concentrations between prematurely senesced plants that failed to produce seeds, and normal *B. tectorum* plants of wild populations. Soluble and total C levels for these *in-situ* populations were similar for prematurely senesced and normal plants, as in the *C. capillus-albis* infection experiment (Figure 4A, B; Table 4). NDSC content was 10% greater for prematurely senesced plants compared to normal plants ($p=0.0048$; Figure 4C). The increased NDSC results from the normal and prematurely senesced field

collections were similar to those in the herbicide application experiment at the Nevada site, providing evidence that prematurely senesced litter contains less insoluble structural carbohydrates such as cellulose and lignin. Lower structural carbohydrate content results in faster decomposition compared to normal *B. tectorum* plants and increased available energy sources for microorganisms. Total N was 2.5 times greater in prematurely senesced plants compared to mature normal plants ($p=0.0053$; Figure 4D; Table 4).

In all three experiments, prematurely senesced plants had increased N undoubtedly because the absence of seed production eliminated seeds as a nutrient sink. Prematurely senesced litter would provide the microbial community with a substrate richer in total N, which may benefit the microbial community as most microbes are limited by the amount of available N (Hodge et al. 2000). The composition of litter decomposed by microbes contributes to the rate of N mineralization, which is the process by which microbes convert organic N to inorganic forms such as ammonium and nitrate (Hodge et al. 2000). Nitrogen is considered one of the most limiting soil nutrients in semi-arid systems; increased available N in prematurely senesced litter may increase soil microbial numbers and species through temporarily decreasing N competition (Adair and Burke 2010; Belnap et al. 2001).

Biomass

Prematurely senesced plants observed in the field are stunted in size compared to normal plants (Meyer et al. 2016). In order to account for the effects of variable growth, we also assessed biomass for the herbicide application experiments at both sites (Figure 5). Aboveground biomass was reduced in all herbicide-treated plots due to ideal weather conditions that resulted in more rapid herbicide death than expected. The reduction in biomass and possible

photodegredation (King et al. 2012) at the Nevada site shortened the sampling period and resulted in insufficient litter to collect for analysis after May 2017. Herbicide application at both sites led to increased soluble C and total N in prematurely senesced plants (Figures 2 and 3) but the total amount of soluble C and total N concentrations available in plant tissues was similar between herbicide-treated and untreated control plants due to decreased biomass of the treated plants (Figure 5A-D). Plants at the Nevada site could potentially contribute approximately 1.6 g m^{-2} soluble C and 0.4 g m^{-2} total N to the soil and the Utah site could potentially contribute 2.9 g m^{-2} soluble C and 0.3 g m^{-2} total N. Delayed herbicide application would likely have resulted in larger plants and greater soluble C and total N concentrations in herbicide-treated plant tissues per unit area. The combination of increased tissue C and N concentrations and decreased biomass for prematurely senesced plants suggest that nutrient contributions per area are similar for both herbicide-treated and untreated plots. Stunted growth did not lead to increased C or N concentrations per unit area from prematurely senesced litter, which was not in support of our hypotheses that prematurely senesced litter would provide larger nutrient pulses to the soil environment.

C:N Ratios

We examined C:N ratios to understand if prematurely senesced litter decomposition rates and litter persistence on soil surfaces differed from that of normal litter. C:N ratios for *C. capillus-albis* infected plants were 3 times lower than uninfected plants with ratios of 147:1 and 501:1 respectively ($p=0.0010$; Figure 6A; Table 1). The C:N ratios for the *C. capillus-albis* infected plants were much higher than those observed in our field experiments. We attribute this to plants being grown in a controlled and protected greenhouse setting and fertilized during growth. The

average C:N ratio of seeds was lower compared to tissues (32:1 vs. 250-500:1), with seeds containing the majority of available C and N (Distelfeld et al. 2014; Fischer 2007; Kitchey et al. 2007). Seeds use stored C and N for germination and early seedling growth, but increased C or N in senesced litter becomes available to the soil community.

C:N ratios were significantly lower for herbicide-treated than untreated control plants at both field sites (Figure 6B). C:N ratios for herbicide-treated plants remained constant at increased concentrations throughout the sampling period compared to untreated control plants (Figure 6C). At the Nevada and Utah sites, C:N ratios were significantly lower for all herbicide-treated plants (41:1 Nevada; 45:1 Utah) compared to untreated control plants (208:1 Nevada; 128:1 Utah) by the final sample dates in May and October, respectively (Figure 6B, C). C:N ratios for the *in-situ* field collected plants were also significantly lower for prematurely senesced plants (38:1) compared to normal mature plants (135:1) (Figure 6D). Therefore, results from all three studies showed that premature senescence leads to increased N concentrations, resulting in lower C:N ratios relative to normal *B. tectorum* plants. We acknowledge that some decreases in tissue C and N concentrations may be associated with leaching following precipitation events that occurred between sampling dates (Figure 7).

C:N ratios of mature *B. tectorum* plants were measured by Evans et al. (2001) to evaluate the influence of litter on an arid grassland. These authors reported an overall average of 150:1. This value is similar to the C:N ratios for normal plants in our field studies, where values ranged from 128-208:1 (128 to 501:1 including greenhouse data) and 38-46:1 (30 to 147:1 including greenhouse data) for prematurely senesced plants. The community of microorganisms that decompose *B. tectorum* litter typically consume higher C:N ratio (150:1) substrates relative to native grasses (50 to 75:1), which would lead to N immobilization in the soil, but prematurely

senesced litter with lower C:N ratios would provide microbes with excess N and potentially increase mineralization rates (Evans et al. 2001). Decomposition is typically slower for *B. tectorum* litter compared to that of native perennials. Slower decomposition coupled with high annual biomass production creates a dense litter cover (Mealor et al. 2013), but decreased C:N ratios could result in a less persistent litter layer. Prolific annual *B. tectorum* litter production, measured to be 125% greater than that of non-invaded native grass communities, can result in complete litter-covered soil surfaces (Mealor et al. 2013). These litter-covered soils have been correlated with successful *B. tectorum* seed germination, credited to seed entrapment, buffered soil temperatures and increased moisture through reduced soil evaporation (Jones et al. 2015; Mealor et al. 2013). Faster decomposition of prematurely senesced litter may reduce litter cover, possibly contributing to greater seedling establishment failure through reduced seed entrapment, less buffered soil temperatures, and increased soil evaporation.

Our study found consistently and significantly lower C:N ratios for litter from prematurely senesced plants compared to normal *B. tectorum* plants. These values are also lower than litter from native bunchgrasses, reported to be 52 to 64:1 (Evans et al. 2001). Lower C:N ratios associated with prematurely senesced litter would accelerate rates of biochemical reactions that cause decomposition. We predict the community of decomposers will also change, as N is the most important nutrient affecting disease development in plants (Akk et al. 2017; Dordas et al. 2009; Johnson et al. 2005). The rates that C and N are returned to the soil is dependent on the C:N ratio of the substrate to be decomposed and the decomposer community composition (Hodge et al. 2000). Fungal pathogens tend to be associated with higher C:N ratios, whereas bacteria are typically associated with lower C:N ratios (Mouginot et al. 2014). The increase in bacteria associated with lower C:N ratio substrates is due to higher N requirements compared to

fungi, which have a higher relative C requirement. Because of higher N requirements, many bacterial communities have been reported to exhibit greater responses to N addition than fungal communities in arid systems (Mueller et al. 2015). Fungi can assimilate substrates more efficiently than bacteria because of their tolerance for broader C:N ratio substrates and lower N requirement (Hodge et al. 2000; Mouginot et al. 2014; Rousk et al. 2009). Furthermore, lower C:N ratios have been associated with greater bacterial population growth in grassland systems (Mouginot et al. 2014).

At C:N ratios greater than 30:1 both fungi and bacteria require additional N to break down organic substrates, possibly leading to immobilization in the soil (Hodge et al. 2000; Johnson et al. 2005). Deficiency of available N associated with high C:N ratio substrates such as *B. tectorum* litter can result in organic N taken up from soils by fungi and bacteria to meet N requirements, in which case N can become temporarily unavailable for plants (Hodge et al. 2000; Johnson et al. 2005).

During growth, *B. tectorum* roots can produce exudates with low C:N ratios, which can favor increased bacterial biomass growth under intact stands (Adair and Burke 2010). Additionally, soils in the western United States are often alkaline, which also increases bacterial growth (Reitstetter et al. 2017; Rousk et al. 2009). Contrastingly, in some scenarios (Belnap and Phillips 2001; Bolton et al. 1993), *B. tectorum* invasion has been found to increase generalist fungal species, which are able to grow faster than specialists and quickly occupy fresh litter or substrates. Previous studies have shown that N addition through fertilization can increase fungal growth, as reported for *Fusarium sp.* (Akk et al. 2017; Guo et al. 2017). Fungal pathogens (e.g., *Fusarium sp*.) are hypothesized to play a role in *B. tectorum* die-offs and have been isolated from soils following large scale seedling establishment failure in the field (Meyer et al. 2014; Meyer et

al. 2016). Furthermore, Mueller et al. (2015) measured the effects of nitrogen addition on microbial communities in an arid system and reported that the fungal class of Sordariomycetes, which includes *Fusarium sp.,* was the only class that increased with N addition. Hawkes et al. (2006) also observed a shift toward saprophytic and pathogenic fungi from mycorrhizal fungi at locations invaded by *B. tectorum*. Panwar et al. (2016) measured *Fusarium sp.* growth at varying temperatures and pH values and reported fungal growth under a wide pH range from 4.0 to 8.0, suggesting that growth in this genera may not be hindered by the alkaline soils in our study. Panwar et al. (2016) also reported different *Fusarium sp*. isolates showed wide variability in their pH requirements and had physiological adaptations to environmental niches. Most fungal pathogens have high phenotypic plasticity, meaning under abiotic or biotic stresses, they can acquire bacterial or eukaryotic genes and perform interspecific hybridization (e.g., genome exchange between related species) (Wrzosek et al. 2017). Hybridization can increase virulence, extend host range, and facilitate successful growth in new niches (Schardle and Craven 2003; Wrzosek et al. 2017). Fungi are also able to perform anastomosis, the fusion of encountered vegetative hyphae, resulting in heterokaryosis, and reproduce parasexually (e.g., exchanging DNA without meiosis or sexual structures) increasing genetic variation and adaptation (Wrzosek et al. 2016). Increased generalist saprophytic fungal growth has been correlated with greater availability of labile or soluble substrates (Belnap and Phillips 2001), and the prematurely senesced litter in our study would likely contribute greater labile C and N concentrations to the soil environment

Premature senescence coupled with a lack of seed production may result in even larger pools of labile C and N due to increased decomposition from litter with lower C:N ratios (Adair and Burke 2010). In a study of herbicide application timing on *B. tectorum* litter accumulation,

Hooker and Stark (2008) varied application dates to measure changes in soil microbial C and N cycling following senescence. These authors reported that earlier senescence stimulated soil C mineralization and N cycling rates and therefore increased microbial growth. Microbes near prematurely senesced litter would be able to begin colonizing plant litter in spring rather than summer, when *B. tectorum* plants typically senesce (Hooker and Stark 2008). *Fusarium sp.* have been reported to grow at much lower soil water potentials than bacteria (Franke et al. 2014; Mueller et al. 2015), which would allow fungal activity during dry periods in summer. Earlier senescence of N enriched litter could promote growth of fungi like the pathogen *Fusarium sp.* and enhance soil organic matter mineralization, resulting in greater available soil N (McTee et al. 2017; Mueller at al. 2015). Litter decomposition is a function of chemical composition, temperature, and precipitation, which mediate the fungal activity and influence the rates of C and N cycling (Gaxiola and Armesto 2015; McTee et al. 2017). Precipitation events that cause soil wetting and drying can increase the availability of substrates such as inorganic N, particularly under *B. tectorum* stands (Saetre and Stark 2005). The microbial decomposition of litter with lower C:N ratios, such as those found in this study, may also contribute additional inorganic N to the soil.

Conclusions

In every instance where *B. tectorum* plants failed to produce seeds, N levels in vegetative tissue were higher than for normal plants. Prematurely senesced *B. tectorum* plants with higher tissue N concentrations and lower C:N ratios likely result in faster decomposition in the field, increased N mineralization, and shifts in the microbial community depending on microbial demand and whether N is immobilized. Soluble C results varied by experiment. Herbicide-

induced senescence resulted in higher soluble C concentration for prematurely senesced plants compared to normal plants. Contrastingly, soluble C concentrations were similar for fungal pathogen-killed or prematurely senesced wild *in-situ* populations compared to normal plants.

Understanding how litter composition affects shifts in microbial populations could assist in efforts to create large-scale die-offs of *B. tectorum* in the field. The ability to manipulate die-off events could provide a key management strategy for improving success of restoration efforts in arid and semi-arid regions of the western United States (Akk et al. 2017; Franke et al. 2014; Guo et al. 2017; Meyer et al. 2014; Sheley et al. 2009). These results illustrate increased N concentrations that led to decreased C:N ratios which could facilitate accelerated decomposition of prematurely senesced litter and potentially terminate fungal pathogen suppression (e.g., fungistasis). Results support the hypothesis that prematurely senesced plants with failed seed production contain increased N concentrations compared with plants that completed their life cycle.

MANAGEMENT IMPLICATIONS

The winter annual *Bromus tectorum* (cheatgrass) is now landscape dominant throughout the semi-arid western United States. Invasion seriously alters biodiversity, species composition, fire frequency and nutrient cycles, which significantly disrupts ecosystem functioning. Understanding changes in *B. tectorum* litter quality and quantity when seed production is prevented may contribute to restoration efforts. Premature senescence caused by epidemics of the crown-root pathogen *Clarireedia capillus-albis* or by herbicide treatment is shown here to significantly affect litter quality. These changes in litter composition may direct successional trajectories and influence the soil microbial community, thereby informing management

strategies for restoring invaded wildlands. Additionally, identifying the influences of prematurely senesced litter on the soil microbial community may allow prediction or triggering of seedling emergence failure in targeted areas. Our study concluded that failed seed production associated with prematurely senesced *B. tectorum* plants consistently resulted in increased litter N concentrations, thereby decreasing C:N ratios, and also increased soluble C concentrations under some scenarios. Prematurely senesced litter may influence the ecology of an invaded wildland through increased N and possibly pulses of available carbon both during decomposition as well as leaching of these nutrients following precipitation. This nutrient pulse could lead to increased fungal pathogen activity in the soil (e.g., *Fusarium* species known to attack germinating seeds) and contribute to large-scale *B. tectorum* die-off events, resulting in a window of opportunity for restoring invaded wildlands.

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FIGURES

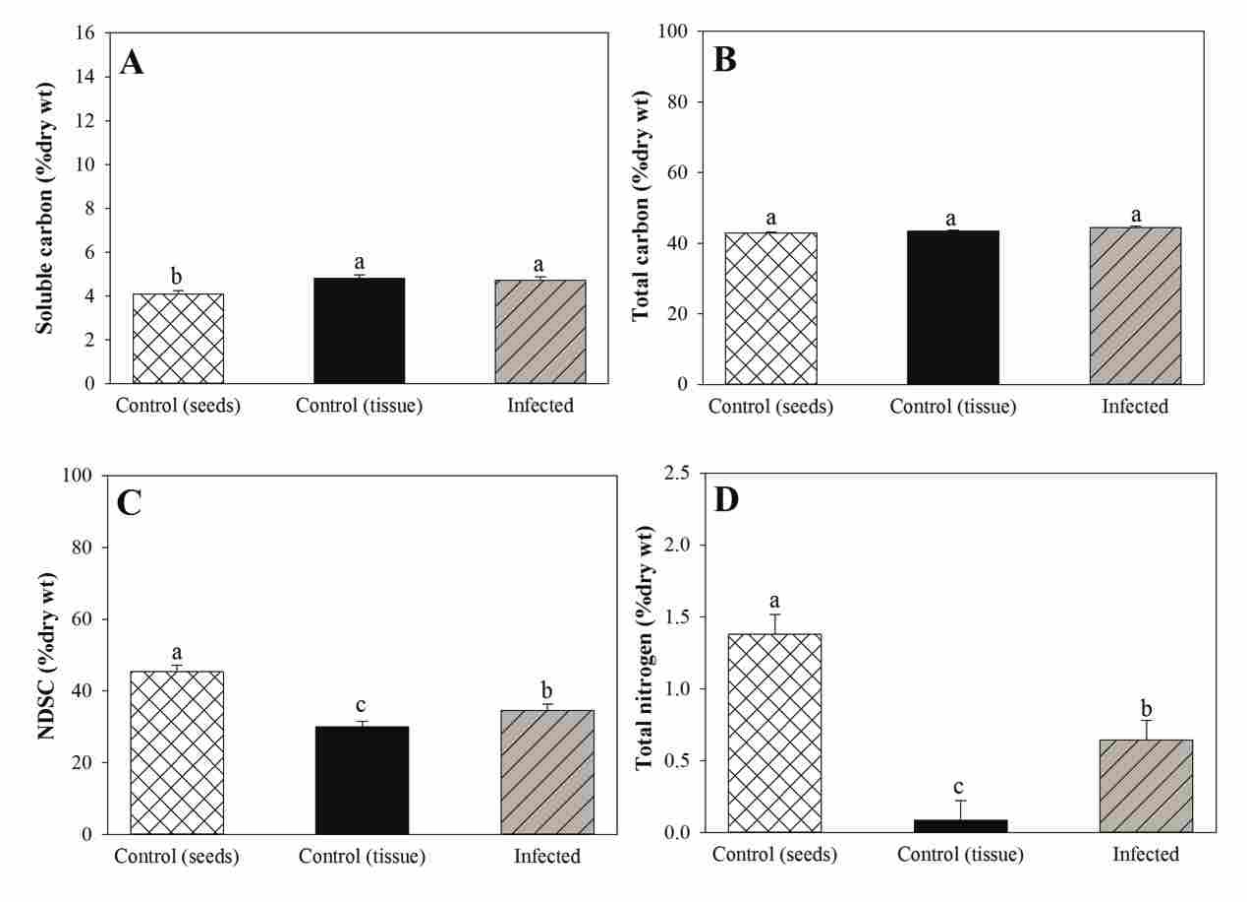

Figure 1. Concentrations of soluble carbon (A), total carbon (B), neutral detergence soluble carbohydrates (NDSC) (C), and total nitrogen (D) in aboveground biomass for control (uninfected) and prematurely senesced (infected) tissue by *Clarireedia capillus-albis.* Different letters above columns for each graph indicate significant differences ($P<0.05$) as determined by a Least Significant Difference mean separation test. Error bars represent standard error of the mean.

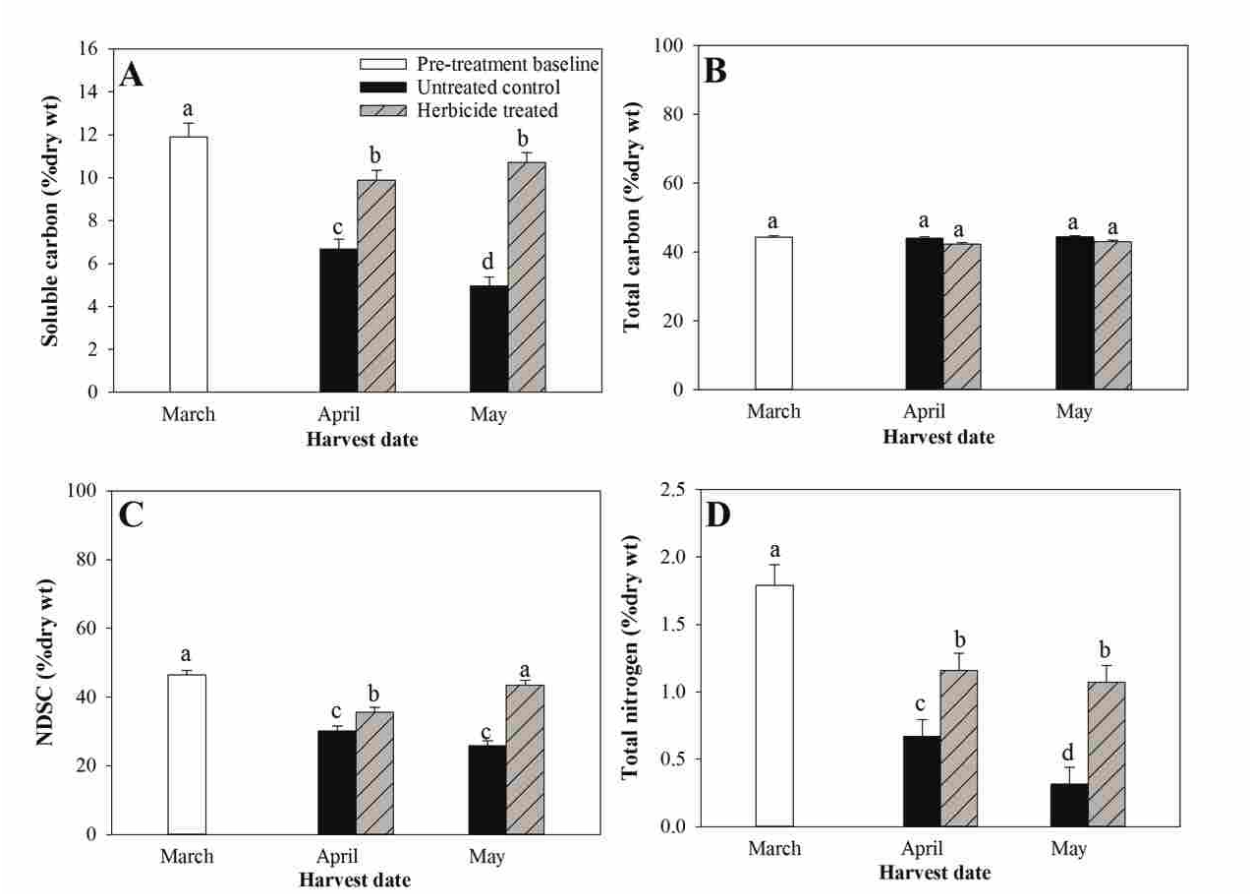

Figure 2. Concentrations of soluble carbon (A), total carbon (B), neutral detergence soluble carbohydrates (NDSC) (C), and total nitrogen (D) at the Nevada study site. Different letters above columns for each graph indicate significant differences ($P<0.05$) as determined by a Least Significant Difference mean separation test. Error bars represent standard error of the mean.

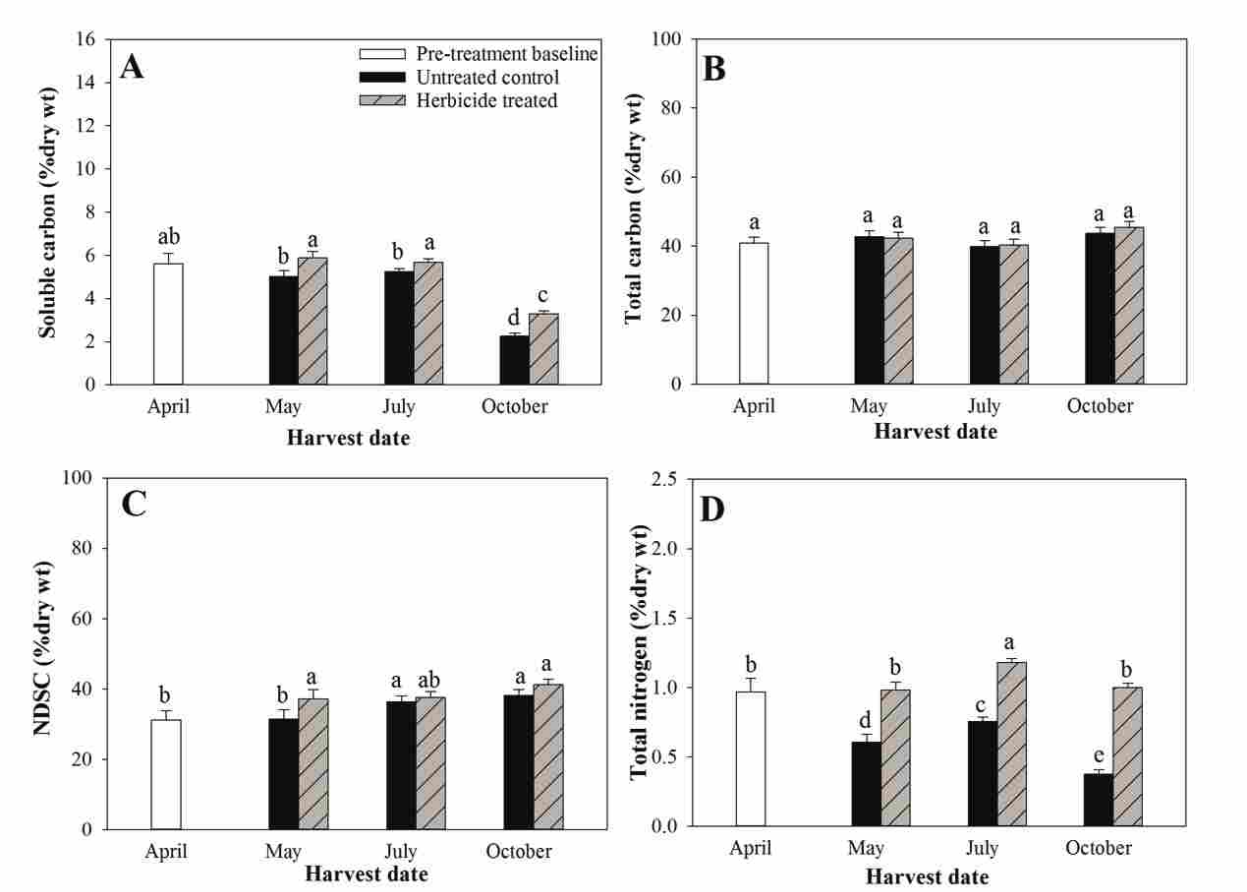

Figure 3. Concentrations of soluble carbon (A), total carbon (B), neutral detergence soluble carbohydrates (NDSC) (C), and total nitrogen (D) at the Utah study site. Different letters above columns for each graph indicate significant differences ($P<0.05$) as determined by a Least Significant Difference mean separation test. Error bars represent standard error of the mean.

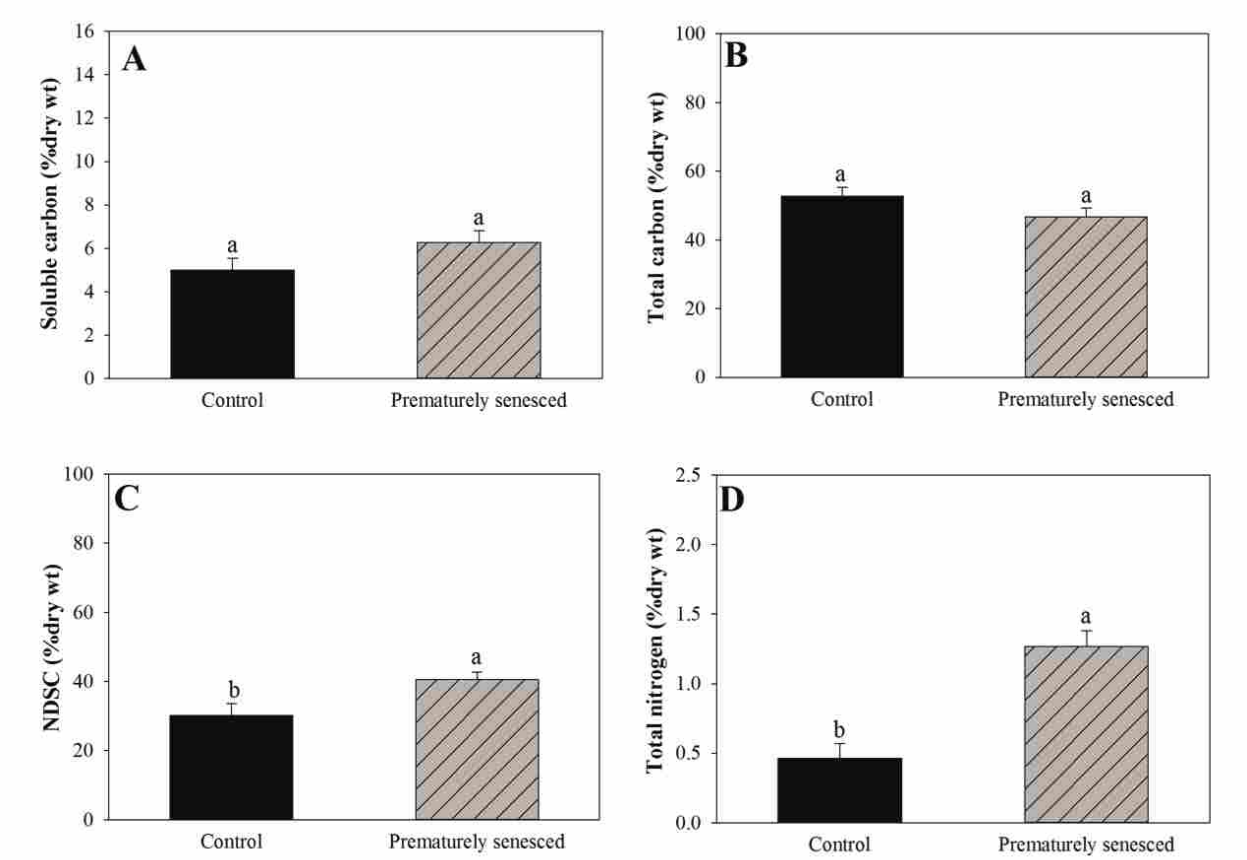

Figure 4. Concentrations of soluble carbon (A), total carbon (B), neutral detergence soluble carbohydrates (NDSC) (C), and total nitrogen (D) in aboveground vegetative tissues of in situ Bromus tectorum populations collected in the field. Plants were collected from wild B. tectorum populations at eight locations throughout northern Utah in summer 2016. Control plants were collected after they had reached maturity and completed seed production. Prematurely senesced plants failed to produce seeds and turned straw colored when healthy plants were still green or purple. Different letters above columns for each graph indicate significant differences ($P<0.05$) as determined by a Least Significant Difference mean separation test. Error bars represent standard error of the mean.

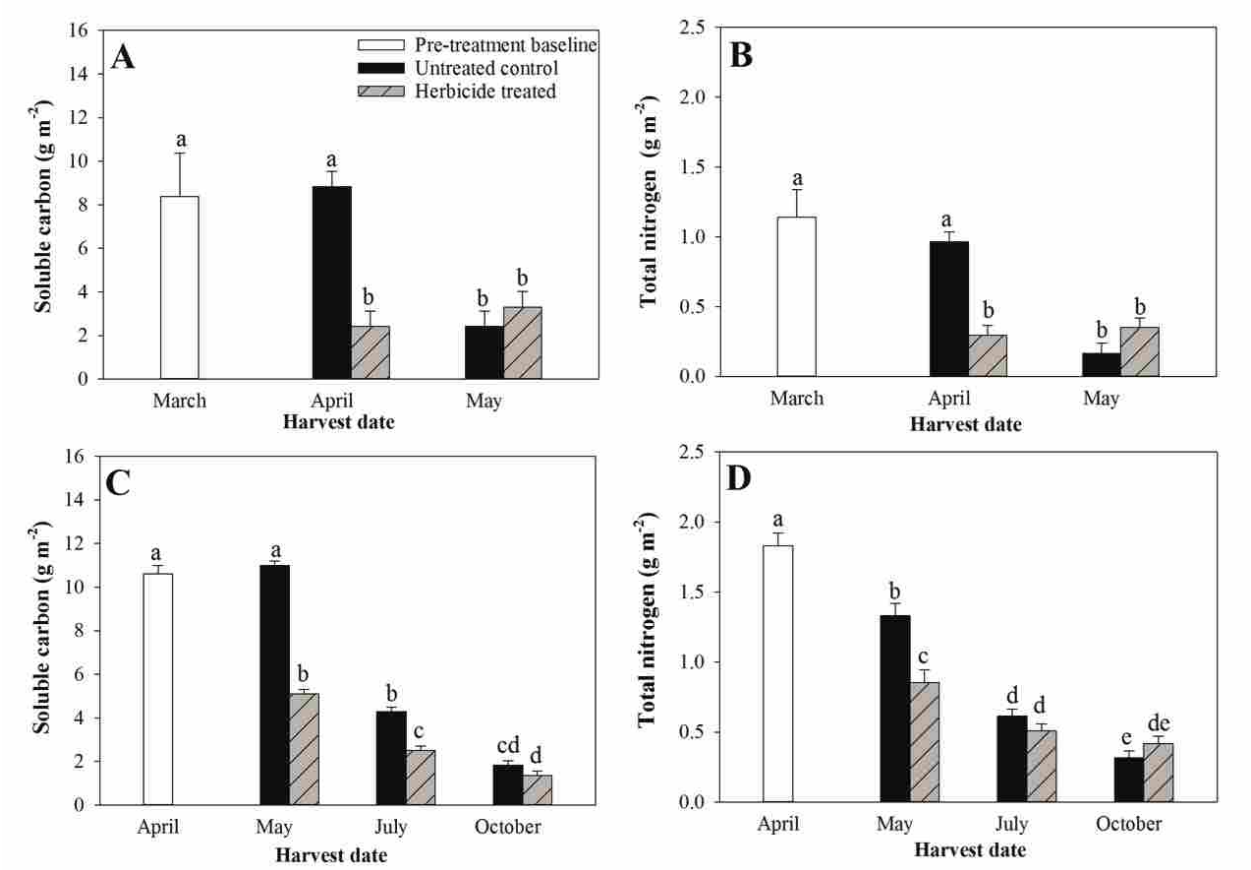

Figure 5. Litter concentrations using tissue biomass for soluble carbon (A,C) and total nitrogen (B,D). Concentrations were calculated for the imposed senescence study at the Nevada site (A,B) and Utah sites (C,D). Different letters above columns for each graph indicate significant differences ($P<0.05$) as determined by a Least Significant Difference mean separation test. Error bars represent standard error of the mean.

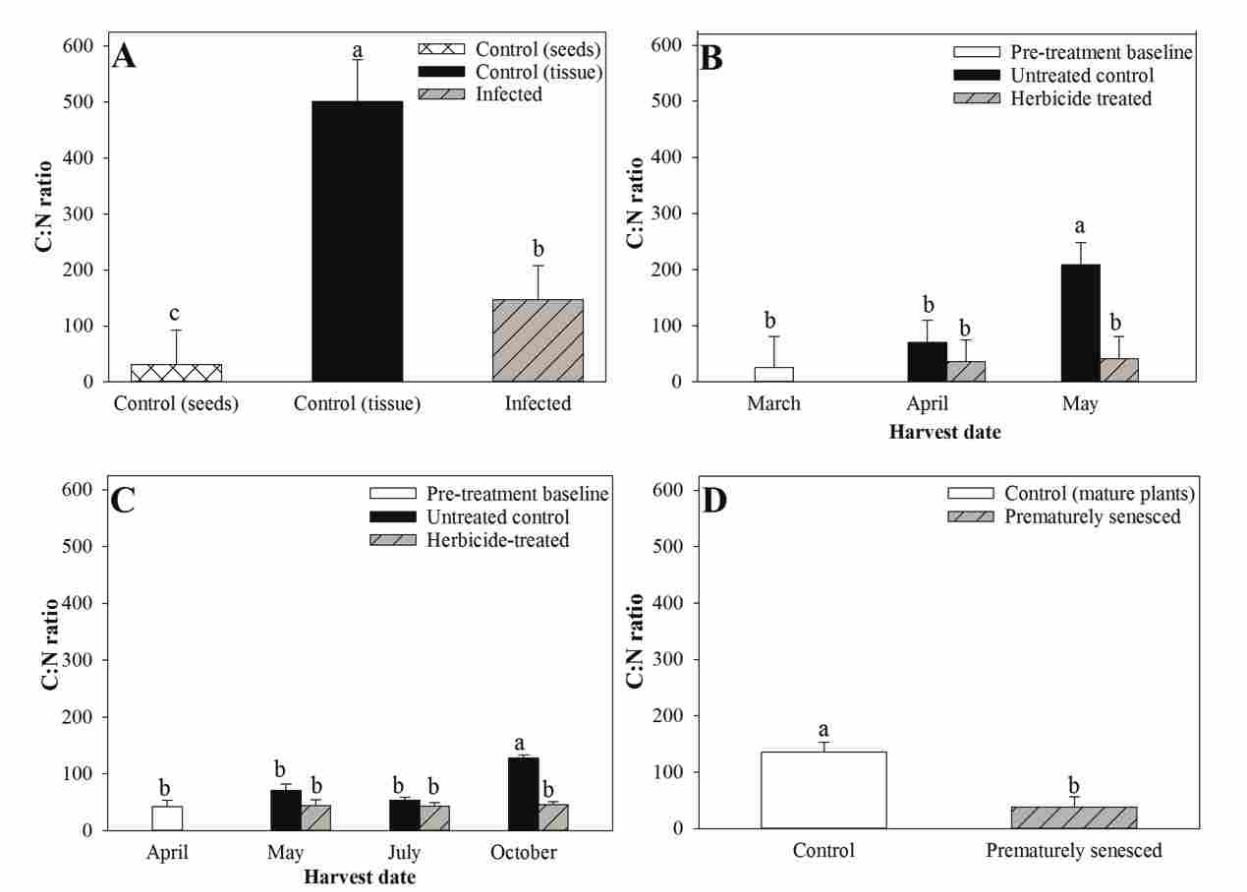

Figure 6. Total carbon to nitrogen ratio (C:N) for *Clarireedia capillus-albis* infection experiement (A), imposed senescence through herbicide application study at the Nevada site (B) and Utah site (C), and the *in situ* field population study (D). For aboveground plant biomass, pre-treatment baseline (in B,C) represents green tissue collected prior to herbicide treatment. Different letters above columns for each graph indicate significant differences ($P<0.05$) as determined by a Least Significant Difference mean separation test. Error bars represent standard error of the mean.

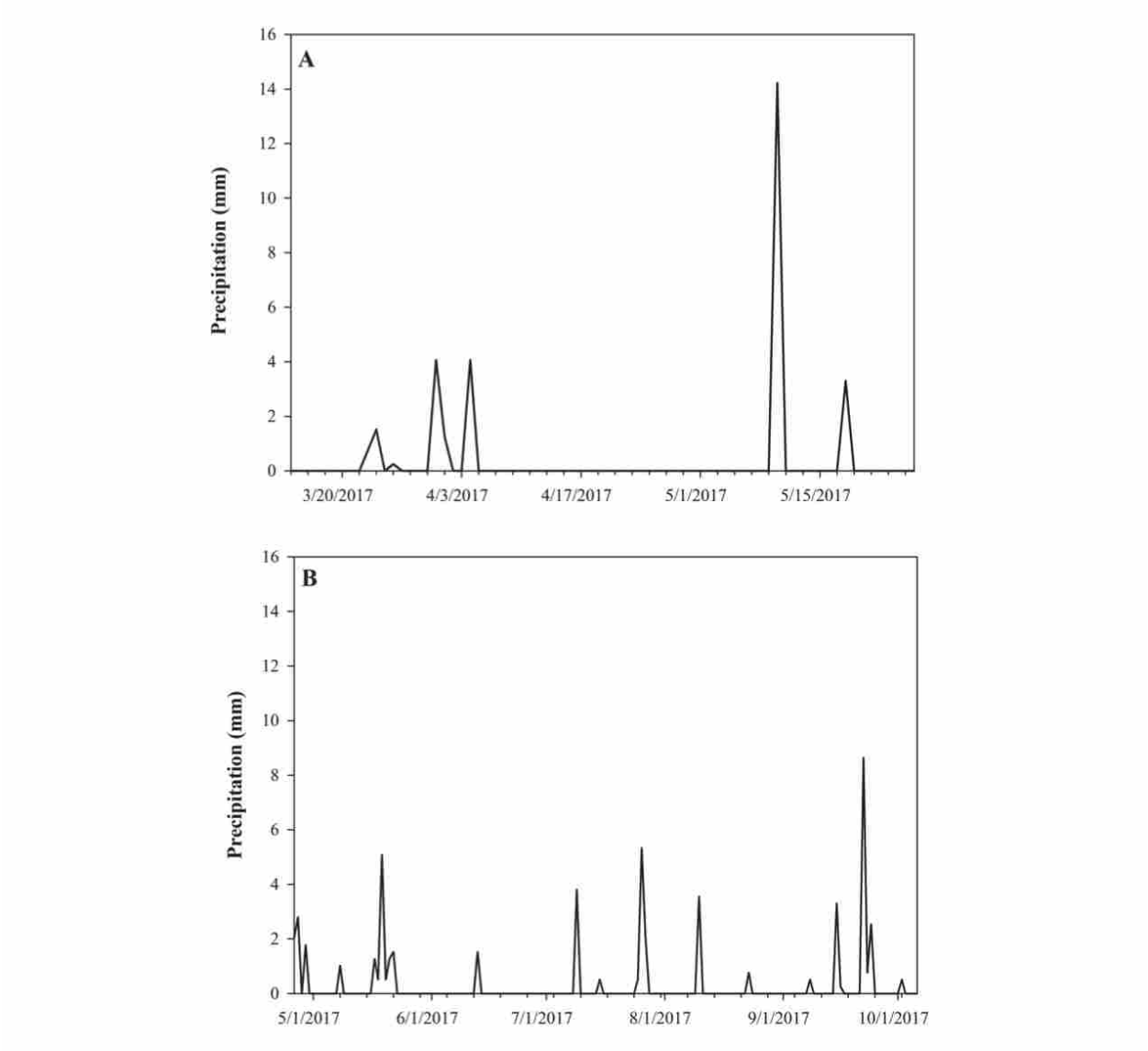

Figure 7. Precipitation estimates for the Nevada (A) and Utah (B) sites during the study period. Estimates based on the Prism Climate Group from Oregon State University (http://prism.oregonstate.edu)

TABLES

Table 1. Statistical summary for concentrations of C and N in aboveground biomass and seeds for prematurely senesced *Bromus tectorum* plants following infection by *Clarireedia capillus-albis.* Nutrients were measured as soluble C, total C, neutral detergent soluble carbohydrates (NDSC), total N, and C:N ratios for infected and uninfected plants and uninfected seeds.

Variable (%dry wt.)	**Effect**	**DF**	**SS**	**F ratio**	**P-value**
Soluble carbon	± *Clarireedia capillus-albis* infection	2	1.8225349	5.4195	0.0169
Total carbon	± *Clarireedia capillus-albis* infection	2	3.7508333	2.5083	0.1149
NDSC	± *Clarireedia capillus-albis* infection	2	588.90931	20.8178	0.0002
Total nitrogen	± *Clarireedia capillus-albis* infection	2	4.0000632	15.5549	0.0004
C:N ratio	± *Clarireedia capillus-albis* infection	2	550674.11	12.3145	0.0010

Table 2. Statistical summary for C and N concentrations in aboveground biomass for the Southern Nevada herbicide application experiment. Samples were harvested in March, April, and May 2017.

Variable (%dry wt.)	Effect	DF	Den DF	F-value	P-value
Soluble carbon	±Herbicide	1	18	112.046	0.0001
	Sample date	1	18	2.9311	0.1041
	±Herb*sample date	1	18	4.3681	0.0525
Total carbon	±Herbicide	1	20	17.0222	0.0005
	Sample date	1	20	2.3324	0.1424
	±Herb*sample date	1	20	0.2749	0.6059
NDSC	±Herbicide	1	18	55.3045	0.0001
	Sample date	1	18	0.2365	0.6326
	±Herb*sample date	1	18	9.7987	0.0058
Total nitrogen	±Herbicide	1	18	63.4328	0.0001
	Sample date	1	18	10.6467	0.0043
	±Herb*sample date	1	18	0.6483	0.4312
C:N ratio	±Herbicide	1	18	6.2760	0.0221
	Sample date	1	18	3.1663	0.0921
	±Herb*sample date	1	18	2.6730	0.1194
Soluble carbon g m^{-2}	±Herbicide	1	20	1.7161	0.2065
	Sample date	1	20	0.0144	0.9036
	±Herb*sample date	1	20	9.7969	0.0052
Total nitrogen g m^{-2}	±Herbicide	1	18	3.4969	0.0773
	Sample date	1	18	0.2401	0.6288
	±Herb*sample date	1	18	8.7616	0.0084

Table 3. Statistical summary for concentrations of C and N in aboveground biomass for the Northern Utah herbicide application experiment. Samples were harvested in April, May, July, and October 2017.

Variable (%dry wt.)	Effect	DF	Den DF	F-value	P-value
Soluble carbon	±Herbicide	1	48	21.1851	0.0001
	Sample date	2	48	187.8432	0.0001
	±Herb*sample date	2	48	2.0280	0.1427
Total carbon	±Herbicide	1	48	0.3068	0.5822
	Sample date	2	48	13.8433	0.0001
	±Herb*sample date	2	48	0.4758	0.6345
NDSC	±Herbicide	1	46	5.7778	0.0203
	Sample date	2	46	5.2190	0.0091
	±Herb*sample date	2	46	0.8226	0.4456
Total nitrogen	±Herbicide	1	48	186.4607	0.0001
	Sample date	2	48	43.4589	0.0001
	±Herb*sample date	2	48	6.8889	0.0023
C:N ratio	±Herbicide	1	48	48.4960	0.0001
	Sample date	2	48	32.2780	0.0001
	±Herb*sample date	2	48	18.4125	0.0001
Soluble carbon g m^{-2}	±Herbicide	1	46	1.5876	0.2134
	Sample date	2	46	8.01	0.0068
	±Herb*sample date	2	46	6.3001	0.0158
Total nitrogen g m^{-2}	±Herbicide	1	46	2.4336	0.1257
	Sample date	2	46	1.7956	0.1864
	±Herb*sample date	2	46	4.9284	0.0312

Table 4. Statistical summary for C and N concentrations in aboveground biomass for *in situ* population sampling of *Bromus tectorum* plants in the field. Prematurely senesced and normal mature plants were harvested and differences in *B. tectorum* aboveground litter were measured for soluble carbon, total carbon, neutral detergent soluble carbohydrates (NDSC), and total nitrogen. Samples were harvested at maturity.

Variable (%dry wt.)	**Effect**	**DF**	**SS**	**F-ratio**	**P-value**
Soluble carbon	±Premature senescence	1	5.7600286	2.7683	0.1220
Total carbon	±Premature senescence	1	62.401905	5.2030	0.0714
NDSC	±Premature senescence	1	306.78831	6.5500	0.0250
Total nitrogen	±Premature senescence	1	1.1194448	22.2307	0.0053
C:N ratio	±Premature senescence	1	16212.358	7.0900	0.0447